ASSESSMENT OF FACTORS INFLUENCING THE POTENTIAL EFFECTIVENESS OF A MATERNAL RESPIRATORY SYNCYTIAL VIRUS VACCINE PROGRAM AMONG WOMEN IN KILIFI AND SIAYA, KENYA

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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DEDICATIONS

I dedicate this thesis to my family, my children Teddy, Kevin, Joshua and Malkia, my husband Kibet and all those who have tirelessly walked with me in this journey.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ANC	Antenatal care
ARI	Acute respiratory infection
CDC	Center for Disease Control and prevention
CGMR-C	Centre for Geographic Medicine Research-Coast
CGHR	Centre for Global Health Research
HDSS	Health and Demographic Surveillance System
KEMRI	Kenya Medical Research Institute
WTRP	Wellcome Trust Research Programme
KCH	Kilifi County Hospital
KHDSS	Kilifi Health Demographic Surveillance System
NPS	Nasopharyngeal swab
RSV	Respiratory syncytial virus
PRNT	Plaque reduction neutralization test
UK	United Kingdom
LRTI	Lower respiratory tract infection
WHO	World Health Organisation
PATH	Programme for Appropriate Technology in Health
LMIC	Low- and Middle-Income Countries
LMP	Last Menstrual Period
LIC	Low Income Countries
IgG	Immunoglobulin G
ILI	Influenza Like Illness

SUMMARY

Introduction

Respiratory syncytial virus (RSV) is a significant cause of severe pneumonia among infants under 6 months of age worldwide and maternal immunization is being considered as a realistic strategy for prevention. Several maternal RSV vaccine candidates are at advanced stages of clinical evaluation, with some showing promising results. However, gaps in knowledge of the factors likely to influence its effectiveness, might hinder the successful implementation of the maternal RSV vaccination program in Kenya.

Objectives

The aim of this study was to provide a comprehensive assessment of three major factors: (i) timing for antenatal care (ANC) attendance, (ii) efficiency of transplacental transfer of RSV antibody and (iii) baseline rates of adverse birth outcomes, and how these are likely to influence the potential effectiveness of a maternal RSV vaccine program in Kenya.

Methods

Data on timing for ANC attendance and birth outcomes was obtained from a sample of 2219 women (594 Kilifi and 1625 Siaya) residents of Health and Demographic Surveillance System (HDSS) areas, who were registered as pregnant in 2017 to 2020 through census rounds and whom by the time of data collection had a birth outcome. These women were traced at home, consented and if willing, data of gestational age at attendance for ANC screening, birth outcomes and uptake of ANC services was abstracted from their mother and child health (ANC) booklets. The efficiency of transplacental transfer of RSV-specific antibodies was determined by cord blood to maternal blood titre ratio (CMTR) using cord-maternal pairs of blood samples from 400 pregnant women (200 Kilifi surveillance cohort and 200 Siaya surveillance cohort). These samples were screened for RSV specific antibodies using plaque reduction

neutralization test (PRNT). Independent predictors of an impaired transplacental transfer were determined using logistic regression models.

Results

From a total of 470/594 (79%) pregnant women, enrolled from Kilifi HDSS, with a card confirmed ANC attendance, the proportion that attended ANC within the required gestational age window for vaccine delivery of 28-32 weeks, 26-33 weeks and 24-36 weeks was 76.6%, 84.5% and 96.2%, respectively. In Siaya HDSS, from a total of 791/1029 (76.9%) women with ANC booklets, the proportion that attended ANC within the proposed gestational age window for vaccine delivery of 28-32 weeks, 26-33 weeks and 24-36 weeks was 76.9% (608/791), 82.4% (652/791) and 90.4% (715/791), respectively. Estimated maternal RSV vaccine coverage among Kilifi HDSS pregnant women, assuming vaccine delivery was only through ANC clinics and 55% of these women within Kilifi per year attended ANC, was, 42.1%, 46.5% and 52.9% within gestational age windows of 28-32 weeks, 26-33 weeks and 24-36 weeks, respectively.

Delayed ANC initiation was significantly associated with being of older maternal age (χ^2, P = 0.022), education below secondary level (χ^2, P = 0.021) and home births (χ^2, P < 0.001). The transplacental transfer of RSV-specific antibodies among pregnant women in Kilifi and Siaya was found to be efficient with a CMTR of 1.02 and did not differ between women from Kilifi and Siaya (1.02 vs 1.02; p=0.946). Women from Kilifi and Siaya HDSS sites were significantly different in most demographic characteristics. Infants born from HIV infected mothers showed a significantly reduced transplacental transfer of RSV-specific antibodies (mean CMTR :0.98 vs 1.03; p=0.015), while prematurity <33 weeks was a strong predictor of an impaired transplacental transfer of RSV specific antibodies (Odds ratio (OR): 0.23, 95% confidence interval (CI)0.06-0.85; p=0.028). About a third of the pregnancies 781/2219 (35%) from Kilifi and Siaya HDSS sites had adverse birth outcomes, most of which occurred at home than in hospital (43.8% vs 34.2 %; p=0.003). Adverse birth outcomes were not significantly different between

Kilifi and Siaya women (38.2% vs 34.1%; p=0,072). Premature births 490/2219(22.1%) were the most common adverse birth outcomes observed in this study. Predictors of adverse birth outcomes were gestational diabetes (aOR 3.01 (1.24-7.30; p=0.015) and home delivery (aOR 2.48 (1.20-5.13); p=0.014). Being multiparous (aOR 0.52 (0.33-0.81); p=0.004) showed a protective effect on adverse birth outcomes.

Conclusion

The differences in characteristics of women from the diverse geographical regions in Kenya might not have a significant effect on vaccine outcomes. The maternal RSV vaccine program will require integration of other strategies that will ensure maximum benefit to infants born from HIV infected mothers and those born premature<33 weeks gestation. Any significant change of adverse birth outcomes from the current baseline rates will be a useful indicator in monitoring safety of the maternal RSV vaccine during implementation. Successful implementation of the maternal RSV vaccine program in Kenya will largely depend on individual's willingness to present for ANC screening and acceptance of utilizing health care services starting from early pregnancy to delivery. Therefore, initiatives which mitigate against delayed ANC attendance and against restricted access to quality obstetric services, are likely to improve maternal RSV vaccines uptake when they become available.

CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 Background Information

Respiratory Syncytial Virus (RSV) is one of the most common causes of acute lower respiratory tract infection among children under 5 years (Li et al., 2022; Nair et al., 2013; Shi et al., 2017b). Globally, RSV has been estimated to cause 25.4 to 44.6 million cases of acute lower respiratory infection, 2.9 to 4.6 million hospitalizations and 15,100 to 49,100 in-hospital deaths in children aged 0-60 months that occurred in 2019 (Li et al., 2022). A previous review of global data has shown that, RSV causes over 30 million new episodes of lower respiratory tract infections (LRTI) and 3 million hospitalisations annually in young children worldwide, with 96% of these occurring in the developing world(Nair et al., 2010). A pneumonia aetiology study, conducted in sub-Saharan Africa and Asia between 2011-2014, in which Kilifi County Hospital was one of the participating sites, showed that nearly 40% of all hospital admissions with severe or very severe pneumonia among infants less than 1 year were caused by RSV (PERCH, 2019). Other pneumonia aetiology studies conducted in Nairobi and Kilifi in Kenya, have shown RSV to be associated with between 12-30% of all viral confirmed pneumonia hospital admissions in children under 5 years of age (Berkley et al., 2010; Nokes et al., 2009).

Severe RSV associated disease predominantly occurs during infancy; between 1-5 months of age (Li et al., 2022; Nokes et al., 2009; Shi et al., 2017a), and this is the primary target age group for disease prevention by vaccination. A systematic review of global data estimated that in 2015, RSV was associated with 1·4 million hospital admissions and 27,300 in-hospital deaths globally among infants less than 6 months of age (Shi et al., 2017a). The burden of RSV disease has drastically increased, with increased RSV associated hospitalizations being experienced in Europe and other

countries since 2021 after easing down covid-19 pandemic control measures such as lockdown and restricted movement (Bardsley et al., 2022). Clinical trials for childhood RSV vaccines began in the early 1960's, but to date no infant RSV vaccines have been licensed. During the early clinical trials which were conducted in the United States of America (USA), participants of the first formalin inactivated RSV vaccine developed enhanced RSV disease which caused two deaths among infants (Kapikian, Mitchell, Chanock, Shvedoff, & Stewart, 1969; H. W. Kim et al., 1969; Murphy, Alling, et al., 1986). Additionally, there has been difficulty in designing an immunogenic live-attenuated vaccine that is well tolerated by the young infant (Karron, Buchholz, & Collins, 2013) due to immature immune system and other infant vaccine candidates have not been able to elicit protective immune response because of interference with maternal antibodies (Anderson et al., 2013; Siegrist & Lambert, 1998).

The focus for preventing the infant from severe RSV disease (Jares Baglivo & Polack, 2019) has therefore shifted to designing alternative strategies. One such strategy in consideration is maternal immunization (Engmann et al., 2020), to boost RSV antibodies during pregnancy, so that the infant is born with a high level of antibodies, which is protective within the first six months of life when most at risk of severe disease (Madhi et al., 2020). The development of maternal RSV vaccines is at advanced stages, and some candidates are showing promising results (Engmann et al., 2020; Higgins, Trujillo, & Keech, 2016). The priority is to introduce these vaccines to low and middle-income countries (LMICs) where the burden of RSV-associated disease is highest (Modjarrad et al., 2016).

Several candidate maternal RSV vaccines are in phase 2 and phase 3 of clinical trials (Higgins et al., 2016; PATH, 2022). A subunit vaccine RSV F prefusion protein, from GlaxoSmithKline (GSK) completed phase 2 of clinical trials (NCT02753413) in Belgium between April and June 2016 involving 102 healthy women (Beran et al., 2018) and

advanced to phase 3 clinical trials in May 2020 (NCT04605159). Early-stage clinical trials (NCT02298179) of the F subunit protein vaccine showed it was safe and immunogenic and vaccine doses were well tolerated within 6 months of follow up (Leroux-Roels et al., 2019). Another RSV F subunit protein vaccine design from Pfizer began phase 3 trials (NCT04424316) in October 2020 after completing phase 2 clinical trials (NCT04032093) among pregnant women in five countries (US, Argentina, Chile, South Africa and New Zealand). Results from a phase IIb (NCT04032093) from Pfizer (http://www.resvinet.org/uploads/2/2/2/7/22271200/abstract_booklet_rsvvw21.pdf), has shown efficacy of 84.7% (95% CI; 21.6%, 97.6%) in preventing medically attended infant RSV LRTI. A maternal RSV vaccine candidate which is of nanoparticle design (NCT02624947) from Novavax completed phase 3 of clinical trials in early 2019. Results of the phase 3 trial, showed that, the vaccine prevented RSV associated disease hospitalization in young infants up to 3 months of age, by 44.4% (95% CI; 19.6 - 61.5%) and mothers immunized <33 weeks of gestational age had higher vaccine efficacy across all endpoints (Engmann et al., 2020; Madhi et al., 2020; Novavax, 2019).

There has been debate surrounding the introduction of the maternal RSV vaccine and its effectiveness in developing countries (Modjarrad et al., 2016). This has been due to the differences in characteristics of maternal populations in developing countries with those of high-income country settings where most clinical trials are currently ongoing. The key factors likely to impact the effectiveness of the maternal RSV vaccine would possibly go beyond the cold chain process and other programmatic factors required for efficient vaccine delivery within the health care system. In addition, unlike the tetanus vaccine, which is the only maternal vaccine that has been in use in Kenya and other developing countries for several decades (Ibinda et al., 2015), maternal RSV vaccine requires delivery within a restricted window of gestational age.

Following concerns about the successful implementation of the maternal RSV vaccine in

LMICs, a convention of RSV experts drawn from clinicians, researchers, policy makers and vaccine manufacturers was done in Geneva in March 2015 (Modjarrad et al., 2016). The primary objective was to provide guidance on clinical endpoints and development pathways for vaccine trials with focus on LMICs (Modjarrad et al., 2016). Through this meeting, several knowledge gaps in implementation were identified. This was followed by a gap analysis report which was coordinated by the Program for Appropriate Technology in Health (PATH) (https://www.path.org/resources/roadmap-advancing-rsv-maternal-immunization/, highlighting critical gaps in evidence that, if left unfilled, could delay maternal RSV vaccine introduction in LMICs (PATH, 2018) some of which this study sought to address.

It is also thought, a maternal RSV vaccine could save thousands of infants' lives, if used optimally and if the key factors likely to impact the effectiveness of the vaccine program in a developing country setting are clearly understood. The transplacental transfer of Immunoglobulin G (IgG) antibodies during the third trimester of pregnancy has been shown to be an efficient active process (Chu et al., 2014; Okoko, Wesumperuma, & Hart, 2001a; R. Suara et al., 1996). Therefore, maximum benefit to the infant requires vaccine delivery at the optimal gestational age, which depends on degree of boosting and rate of subsequent decay of antibodies following vaccination. This timing for vaccination in relation to delivery of the baby is very critical and suggests a window of opportunity when it is best to deliver the maternal RSV vaccine. Thus, distribution of gestational age at attendance for ANC screening by pregnant women in Kenya is critical information required to advise on the timing for a maternal RSV vaccine delivery.

The level of antibody transferred to the infant will also depend on other factors such as hypergammaglobinaemia, placental malaria, and possible adverse events occurring during the third trimester of pregnancy (B. J. Okoko, L. H. Wesumperuma, M. O. C. Ota, et al., 2001; J. B. Okoko et al., 2001a). Vaccine effectiveness may also be influenced by other confounding factors, whether socio-demographic, cultural or other beliefs and

perceptions on its safety (Fabry, Gagneur, & Pasquier, 2011). Currently, the factors that hinder pregnant women from utilizing health care services in Kenya are not clearly understood.

At present, there is little data in Kenya or sub-Saharan Africa in general on the distribution of gestational age at presentation for antenatal care (ANC) screening. The national Demographic Health Surveys (DHS) (GOK, 2014) only record the first ANC visit and therefore, there is no information available on gestational age distribution for women attending the subsequent visits. Currently, WHO suggests a vaccination window for maternal RSV vaccine delivery of between 28-32 weeks of gestation. Another proposed gestational age window for maternal RSV vaccine delivery is 24-36 weeks (Baral, Li, et al., 2020). The gestational age window of 28-32 weeks has also been found to have higher anti-pertussis IgG avidity for maternal Tetanus-Diphtheria-acellular pertussis vaccine when administered during this period (Abu-Raya, Giles, Kollmann, & Sadarangani, 2019). However, vaccine coverage and effectiveness will depend on whether a majority of the women attend ANC within this period in Kenya. There is also scanty information on the frequency of RSV infections among pregnant women and how these will influence the level of RSV antibody at birth. Little is also known about the type and incidence of adverse events occurring during the 3rd trimester of pregnancy that might coincide with the time of vaccine delivery and affect the efficiency of transplacental transfer. Additionally, there is scanty data about pregnancy outcomes of births occurring at home and their impact in validating safety outcomes of a maternal vaccine program.

Through this thesis, a comprehensive assessment of the factors that would impact successful implementation of a maternal RSV vaccine program in Kenya has been provided. The study utilized existing Health and Demographic Surveillance System (HDSS) platforms run by KEMRI-Centre for Global Health Research (CGHR) in Siaya (western Kenya) and KEMRI-Centre for Geographical Medicine Research-Coast

(CGMRC) in Kilifi (coastal Kenya) to obtain data. The findings in this study provide critical guidance in policy development towards implementing a maternal RSV vaccine program and provide important baseline data for clinical trials of new maternal vaccines in Kenya.

1.2 Statement of the Problem

Respiratory syncytial virus is a significant cause of severe pneumonia among infants less than 6 months of age worldwide. Development of childhood vaccines to provide direct protection against RSV disease in this age group has not been successful. An alternative strategy being considered is to vaccinate mothers during pregnancy so that their infants are born with increased levels of RSV specific antibodies that can protect them during the period when they are most at risk. There are plans to introduce these vaccines to low and middle-income countries like Kenya in the near future through the ANC clinics. However, there is no baseline data to guide successful implementation of a maternal RSV vaccine program in Kenya. This study aimed to assess three major factors that are likely to influence the effectiveness of the maternal RSV vaccine program in Kenya to address the existing knowledge gap.

1.3 Justification

Maternal immunization to boost RSV-specific antibodies among pregnant women to protect young infants from severe RSV associated disease is being considered as a realistic approach. Candidate maternal RSV vaccines are undergoing late-stage vaccine trials in developed countries. Unlike developed countries, a developing country setting has a maternal population that is affected by significant comorbidity such as malaria, HIV and undernutrition that could potentially impact the efficacy of a maternal vaccine program. Factors such as prematurity or low birth weight, hypogammaglobinemia, placental malaria and RSV season are known to have a potential impact on transplacental transfer of RSV specific antibodies (Nyiro et al., 2015; B. J. Okoko, L. H.

Wesumperuma, M. O. C. Ota, et al., 2001; J. B. Okoko et al., 2001a). However, data on other key factors are scant. At present, there is little data collated on the gestational age distribution of pregnant women attending antenatal care in Kenya. The efficiency of transplacental transfer of RSV specific antibodies among pregnant women in this population is not known. Little is also known about the adverse events during late pregnancy that might occur at around time of maternal RSV vaccine delivery, and how these can affect the level of RSV specific antibodies transferred to the infant. A maternal vaccine is intended to target all pregnant women, but in the low income resource setting most births still occur at home (47% in Kilifi and 27% Siaya of births) (GOK, 2014). Premature births are likely not to benefit from this program and might require consideration of other preventive strategies but currently, little is known about these pregnancy outcomes or the gestational ages of births occurring at home. These gaps in our current knowledge can confound observations in future maternal RSV vaccine trials and quantifying these ahead of the trials could lead to improved vaccine outcome.

1.4 Research Questions

The key question asked through this research project was, how will a set of factors influence the degree to which newborns benefit from the maternal RSV vaccine program in Kenya? Specific questions on the relevant factors were:

i. What is the distribution of gestational ages at presentation for antenatal screening among pregnant women in Kenya that could help in making decisions about the appropriate time to give a maternal RSV vaccine?

ii. How efficient is the transplacental transfer of RSV specific antibody among pregnant women in Kenya?

iii. What are the main complications/illnesses during late pregnancy that would occur at around the same time as vaccine delivery? How do these complications/illnesses influence the level of RSV antibody transferred to the baby?

iv. What are the differences in birth outcomes and distribution of gestational ages at birth between those occurring at home and those in the hospital?

v. What factors influence the choice of a place to deliver a baby or uptake of an intervention?

vi. What proportion of infants in Kenya is likely not to benefit from the maternal RSV vaccine program because they are born premature?

1.5 Hypothesis

This is a descriptive study therefore null hypothesis is not applicable.

1.6 Objectives

1.6.1 General Objective

To assess factors that are likely to influence the potential effectiveness of a maternal RSV vaccine program in Kenya.

1.6.2 *Specific Objectives*

1. To describe the distribution of gestational ages at presentation for antenatal care screening, among pregnant women in Siaya and Kilifi, Kenya.

2. To determine the efficiency of transplacental transfer of RSV-specific antibodies among pregnant women in Kenya.

3. To quantify birth outcomes among pregnant women in Siaya and Kilifi, Kenya.

CHAPTER TWO

2.0 LITERATURE REVIEW

This chapter contains review of literature on RSV occurrence and epidemiology, the burden of RSV disease, strategies for preventing RSV disease among infants, evidence of protection by maternal antibodies, progress in maternal RSV vaccines development and factors likely to affect the potential effectiveness of a maternal RSV vaccine.

2.1 Respiratory Syncytial virus occurrence and epidemiology

Respiratory Syncytial Virus (RSV) is a negative sense single stranded RNA virus and ranges in size between 150-300nm. The virus has 2 important glycoproteins on its surface: the attachment (G) and the Fusion (F) protein. The G protein targets the ciliated cells of the airways, while F protein controls the initial phases of infection and causes the virion membrane to fuse with a target cell membrane (McLellan, Ray, & Peeples, 2013). The F glycoprotein is highly conserved in all RSV strains and is a target of a large proportion of the neutralizing antibodies. For this reason, the F protein is being considered suitable for a vaccine antigen (McLellan et al., 2013; McLellan, Yang, Graham, & Kwong, 2011; Taleb, Al Thani, Al Ansari, & Yassine, 2018).

Transmission of RSV occurs through contact with respiratory droplets from an infected person either through coughing, sneezing or by touching contaminated surfaces (Hall, 1982). Pathogenesis of RSV associated disease has been shown to begin when the virus enters onto the nasopharyngeal epithelium and gets transmitted to the lower respiratory tract through intercellular spaces (Piedimonte & Perez, 2014; Taleb et al., 2018). Co-infection of RSV with bacteria has been shown to enhance the severity of RSV LRTI disease and development of bacterial pneumonia among infants (Thorburn, Harigopal, Reddy, Taylor, & van Saene, 2006). This is because respiratory virus infections including RSV have also been found to alter the microbiome in the respiratory airways, which

impairs the immune system and consequently predisposing patients to secondary bacterial super-infections (Hanada, Pirzadeh, Carver, & Deng, 2018).

RSV was first isolated from chimpanzees with coryza and in 1956 it was recovered from a child with pneumonia in the USA (Chanock et al., 1961). In 1961, Chanock and group associated the virus with bronchiolitis and lower respiratory tract infection through a surveillance conducted in a children's hospital in Washington DC. In this surveillance, the virus was observed to occur in sharp outbreaks each year lasting for about 3 to 5 months which was coincident with serious acute respiratory illness among infants. It is through this surveillance, where RSV was identified as a respiratory pathogen of major significance in early life (Chanock et al., 1961). In Japan, RSV has been known as a major pathogen for respiratory infections in older adults >/=65years with a median duration of RSV-acute respiratory disease of 18 days (Kurai et al., 2022). Currently, only two RSV groups are known: A and B, which alternate between epidemics. In Cheonan, Korea, RSV prevalence was found to be different among the two subtypes and the average age of RSV-B-positive patients was higher than that of RSV-A among elderly patients above 80 years. The highest RSV detection rate (36.5%) was observed in December of each year (G. Y. Kim, Rheem, Joung, & Kim, 2020). The co-infection rate between the two RSV sub-types with other respiratory viruses such as rhinoviruses was found to increase every year (G. Y. Kim et al., 2020). A study on genomic epidemiology of RSV which was conducted in coastal Kenya, found that, a cluster of viruses emerged in the 2016/17 epidemic, carrying distinct amino-acid signatures including a novel nonsynonymous change (K68Q) in antigenic site slashed circle in the Fusion protein, highlighting possibilities of recent emergence of new antigenic variants for RSV B (E. Kamau et al., 2020).

In Western Kenya, RSV epidemics occur from March to August of each year. Whereas, in the coastal part of Kenya, through a long-term RSV surveillance which has been going on in the pediatric ward of Kilifi County Hospital (KCH) since 2002, RSV epidemics

have been found to occur from October to May with notable variation in the actual start date, peak month and duration of the circulation (Figure 1).

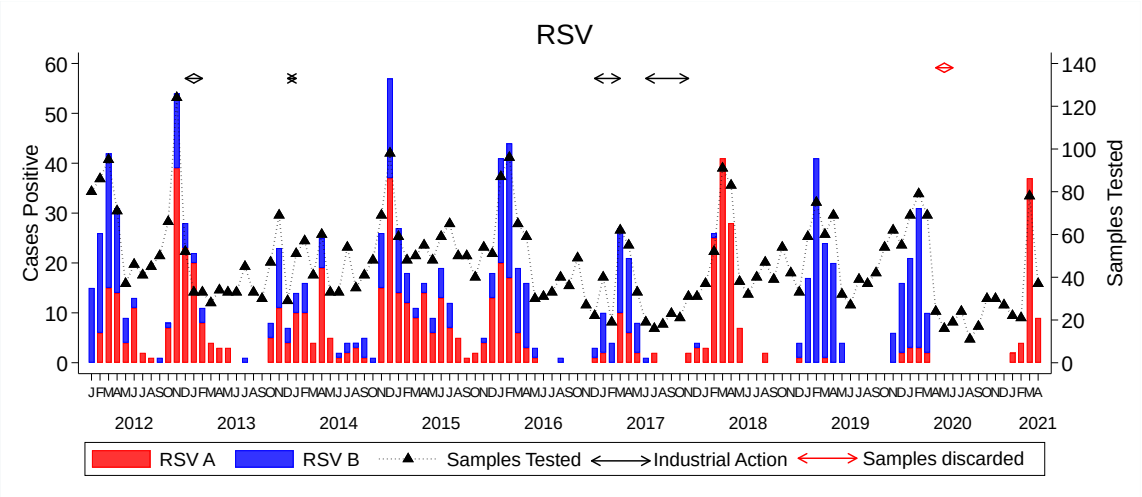

Figure 1. Seasonal occurrence of RSVA and RSVB in Kilifi, Coastal Kenya: Data from Kilifi County Hospital Pediatric inpatient surveillance 2012-2021 (Source: Long term RSV surveillance study).

Infection with RSV has been found not to induce an effective immunological memory. However, the virus is thought to modulate both humoral and adaptive immune responses. A study to investigate the association between age and the development of RSV antibody responses following natural infection among infants in coastal Kenya found that, neutralizing antibody decline rapidly to pre-infection levels following primary infection (Sande, Mutunga, Okiro, et al., 2013), and this leads to repeated infections occurring throughout life among individuals. Seroprevalence studies have also found that, the majority of primary RSV infections are acquired early in life (Faneye, Motayo, Adesanmi, & Onoja, 2014; Nyiro et al., 2017). A RSV transmission study conducted in the rural coast of Kenya by Munywoki and Colleagues, found that, the source of early life

or infant infections are older siblings within the household (Munywoki et al., 2014). In another study, the incidence of RSV has been found to decrease with age for infants older than 1 year (Poletti et al., 2015). Other data from age-specific RSV antibody acquisition studies conducted in Kenya show that, by 2 years of age, everyone shows to have experienced at least one RSV infection (Nyiro et al., 2017; Poletti et al., 2015). Due to repeated exposure to RSV infections, older children (>5 years) and adults show 100% acquisition of RSV-specific antibodies (Nyiro et al., 2017), resulting to less severe disease being experienced in this age group.

2.2 The Burden of RSV Disease

Respiratory Syncytial Virus is a significant cause of lower acute respiratory infection among young children under 5 years of age worldwide (Nair et al., 2010). Globally, RSV is estimated to result to about 3.2 million (2.7-3.8) hospital admissions and 59 600 million in-hospital deaths per year among children under 5 years with 99% of these occurring in the developing countries (Shi et al., 2017b). Through a review of global data, RSV disease was associated with 1.4 million hospital admissions and 27,300 in-hospital deaths among infants under 6 months of age that occurred in 2015 (Shi et al., 2017b). A multi-site aetiology study conducted in 2011-2014 in sub-Saharan Africa and Asia, revealed that nearly 40% of all hospital admissions with severe or very severe pneumonia among infants under 1 year were caused by RSV (PERCH, 2019). Numerous studies in Kenya have associated RSV with between 12-30% of all viral confirmed pneumonia hospital admissions in children under 5 years of age (Berkley et al., 2010; Hazlett et al., 1988; Nokes et al., 2009). Through an ongoing inpatient surveillance for respiratory viruses since 2002 at Kilifi County Hospital (KCH), RSV has been associated with annual rate of pneumonia hospitalization of 11.1 per 1000 for infants and 2.9 per 1000 for children under 5 years (Nokes et al., 2009).

Severe RSV associated disease occurs primarily in infancy, particularly in infants 0-5 months of age (Chanock et al., 1961; Li et al., 2021; Nokes et al., 2009). A study conducted in Houston, US (1975-1979), found that over 85% of infants hospitalized with RSV associated disease were infants less than 6 months (Glezen, Paredes, Allison, Taber, & Frank, 1981). Another respiratory disease surveillance done in 3 US counties surrounding Nashville, Rochester and Cincinnati from 2002-2004, found the rate of hospitalization with RSV associated disease among infants under 6 months as 17 per 1000 (Hall et al., 2009). Through a birth cohort study conducted in 2002-2007 in rural areas of coastal Kenya, it was observed that, of the 6026 children hospitalized with severe or very severe pneumonia, 65% of the case patients were aged 3 months or younger and RSV prevalence was 32% among these infants during epidemics (Nokes et al., 2009). RSV disease has also been found to be highest among infants (86.9 and 62.8 episodes per 1000 person-years of observation) in rural and urban sites of Kenya, respectively (Bigogo et al., 2013). Consequently, strategies to prevent RSV disease targeting this age group would save thousands of infants lives.

2.3 Strategies for Prevention of RSV Disease among Infants

Vaccination would have been the most effective strategy for the prevention of RSV associated disease among infants. However, to date there are no licensed vaccines for prevention of RSV disease among infants and young children. Development of childhood vaccines that could provide direct protection have been a challenge in this age group. Vaccination of young infants has been complicated by interference of maternal antibodies (Anderson et al., 2013; Siegrist & Lambert, 1998), and their immature immunological systems, hence difficulty in responding to antigens (Broadbent, Groves, Shields, & Power, 2015) among other factors.

Clinical trials of the first RSV vaccine candidate targeting infants were conducted in the 1960s using a formalin inactivated and aluminium precipitated vaccine from Pfizer,

administered intramuscularly to children who were residents of Harrison and Arthur cottages in Junior village, which was a District of Columbia welfare institution of the homeless (Kapikian et al., 1969). Participants in the vaccine trial developed enhanced respiratory disease on subsequent RSV infection, resulting in two deaths of children 14 and 16 months old (Kapikian et al., 1969; Murphy, Prince, et al., 1986). It was later found that, inactivation of the RSV virus with formalin altered the epitopes of F and G glycoproteins leading to production of non-functional neutralizing antibodies (Murphy, Prince, et al., 1986). As a result of the adverse events experienced with this vaccine, there was no further progress in clinical trials. Another attenuated vaccine MEDI ΔM2-2, developed by the use of reverse genetics systems, has been shown to be highly restricted in replication and more immunogenic in RSV seronegative children than the previous lead live attenuated RSV vaccine candidates (Karron et al., 2015). Other live attenuated RSV vaccines have also shown promising results (Karron et al., 2005; Wright et al., 2007; Wright et al., 2000) but currently there is no candidate in late-stage clinical trials. Developing RSV vaccines targeting infants has been hampered by failure of infants to mount strong immune response from the live attenuated RSV vaccine, partly due to immature immune system and interference with maternal antibodies (Esposito et al., 2016; Sande, Cane, & Nokes, 2014). As a result, a live attenuated vaccine which is at an advanced stage of clinical development in Sanofi (PATH, 2022), is planned for administration to infants and children between 6 to 18 months of age (NCT04491877).

Alternative strategies for RSV disease prevention among infants are currently in focus (Jares Baglivo & Polack, 2019). A study to investigate the source of RSV infection among infants, found that school going siblings are responsible for introducing RSV into households, leading to the infection of infants (Munywoki et al., 2014). Consequently, there are considerations to have vaccines targeting older children (Poletti et al., 2015). Several live attenuated vaccines (NCT03213405; NCT03213405) and vectored vaccines targeting older children are in different phases of clinical trials (Majhen et al., 2014;

McFarland et al., 2018; PATH, 2022; Rey-Jurado, Soto, Galvez, & Kalergis, 2017). Another strategy to protect infants is the use of non-pharmacological strategies like social distancing (e.g. school closures) during RSV epidemics, to reduce spread and transmission of the virus.

A more plausible strategy under consideration is to vaccinate the mother during pregnancy so that the infant is born with a higher level of RSV specific antibodies, which can provide protection during the time when the risk of severe disease is highest (Engmann et al., 2020; Madhi et al., 2020; Munoz, 2015) vaccine boosting of maternal antibodies is likely not to benefit infants born prematurely. This is because, the expression of Fc gamma RII (FcgII) receptor responsible for materno-foetal transfer of antibodies occurs during the third trimester of human pregnancy (Kameda et al., 1991) and these infants are born early before the transplacental transfer of IgG antibodies begins.

To prevent high risk infants such as those born premature from severe RSV disease, use of passive prophylactic monoclonal antibodies is under consideration. A prophylactic monoclonal antibody (Palivizumab) has been in use in developed countries and has been shown to prevent RSV associated hospitalization to a certain extent among high risk infants. A monthly prophylaxis with Palivizumab^{TM}, conducted at 139 centers in the United States, the United Kingdom, and Canada, among premature infants and infants with bronchopulmonary dysplasia was associated with a reduction in RSV associated hospitalization of 55% during a randomized placebo-controlled trial (IMpact & group, 1998). However, this monoclonal antibody has a short half-life, requires multiple injections and it is too expensive for the global market.

Another prophylactic monoclonal antibody developed using stabilized pre-fusion conformation of the RSV F protein (Domachowske et al., 2018; Krarup et al., 2015) is in phase 3 of clinical trials (NCT03959488) in 131 sites with South Africa as one of the

participating centers. This clinical trial started on 30th July 2019 and completed on 3rd May 2021. Plans are underway to introduce this high potency prophylactic immunoglobulin vaccine (MEDI8897) to high risk infants at birth (Krarup et al., 2015) and to all infants at birth or before the start of RSV season. In an earlier randomized clinical trial of the RSV monoclonal antibody involving 1, 417 healthy preterm infants from 23 northern and southern hemisphere countries, MEDI8897 immunoprophylaxis provided a 70.1% (95% CI: 52.3%, 81.2%; *P* <0.0001) reduction in RSV medically attended LRTI and 78.4% (95% CI: 51.9%, 90.3%; *P* = 0.0002) reduction in hospitalization (Griffin et al., 2019). Despite these efforts, there is no data in Kenya about pregnancy outcomes or gestational ages of births occurring at home to inform on the proportion of infants likely not to benefit from the maternal RSV program, who would require the high potency RSV immunoglobulin vaccine.

2.4 Evidence of Protection by Maternal Antibodies

There is enough evidence to suggest that maternal RSV specific antibodies protect against severe RSV disease (Glezen et al., 1981; Lamprecht, Krause, & Mufson, 1976). Using wild type RSV infection, Glezen et al in 1981, demonstrated that infants born with higher levels of antibody develop infection at a later age, and infants infected in the presence of moderate levels of serum antibody have milder illnesses than infants infected with lower or undetectable levels of antibody. In rural Mozambique, a case control study showed that high levels of maternal antibodies were associated with protection against RSV disease(Roca et al., 2002). Another case control study conducted in Coastal Kenya demonstrated that maternal antibodies from wild type RSV infection provide partial protection of about 30% (Nyiro et al., 2016), which decreases as the antibody level declines (Nyiro et al., 2016).

Other studies have provided evidence that boosting of maternal RSV antibodies occurs following challenge with a wild type RSV infection (Nyiro et al., 2015; Sande, Mutunga,

Okiro, et al., 2013; Stensballe et al., 2009). This has been demonstrated by a rise and fall in the cord level of RSV specific antibodies tracking boosting and waning of RSV specific antibodies with RSV seasonality, suggesting that vaccine boosting of maternal RSV specific antibodies is possible (Nyiro et al., 2015; Sande, Mutunga, Okiro, et al., 2013; Stensballe et al., 2009).

Mathematical modelling using household RSV transmission data has shown that vaccination of pregnant women is estimated to reduce RSV infection among infants by 31% (Poletti et al., 2015). Through another mathematical modelling of data to assess the impact of maternal RSV vaccine in 73 low- and middle-income countries (duration 2023-2035) showed that a vaccine with 60% efficacy could avert 10.1-12.5 million cases and 2.8-4.0 million hospitalizations among infants under 6 months of age (Baral, Li, et al., 2020). All these findings imply that a maternal RSV vaccine has the potential to prevent RSV associated disease among infants.

2.5 Progress in Maternal RSV Vaccines Development

The idea of maternal immunisation is not new; this method has been in practice in the US since 1957 ((Englund, Glezen, & Piedra, 1998). The approach of maternal immunisation has been used previously to effectively prevent other viral infections like poliovirus and influenza among infants (Englund et al., 1998) and pertussis in the US(Baxter, Bartlett, Fireman, Lewis, & Klein, 2017) or, in the case of rubella, in the foetus (Englund et al., 1998). Maternal immunisation has been carried out in Kenya since 1980s (Brabin, Nagel, Hagenaars, Ruitenberg, & van Tilborgh, 1984), and has been used to successfully reduce the burden of neonatal tetanus (Ibinda et al., 2015).

The view that sufficient levels of RSV specific antibody might protect infants from severe disease has been the basis for exploring the strategy of vaccinating pregnant women with subunit RSV vaccines. Currently there are 60 candidate RSV vaccines in development, of which 10 are maternal RSV vaccine candidates (Higgins et al., 2016; PATH, 2022). Two of the maternal vaccines progressed to phase 3 of clinical trials in the year 2020 (PATH, 2022).

In the early phase 1 clinical trials of maternal RSV vaccines, a purified fusion protein sub-unit vaccine (PFP-2 from Wyeth Lederle Vaccines, NY) was administered to 20 healthy women 2 weeks after delivery. The vaccine was found to be minimally reactogenic and immunogenic among women of child bearing age (Englund et al., 1998). A phase 2 clinical trial to determine safety and immunogenicity of the vaccine among 35 healthy women in the third trimester of pregnancy and their offspring was initiated. The vaccine was found to be safe but with a half-life of the maternal antibodies in infants lasting to about 3 weeks (Munoz, Piedra, & Glezen, 2003). This vaccine, therefore, never advanced to late-stage clinical trial.

The first candidate maternal RSV vaccine to advance to phase 3 of clinical trials is an F protein vaccine of nanoparticle design (Novavax). Preclinical stages of this vaccine involved an insect cell derived RSV F nanoparticle vaccine intramuscularly injected to cotton rats. The vaccine protected lower and upper respiratory tract against both RSV A

and B strain infection and induced polyclonal palivizumab competing antibodies similar to but potentially more broadly protective against RSV than palivizumab (Raghunandan et al., 2014). These findings led to progress of the vaccine to clinical trials in human subjects.

A phase 1 trial to evaluate safety and immunogenicity of the RSV Fusion protein vaccine particle was conducted in Texas, USA, among 150 healthy adults between 18-49 years of age from December 2010 to December 2011 (NCT01290419). The vaccine was found to be well tolerated without dose-related increases in adverse events (Glenn et al., 2013). From October 2013 to April 2014, a phase II clinical trial was conducted in nine states in the US, among 720 healthy women of childbearing age to evaluate the immunogenicity and safety of multiple formulations of the vaccine with aluminum (NCT01960686). This study showed that, RSV F nanoparticle vaccine formulations were well tolerated and immunogenic (August et al., 2017).

The RSV F nanoparticle maternal RSV vaccine progressed to phase 3 clinical trials (NCT02624947), to determine the safety and efficacy of the vaccine to protect infants via maternal immunization, which started in December 2015 and trial results were released in February 2019. The trial (NCT02624947) involved, 4, 636 pregnant women in 87 sites from 11 countries (USA, Argentina, Australia, Bangladesh, Chile, Mexico, New Zealand, Philippines, South Africa, United Kingdom and Spain), randomized into a ratio 2:1 vaccine or placebo. Results of the phase 3 clinical trial showed some promising efficacy against hospitalization in young infants (Madhi et al., 2020). Some of the significant results include observed efficacy against hospitalization of 44.4% (95% CI; 19.6 - 61.5%) and day 90 vaccine efficacy of 57% (95% CI; 32.7%-72.5%) (Madhi et al., 2020) in one country which was South Africa. This vaccine has not progressed to licensure (PATH,

2022) because it did not meet its primary endpoint of preventing medically significant RSV LRTI. This was mostly observed in the US population, where at day 90, vaccine efficacy against medically significant LRTI was -9.7% (95%CI;-259.2-66.5%) for women immunized <33 weeks gestation.

There are other maternal vaccines (RSV F subunit protein) which have also advanced to final stages of vaccine trials in high income countries. A subunit RSV F protein vaccine from GlaxoSmithKline (GSK) completed phase 2 of clinical trial (NCT02753413) (Beran et al., 2018) and a phase 3 clinical trial (NCT04605159) which will involve 20, 000 pregnant women started in November 2020 in the developed countries and is expected to complete in February 2024. Phase 1 randomized controlled clinical trial (NCT02298179) of the F subunit protein vaccine from GSK, conducted in Belgium among 288 healthy adults, found it to be safe and immunogenic through six months of follow-up (Leroux-Roels et al., 2019). The National Institute of Allergy and Infectious Diseases (NIAD) subunit RSV F protein maternal vaccine also completed phase 2 of clinical trials (NCT03049488) and has progressed to phase 3 of clinical trials. A subunit RSV F maternal vaccine from Pfizer completed phase 2 of clinical trials (NCT04032093) and an efficacy trial (NCT04424316) aiming to enrol 6, 900 pregnant women from 14 countries (USA, Argentina, Australia, Brazil, Chile, Canada, Denmark, Finland, Japan, Netherlands, New Zealand, South Africa, Spain and Taiwan) started in June 2020 and is expected to complete in August 2023. Topline results of the phase3 clinical trial (NCT04424316) released on 1st November 2022 showed the vaccine to have an efficacy of 81.8% against RSV associated severe medically attended lower respiratory tract illness in infants within the first 3 months of life and an efficacy of 69.4% through the first six months of life.

With the quick progress of maternal RSV vaccines advancing towards licensure, it is viewed that a maternal RSV vaccine will be available in the near future and these

vaccines will be more beneficial to low-income country setting due to the high RSV disease burden. However, it is still unclear how a maternal RSV vaccine will be successfully implemented in a LMIC setting such as Kenya. This is because, maternal characteristics of pregnant women in high income countries where vaccine trials are being conducted is different from those of low-income countries. Patterns of ANC attendance are also different. The maternal population in Kenya is also affected by comorbidities such as malaria and HIV which are known to impact negatively the efficacy of a maternal vaccine, since these infections adversely affect placental function (Greenwood, 2003). Furthermore, an intervention that is effective in developed countries might not necessarily have the same effect in Kenya or other developing countries. All maternal RSV vaccines in development are likely not to provide protection to infants born premature especially those born <33weeks of gestation due to the short interval between onset of transplacental transfer and birth. This means, there is need to consider alternative strategies for RSV prevention among preterm infants such as monoclonal antibodies for prophylaxis at birth. However, their consideration will require knowledge on rates of preterm births in a setting. Thus, assessment of the factors likely to influence the effectiveness of these vaccines ahead of introduction is urgently required to provide guidance on their implementation.

Due to the existing knowledge gap around the delivery and implementation of the maternal RSV vaccine program in LMICs, the World Health Organisation (WHO) (Modjarrad et al., 2016) conveyed a consultative meeting in Geneva in 2015 with RSV experts. The goal was to obtain advice on way forward towards clinical development of RSV vaccines for use in LMICs. One of the key objectives during this convention was to identify key priority areas and knowledge gaps in LMICs that need to be addressed, for defining a roadmap to vaccine licensure with additional considerations to be given to the specification of target populations such as pregnant women (Modjarrad et al., 2016). This meeting was then followed by a gap analysis report (PATH, 2018) on advancing maternal

immunisation in LMICs with focus on RSV which was coordinated by PATH. The WHO agenda of 2015 which highlighted some of the knowledge gaps in implementation of the maternal RSV vaccine program in LMICs therefore informed part of the basis for conducting this study.

2.6 Factors associated with effectiveness of a Maternal RSV Vaccine Program

2.6.1 Gestational age at attendance for antenatal care screening

The current maternal RSV vaccines in advanced stages of clinical trials have restricted timing for administration. This is because, RSV-specific antibodies have been known to wane rapidly with time (Sande et al., 2014). The phenomenon of rapid decay of RSV antibodies, has also been observed during a maternal RSV vaccine trial, where maternal antibody boosting was found to reach peak level within 14 days after vaccination (August et al., 2017), and declined to unvaccinated levels within a period of 3 months (Madhi et al., 2020). The level of protective RSV antibodies passively transferred to an infant will therefore depend on the gestational age at which pregnant women receive the vaccine.

The proposed plan for administration of maternal RSV vaccines is through the ANC clinic (https://www.path.org/resources/roadmap-advancing-rsv-maternal- immunization/). This therefore implies that, it is only those pregnant women who present for ANC who will be reached for vaccination. A Mathematical model developed to infer potential maternal RSV vaccine coverage which included Demographic Health Survey (DHS) data from 69 LMICs showed that, countries with higher first ANC coverage were predicted to have higher vaccination coverage (Baral, Fleming, et al., 2020). Currently, the Kenya national guidelines for quality obstetric care recommend at least 4 ANC visits during pregnancy (MOH., 2012), with initiation expected before 16 weeks of gestation. The WHO on the other hand recommends 8 contacts with a health care provider for women during pregnancy. With the first contact expected to occur within the first 12 weeks of

pregnancy (WHO, 2016). Consequently, the maternal RSV vaccine coverage will depend on the number of women in a setting, who attend ANC during pregnancy and are within the required gestational age window period for vaccine delivery. Before this study was conducted, there was no published data on subsequent ANC visits in Kenya and other countries in sub-Saharan Africa. Only the first ANC visit was available (GOK, 2014).
Currently, there are two gestational age windows 28-32 weeks and 24-36 weeks (Baral, Fleming, et al., 2020) for maternal RSV vaccine delivery under consideration. The gestational age window period of 28-32 weeks has been proposed by WHO as an optimal window for maternal RSV vaccine delivery. The window period of 28-32 weeks has also been found to have higher anti-pertussis IgG avidity (Abu-Raya et al., 2019). The window period of 24-36 weeks is undergoing evaluation with the sub-unit maternal RSV vaccine from Pfizer. Thus, knowledge on the proportion of pregnant women who attend ANC and can be reached for vaccination within the proposed gestational age window period for vaccine delivery will be critical to inform expected vaccine coverage.

Other factors such as, maternal age, marital status, religion, parity, associated implications for pregnancy disclosure, cost of ANC screening, education level and interactions with healthcare workers (Abeje, Azage, & Setegn, 2014; Pell et al., 2013; Rurangirwa, Mogren, Nyirazinyoye, Ntaganira, & Krantz, 2017) which affect ANC attendance and utilization of ANC services are also likely to influence the effectiveness of a maternal RSV vaccine. A population-based study in Rwanda found that older maternal age, being single, divorced or widowed, and poor social support were associated with poor utilization of ANC services (Rurangirwa et al., 2017). In Ghana, socio-cultural interpretations about threats during pregnancy forced pregnant women to seek multiple care choices, including the use of herbalists and traditional birth attendants. This disrupted their continued use of ANC care from skilled health providers (Dako-Gyeke, Aikins, Aryeetey, McCough, & Adongo, 2013).

2.6.2 Transplacental transfer of RSV-specific antibodies

Efficient transplacental transfer process of antibody is very critical during maternal immunization. A study by Chu and group in Bangladesh demonstrated that the transfer of RSV-specific antibodies from mother to foetus during the 3rd trimester of pregnancy to birth is efficient (Chu et al., 2014). A study in Nepal also showed that there is efficient transfer of RSV-specific antibodies from mother to infant, irrespective of the age of the mother (Chu et al., 2017). Among pregnant women from rural Nepal, the efficiency of transplacental transfer using cord-maternal transfer ratio (CMTR) was estimated to be 1.03 (0.88-1.19) (Chu et al., 2017).

Transplacental transfer of RSV antibodies occurs via an active, receptor-mediated process across all three layers of the placenta maternal-fetal interface(Clements et al., 2020). Of the five known immunoglubulins, only IgG antibody is transported across the placenta in meaningful quantities. The four subclasses of IgG antibodies have different roles in protection (Schroeder & Cavacini, 2010). For instance, IgG1 which is important in mediating antibody responses against viral pathogens have been shown to have the greatest affinity for the FcRn receptor (Schroeder & Cavacini, 2010) and as a result it is the RSV-specific antibody detected in infants in high concentrations. Other subclasses such as, IgG3 are a minority while IgG4 and IgG2 are not common (Atwell, Lutz, Sparrow, & Feikin, 2022). The hierarch of transfer efficiency has been reported as IgG1>IgG3>IgG4=IgG2. IgG1 are transferred more efficiently than IgG4 and IgG3,while, IgG2 have the lowest transfer efficiency (Clements et al., 2020). The differences in transplacental transfer are thought to be governed by glycosylation patterns (Atwell et al., 2022) and clinical characteristics such as Rhesus-negative pregnancies have been shown to reduce IgG2 levels, while maternal influenza vaccination have been found to elevate IgG1 and IgG4 antibody levels (Clements et al., 2020). Thus, knowledge on the factors likely to impair transplacental transfer of IgG antibodies and mitigating against those factors is likely to improve vaccine outcomes.

Placental malaria and hypergammaglobulinemia has been found to reduce transfer of RSV-specific antibodies by 58% and 90% respectively in The Gambia (Jallow et al., 2018; B. J. Okoko, L. H. Wesumperuma, M. O. C. Ota, et al., 2001). Other studies have also shown evidence in reduction of transplacental transfer of antibodies by HIV (Jallow et al., 2018). A study in Gaborone, Botswana found that the placental transfer ratios for RSV antibodies among infants of HIV infected mothers was lower (1.02 vs 1.15) than that of HIV-unexposed, uninfected infants (S. M. Patel et al., 2020). In a safety and immunogenicity clinical trial of the RSV F protein nanoparticle design maternal vaccine conducted in the US among third trimester women, efficiency of transplacental antibody transfer was 90%-120% for infants of vaccinated women (Munoz et al., 2019).

The level of antibodies transferred to infant will also depend on gestational age at birth and birth weight (Okoko, Wesumperuma, & Hart, 2001b; Wesumperuma, Perera, Pharoah, & Hart, 1999). Some studies conducted in Kenya and Sri-Lanka have shown a strong association between the cord level of RSV specific antibodies and gestational age at birth (Nyiro et al., 2016; Wesumperuma et al., 1999). Premature infants demonstrate low levels of RSV antibody at birth compared to full term infants (J. B. Okoko et al., 2001b; Wesumperuma et al., 1999). This suggests that short intervals to birth of <30 days following or before onset of transplacental transfer of antibodies (beginning 28 weeks gestation) will lead to low levels of antibody transferred to the infant. According to Kenya Population-Based HIV Impact Assessment (KENPHIA 2018) report, Siaya county has the highest adult HIV prevalence of 21%. Similarly, the coastal region is known to be endemic for malaria. With these existing comorbidities among the Kenyan maternal population, little has been known about the efficiency of transplacental transfer of RSV specific antibodies and the degree to which factors such as placental malaria and HIV or illnesses occurring during the third trimester of pregnancy would impact the effectiveness of a maternal RSV vaccine program.

2.6.3 Baseline rates of birth outcomes

Successful delivery of the maternal RSV vaccine will depend on pregnant women's willingness to accept the vaccine when available. Any safety concerns or risks associated with the vaccine to pregnancy outcomes might result into high rates of vaccine refusals or hesitancy which might affect the overall effectiveness (Fabry et al., 2011). Thus, to build confidence among pregnant women, there will be need to have a robust tracking system for adverse birth outcomes during implementation of the maternal RSV vaccine program (PATH, 2018).

It is also hypothesized that, since women from some LMIC's experience comorbidities of HIV, malaria and undernutrition which are associated with adverse birth outcomes such as preterm births, low birth weight and still births (Adane, Ayele, Ararsa, Bitew, & Zeleke, 2014) are likely to have obscured safety outcomes during a maternal vaccine implementation program (Heyderman et al., 2016). Having prior knowledge of the baseline prevalence of adverse birth outcomes before introduction of the vaccine is very important in evaluating safety and effectiveness of the maternal RSV vaccine program in Kenya. However, monitoring of these adverse birth outcomes will only be possible if pregnant women utilize health care services during delivery.

There are initiatives by the government of Kenya to encourage women to utilize health care services during pregnancy and to reduce maternal and child mortality. These initiatives include 'Linda Mama'(MOH, 2016), which is a public funded health scheme to ensure that pregnant women and infants have access to quality and affordable health services (http://www.nhif.or.ke/healthinsurance/lindamamaServices). The other initiative is 'Beyond Zero', launched in 2014, which aims to improve maternal and child health in Kenya, and to reduce new HIV infections among children (Beyondzero, 2014). Through these initiatives, pregnant women have access to free maternal health services. According to the 2014 demographic health survey which was conducted prior introduction of these initiatives, a high proportion of births (e.g. 47% in Kilifi) were found to have occurred at home (GOK, 2014).

In this study, data collection from HDSS sites started on 1st October 2018 and completed on 18th April 2021. There was a pause to all study activities in the year 2020 due to SARS-CoV-2 pandemic. Through this study, a comprehensive description of the factors likely to influence the effectiveness of a maternal RSV vaccine program in Kenya is provided.

CHAPTER THREE

3.0 MATERIALS AND METHODS

This chapter provides a detailed description of the geographical area where this study was conducted, the characteristics of the study population, study design, determination of sample size, sampling procedures and the laboratory procedures. The chapter also describes how data management and ethical concerns for involvement of human subjects in research were addressed.

3.1 Study Sites (geographical)

This study was conducted in two geographical sites: Kilifi health and demographic system (KHDSS) area in Coastal Kenya and Siaya Health and Demographic Surveillance System (HDSS) area in Western Kenya. All serological assays and data analysis were done at KEMRI-Wellcome Trust Research Programme (KWTRP), Kilifi.

3.1.1 The Kilifi health and demographic surveillance system (khdss) area

The KHDSS area (Scott et al., 2012), is located within Kilifi County and was established in 2000 by KWTRP for the purposes of demographic surveillance and epidemiological research. The KHDSS area comprises administratively 15 locations and 40 sub-locations, covering an area of 890 km^2, extending 35 km north and south of Kilifi County Hospital (KCH) in Kilifi town (Scott et al., 2012). This surveillance area experiences two rainy seasons (March to June and October to December) per year. However, over recent decades the rainfall has become increasingly unpredictable. The main activity is subsistence farming (of crops such as maize, rice, cassava, beans and peas), horticulture, fishing as well as keeping of livestock. Main source of employment is in tourism-related activities, civil service and from academic and research institutions situated within Kilifi town. The local community residing in this area belong to Mijikenda ethnic group with majority of the residents speaking the Giriama or Chonyi languages although other ethnic

groups from different parts of Kenya also reside in this area, particularly within the township. According to Kenya Health Policy (KHP 2015), the ratio of female to male in Kilifi is 1:1.

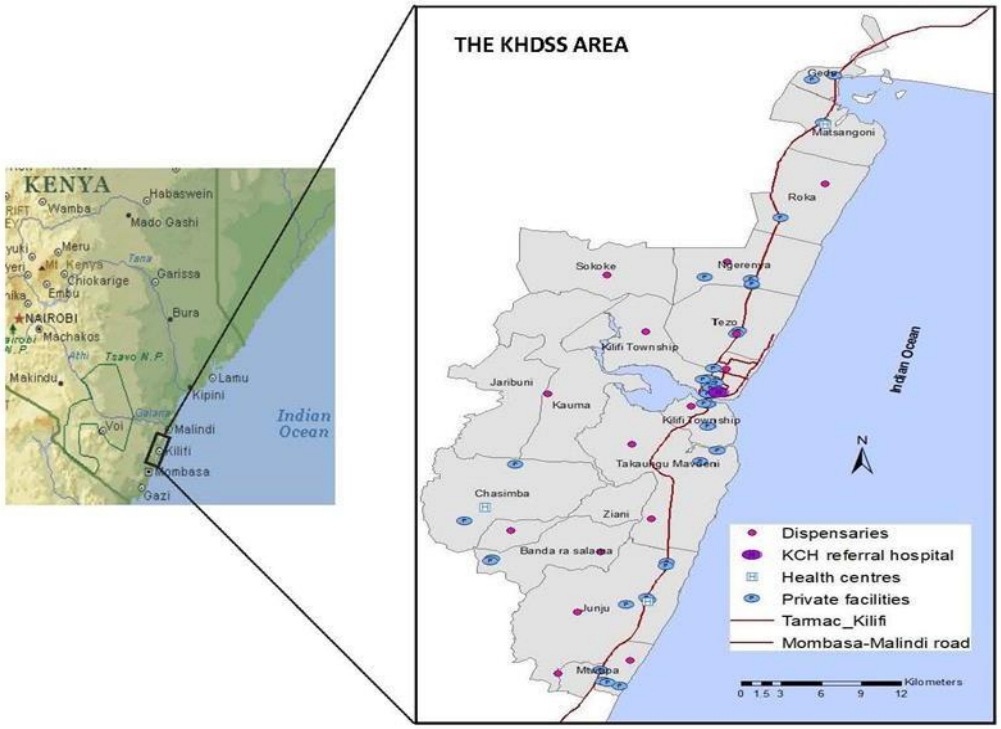

Figure 2. KHDSS map showing administrative locations and health facilities where pregnant women residents attend for antenatal care. *(Source: Map generated by Christopher Nyundo, KEMRI-WTRP, Kilifi, December 2020).*

Currently, the surveillance system monitors a population of around 300,000 residents through enumeration rounds conducted three times a year. The surveillance records about 8,000 pregnancies per year. Records of in-migration and out-migration, births and deaths occurring in this area are collected during these enumeration rounds. Malaria has been endemic in this region with prevalence declining overtime (Njuguna et al., 2019). RSV epidemic occurs between October to May of the subsequent year (Nokes et al., 2009).

According to Kilifi County health statistics for 2015, HIV prevalence among women is

6.7%. The area is served by about 60 health facilities both private and public and approximately 60% of deliveries within this area occur at Kilifi County hospital which serves as the referral health facility.

3.1.2 The Siaya Health and Demographic Surveillance System area

The Siaya HDSS is located in Rarieda, Alego-Usonga and Gem Sub-Counties, lying northeast of Lake Victoria in Siaya County, western Kenya run by KEMRI-Centre for Global Health Research (CGHR) with financial support from US Centre for Disease Control and Prevention (CDC). This HDSS was established in 2001 as part of a large trial of insecticide-treated bed nets (ITN), and the HDSS continued even after completion of this trial (Odhiambo et al., 2012). The Siaya HDSS monitors a population of about 259,000 people, living in approximately 65,000 households, and records about 6000 pregnancies per year. The population is culturally homogeneous with over 95% of the population being members of the Luo ethnic group who live through subsistence farming and fishing.

The Siaya HDSS site has a high burden of pneumonia and diarrhea and is used to conduct longitudinal population based infectious disease surveillance. The HDSS has also been used to evaluate several interventions including social and behavioral interventions (Odhiambo et al., 2012). Malaria is holoendemic in this area accounting for 30% of diagnosed cases at peripheral health facilities. HIV prevalence among women in this area is 26.4% according to 2015 HIV estimates for Siaya. In this surveillance system, routine home visits to collect morbidity data have been conducted twice per year since 2015; residents are given free care at the study's referral clinics for all potentially infectious disease syndromes.

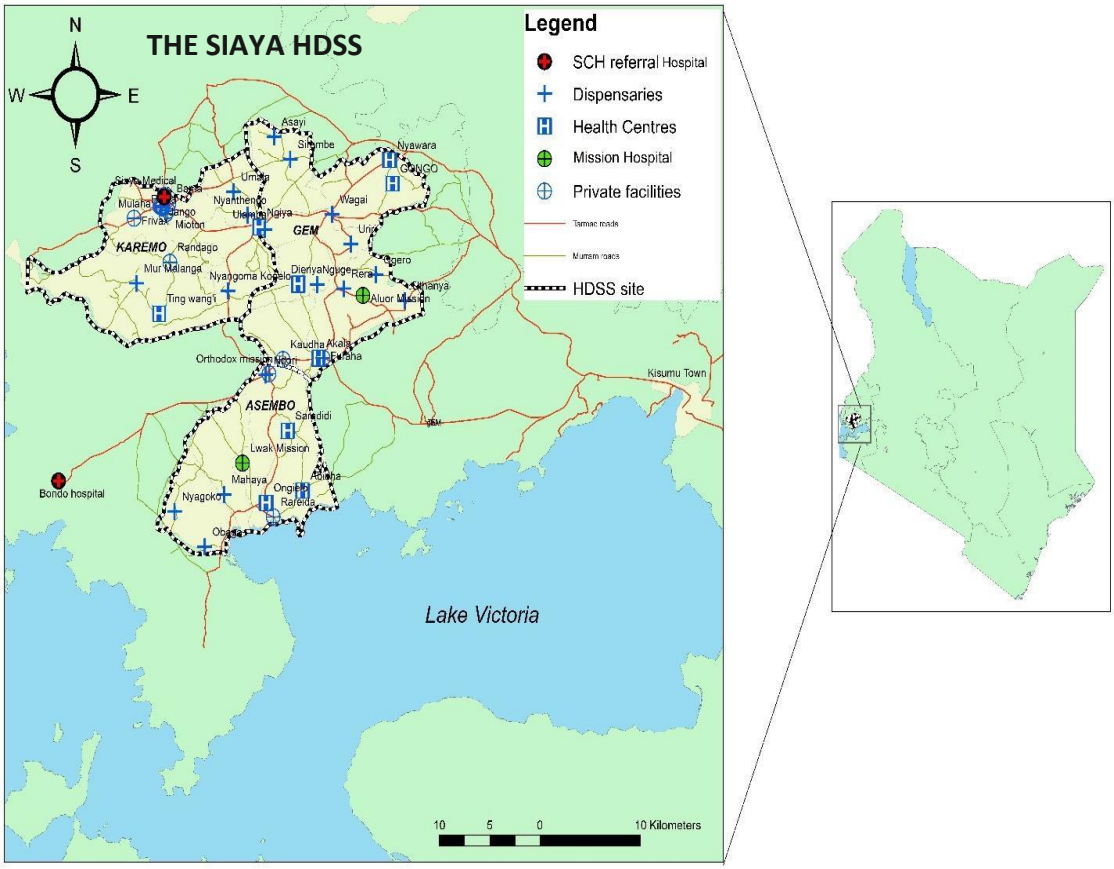

Figure 3. Map of the Siaya Health and Demographic Surveillance System showing Health Facilities. (Source: Map generated by Maurice Ombok, KEMRI-CGHR, Kisumu, April 2021)

3.2 Study Design

This project was nested in ongoing surveillance projects involving a hospital cohort of pregnant women and a surveillance of women from the general population within the health and demographic surveillance system areas in Kilifi and Siaya Counties. This study obtained data and samples using: (i) A cohort study in which data and blood samples were obtained from two cohorts of pregnant women (Siaya and Kilifi) and (ii) used a cross sectional study design to collect data from women with birth outcomes for pregnancies registered during the year 2017 to 2020 through census rounds conducted within Kilifi and Siaya HDSS areas.

The selection of the study sites was purposive. The cohorts of pregnant women were available to provide the required data and samples to answer the questions in this study and HDSS census registers were available as sampling frames for study participants with a registered pregnancy and a birth outcome. The HDSS areas from where a random sample of women were traced to collect data on ANC attendance and birth outcomes had population denominators available and the prevalence for infectious diseases was known. The choice of two cohorts instead of one enabled a comparison between pregnant women from two diverse geographical regions in Kenya which fit with the main objective of the study.

3.3 Study Population

The study population comprised of pregnant women who were:

(i) Residents of the HDSS area in Kilifi and Siaya.

(ii) Participants in the maternal influenza (MATFLU) study – a cohort of pregnant women in Siaya HDSS sites.

(iii) Participants in the KIPMAT study – a cohort of pregnant women in Kilifi investigating risk factors for severe morbidity and mortality in mothers and neonates in Kilifi HDSS.

3.3.1 The Inclusion criteria

This study involved:

(i) Pregnant women from Siaya HDSS MATFLU surveillance and Kilifi HDSS KIPMAT study with maternal and cord blood samples, complete meta-data and who had given consent to have their samples stored and tested for other causes of illness.

(ii) Pregnant women residents of Kilifi and Siaya HDSS who were registered in 2017-2020 census rounds, were traced at home or they presented at Siaya or Bondo hospital maternity ward for delivery, had a birth outcome at the time of data collection and who had given a written consent to participate in the study.

(iii) Participants from Siaya HDSS MATFLU surveillance cohort and Kilifi HDSS KIPMAT cohort missing meta-data, maternal or cord blood samples.

3.3.2 The exclusion criteria

(i) Participants from Siaya HDSS MATFLU surveillance cohort and Kilifi HDSS KIPMAT cohort missing meta-data, maternal or cord blood samples.

(ii) All selected participants from HDSS areas who declined consent for participation in the study.

(iii) All selected pregnant women from HDSS areas of Kilifi and Siaya, whom by the time of data collection were living outside the HDSS areas.

3.4 Sample Size Determination

The sample size for this study was determined by the objectives that each proposed population of pregnant women was set to achieve and is described below.

3.4.1 Sample size to determine distribution and proportion attending ANC by gestational age

Collection of data on gestational age at attendance for each ANC visit was through review of records from mother and child health booklets (ANC booklets) for women registered pregnant during enumeration rounds in the year 2017 to 2020. The sample size was therefore, determined to estimate the proportion of women who attended ANC within Kilifi HDSS in a specific gestational age week within a 5% degree of precision. By using the formula for proportion; $nr = z^2pq/d^2$ (Charan & Biswas, 2013),

where nr = required sample size,

z = abbisca of normal distribution (1.96),

p = proportion of population attending ANC visit at a specific gestational age week (50%),

q = 1-p and d = the degree of precision i.e. desired margin error (0.05).

A sample size of 384 pregnant women ensured that the 95% confidence interval of the median gestational age estimate at attendance for ANC screening was within 0.05 margin of error.

A piloting study to assess the proportion of pregnant women within the KHDSS area who attended ANC and had ANC booklets during the 2018 census rounds, found that about 60% of the women did not have booklets. To adjust for the missing booklets initially estimated at 60%, 1000 women were sampled from the KHDSS from whom at least 400 participants were estimated would provide complete gestational age data. A further 1000 women were sampled and uploaded to the study database to replace women not found at home during tracing. A similar sample size of 1000 women were selected from Siaya. HDSS for collection of data on birth outcomes, demographic characteristics, and pregnancy history.

The median age for first ANC attendance in Western Kenya during the 2014 Kenya Health Demographic Survey was similar with that from the coast and therefore both sites used the same sample size determination method. Following the challenges experienced with tracing of women in Kilifi HDSS area, where only 60% of the target was met, an adjustment in the sample size selection was made for Siaya women. An additional sample set of 1000 women for replacement was selected and uploaded to the computer tablets and issued to fieldworkers for collection of data.

3.4.2 Sample size to determine efficiency of transplacental transfer of RSV-specific antibodies

The sample size to determine efficiency of transplacental transfer of RSV specific antibodies was based on cord-to-maternal transfer ratio (CMTR) using Edgar C. Fieller methods of calculating confidence intervals for the ratio of two means. In this study, the sample size was calculated using estimates for CMTR from women in rural Nepal of 1.03

(0.88-1.19) with a cord mean titre 11.3 and maternal mean titre 10.7 (Chu et al., 2017). Assumption was made that pregnant women in Kenya would have similar antibody levels as those of Nepalese women. Thus, if the mean log2 RSV antibody titre was 11.3 [SD: 1.2] in infants and 10.7 [SD:1.3] in mothers, and both these variables followed a Gaussian distribution, 200 mother - infant pairs would be able to detect a CMTR of 1.03 with a 95% interval of 1.01-1.06.

A total of 200 women with a pair of cord and maternal blood samples were randomly selected from the registers of each pregnant cohort (Siaya MATFLU cohort and Kilifi KIPMAT study) for this objective.

The reduction of transfer of RSV specific antibodies by transplacental malaria and hypergammaglobulinaemia of 58% and 90% respectively found in The Gambia (B. J. Okoko, L. H. Wesumperuma, M. O. C. Ota, et al., 2001) was applied to estimate the sample size to assess predictors of an impaired transplacental transfer of RSV specific antibodies. A sample of 160 pregnant women with RSV infection, was estimated to give the study 82.8% power to detect 12.5% reduction in transplacental transfer of RSV specific antibody by any of the covariates such as, anaemia, malaria, ARI, diabetes, high blood pressure, HIV status, maternal age and parity. However, participants with characteristics such as HIV infection, malaria and acute RSV infection during pregnancy and who had paired cord and maternal blood samples at birth were very few. Therefore, an exploratory analysis of the factors that would have influenced transplacental transfer was done using the sample size of 200 women selected to determine efficiency of transplacental transfer of RSV-specific antibodies.

3.4.3 Sample size to determine prevalence of birth outcomes

One of the birth outcomes of major significance during implementation of the maternal RSV vaccine was preterm births. These form the group of infants likely not benefit from the maternal RSV vaccination program besides being significant in monitoring safety of

the vaccine. An assumption was made that the national prevalence of preterm births would be the same in both Kilifi and Siaya counties and similar to that observed at Kenyatta National Referral Hospital of 18.3% (Wagura, Wasunna, Laving, Wamalwa, & Ng'ang'a, 2018), using the binomial formula for the 95% confidence interval on a simple proportion [Mean +/- 1.96*21SQRT(p*(1-p)/N)] the 95% confidence interval prevalence of preterm births in a sample of 1000 women could be estimated with a width of 4.8 percentage points.

3.5 Sampling Method

3.5.1 Sampling from pregnant women residents of Kilifi and Siaya HDSS area

A computer-generated random sample of 25 women from each of the 40 sub locations in Kilifi HDSS, who had a birth outcome, were selected from the census register within the KWTRP integrated database. This ensured the required sample size of 1000 women from the Kilifi HDSS population to provide at least 400 women with complete gestational age data at ANC attendance was achieved. The same sample of women was to provide data on birth outcomes. The selected women were visited at their homesteads by trained fieldworkers/interviewers for collection of data on gestational age at ANC attendance, pregnancy history and birth outcomes. The women selected, if were willing to participate in the study, but did not have ANC booklets were enrolled to provide data on birth outcomes. In Siaya HDSS area, a sample of 1000 women registered pregnant during 2017 to 2020 enumeration rounds, were randomly selected from the computer census registers to provide data on birth outcomes. More details on the sampling procedures within the HDSS sites, for each specific objective have been provided in Chapters Four and Six.

3.5.2 Sampling from pregnant women participants of influenza surveillance cohort in Siaya HDSS area

These participants were selected from a surveillance for influenza virus infections in a cohort of pregnant women in Siaya, Western Kenya conducted by KEMRI-Centre for Global Health Research (CGHR), Kisumu in collaboration with CDC-Kenya. This surveillance cohort has been ongoing since 2015. The specific procedures involved in the influenza surveillance cohort are described in detail in Chapter Five.

In brief, pregnant women in this surveillance were residents of Siaya HDSS area and were recruited either from their home or when they attended ANC at Bondo or Siaya County Hospital. All pregnant women presenting to these hospitals were from the general population attending ANC for care. Participants were enrolled into the study if the gestational age which is measured by fundal height or last menstrual period (LMP) and confirmed by ultrasound was less than 20 weeks. These pregnant women in the cohort were followed up weekly through a phone call or by home visit to record the occurrence of any adverse events or illness. Women who developed symptoms of acute respiratory tract infection had a nasopharyngeal swab sample collected to test for influenza and RSV viruses by molecular methods. All sick women during the period of follow-up were provided with medical care at either Bondo or Siaya County Referral Hospital. A blood sample was collected at enrolment and a maternal and cord blood collected at birth.

A computer-generated random sample of 200 participants stratified by covariates such as malaria, anemia, HIV were selected from the surveillance cohort. Data and aliquots of 100 microliters archived serum samples (maternal and cord blood collected at birth) were obtained from the selected women.

3.5.3 Sampling from pregnant women participating in a cohort to investigate risk factors for severe morbidity and mortality in mothers and neonates in Kilifi HDSS area

Sampling for these participants applied similar methods to those used in sampling from

women participants of an influenza virus infection surveillance. The surveillance to investigate risk factors for severe morbidity and mortality in mothers and neonates was conducted by KWTRP and has been ongoing since 2011 at Kilifi County Hospital maternity ward and the KHDSS area. In this study consent was sought (Appendix III) from pregnant women presenting at KCH maternity ward for delivery to collect cord and maternal blood samples after birth. Details of the specific procedures involved in this surveillance are provided in Chapter Five.

From the KIPMAT study, a sample of 200 women who delivered at KCH in 2017 and 2018, residents of KHDSS with a cord and a maternal blood sample available, were randomly selected from the integrated study database for this project.

3.6 Laboratory Procedures

All serum samples obtained from the MATFLU (Siaya) and KIPMAT (Kilifi) pregnancy cohorts were screened for the concentration of RSV specific antibodies using plaque reduction neutralization test. The 200 pairs of cord- maternal blood samples from Kilifi were retrieved and a volume of 100 microliters from each participant sample aliquoted at KWTRP laboratories. Aliquots of serum samples obtained from the MATFLU cohort in Siaya were transported to KWTRP for screening. A total of 400 cord-maternal pairs of blood samples collected from pregnant women cohort participants (200 Siaya and 200 Kilifi) were screened for RSV antibodies. Results for nasopharyngeal swab specimen screening were extracted from the pregnancy cohort databases of the parent studies.

3.6.1 Plaque Reduction Neutralisation Test (PRNT) Assay

The level of RSV specific antibodies in serum samples was determined using PRNT assay. Currently, PRNT is the preferred test for use to quantify protective immunity correlates for RSV in vaccine studies. The PRNT assay used in this study was developed inhouse at KWTRP laboratories. The detailed procedure for this assay has been provided in Appendix VII. This inhouse PRNT assay (Nyiro et al., 2019) determines the

concentration of functional antibodies from a human serum sample (or antibody preparation) required to induce 50% neutralisation of a known titration of RSV virus (Sande, Mutunga, Okiro, et al., 2013). The detection of RSV is usually done using a micro-plaque assay technique in which micro-RSV plaques are stained brown by immunoperoxidase which could then be counted under a microscope or with an ELISPOT reader. The neutralization titre which is the fifty percent end point titre (Neutralizing Dose, ND50) is then calculated using Spearman-Karber formula (Cohen, Audet, Andrews, Beeler, & test, 2007).

3.6.2 Quality assurance and control

Standard procedures for quality control of assays and apparatus were operational in the KWTRP laboratory and were monitored throughout the period when screening of samples was conducted. The KEMRI/CDC labs are now ISO 15189 accredited. All laboratory personnel in KEMRI-CGHR/CDC-Kenya and KWTRP were trained in Good Laboratory and Clinical Practice (GCLP).

For quality assurance and control during laboratory screening of maternal and cord blood samples for RSV-specific antibodies, an inhouse pooled adult sera and a RSV group A human reference standard (RSV IS 16/284) (McDonald, Rigsby, Dougall, Engelhardt, & Study, 2018), obtained from National Institute for Biological Standards and Control (NIBSC), Potters bar, UK, were incorporated into each assay run to check for antibody deterioration, standardization of sample titres and monitor day to day or plate to plate variation.

3.7 Data Management

3.71. Data collection

Clinical and demographic data about the participants in this study were extracted from existing data capture systems of the KEMRI-CGHR/CDC-Kenya and KWTRP. The

clinical and demographic data collection tools from participants of the influenza surveillance are shown in Appendices V and VI. A standardised questionnaire (Appendix IV) was used for collection of clinical, demographic and socio-cultural factors data from study participants who were residents of Kilifi and Siaya HDSS areas. Data on uptake of tetanus vaccine during pregnancy, gestational age at attendance for ANC screening, birth outcomes, demographic and obstetric details for women in Kilifi and Siaya HDSS was abstracted from ANC booklets of the participants (Appendix IV).

Data from KEMRI-CGHR/CDC-Kenya was collected using computer tablets loaded with questionnaire surveys. Standard questionnaires (Appendices V and VI) were used to collect demographic, obstetric and clinical data from study participants during enrolment, follow up and delivery. An anonymised dataset was requested from the database administrators from KEMRI-CGHR/CDC-Kenya. In Kilifi, an Integrated Data Management System (KIDMS) exists which has demographic data from all residents of the KHDSS area linked with both hospital and laboratory data (Scott et al., 2012). All data collected was de-identified.

3.7.2 Data Storage

In both study sites, data on ANC attendance and birth outcomes from the HDSS sites was collected electronically. Field supervisors collected all tablets and laptops from the fieldworkers daily when they came back from the field. They conducted verification and quality checks on the data collected and they synchronised the data from the tablets into the main database on the local server which was stationed in Kilifi. In Kilifi, all databases were backed up to a local alternative hard-drive on a weekly basis and to a remote hard-drive at the KWTRP in Nairobi on a monthly basis. All data obtained for this study was stored on the local server with an external back up. Extraction of data for analysis was via password-protected access to the study principal investigator and computer services manager.

3.8 Data analysis and Presentation

All data collected in this study were analysed in STATA version 15.0 (College Station, Texas). In each chapter, different analysis was performed. The specific analysis is described in chapters Four, Five and Six. To determine predictors of impaired transplacental transfer of RSV specific antibodies, all variables with a statistically significant association (P value <0.05) on univariant analysis, were selected and included in a multi-variate analysis model. Similar methods were used to determine predictors of adverse birth outcomes.

3.8.1 Assessment for Bias and Confounding effect

To assess for any evidence of confounding in this study, stratification and multi-variate model of analysis was used at analysis stage. Selection bias was overcome by use of random sampling of the HDSS participants. Comparison of the demographic characteristics of the selected women with those not selected from the census register were not significantly different. Additionally, comparison of women with MCH booklets with those without were not significantly different in demographic characteristics.

3.9 Ethical Considerations for Research Involving Human subjects

Before any project activities were initiated, ethical approval to conduct this study was obtained from KEMRI-Scientific and Ethical Review Unit (SERU).

3.9.1 Direct benefits to study subjects and community

There were no immediate, direct benefits to the participating individuals in this study since there is no established treatment for respiratory syncytial virus infections. The results are aimed to lead to development of policy to guide implementation of a potential maternal RSV vaccine program which will benefit the general population, of which the study participants may be a part in the future.

3.9.2 Informed consent by subjects and including possible benefits and risks

Informed Consent

Consent was sought in writing for all participants from the HDSS areas of Kilifi and Siaya (Appendix I). Trained study fieldworkers visited the homes of all women selected from the census register eligible for this study from whom they sought their consent for participation. There was no new consenting to participants of the MATFLU pregnancy cohort conducted by KEMRI-CGHR/CDC-Kenya and the KIPMAT study, from whom archived data and samples were used for this study. Individuals from MATFLU and KIPMAT study cohorts were consented through protocol for MATFLU surveillance (Appendix II) and protocol for KIPMAT study (Appendix III). However, approval to use the data and samples for this research was obtained from KEMRI-Scientific and Ethical Review Unit.

Community benefit

Community benefits are general, and the effects will not be immediate. The study aimed to generate information that could be useful to public health policy makers. The results of this study may have the potential to guide in policy development towards implementing a maternal RSV vaccine program in Kenya and provide important baseline data for trials of maternal vaccines in developing countries.

Risks.

There were no risks to participants through this study for whom data was obtained retrospectively from surveillance cohorts. There might have been some inconveniences about retrieval of ANC booklets for the Kilifi and Siaya HDSS participants and time taken for consent and review of records, but this did not pose any risk. The field staff ensured that minimal time of not more than 30 minutes was taken to review ANC records of a participant and filling in of the questionnaire.

3.9.3 Confidentiality

Participant confidentiality was and will be protected. All personal identifiers were delinked from the clinical and demographic data requested for this study. Each participant was assigned a unique study identification number (ID) which was used on the sample labels. All records for results of this study were securely stored in a password-protected database at KEMRI-WTRP, accessible only to the study principal investigator.

3.9.4 Data sharing

A data repository was created in Harvard Dataverse with the replication data set and cleaning analysis code. The de-identified dataset (following KWTRP data protection policy guidelines) if already used in a publication is on Open Access. The datasets and analysis codes for Chapters Four, Five and Six with complete manuscripts are available at https://doi.org/10.7910/DVN/TM8YHW, https://doi.org/10.7910/DVN/XOKFFK and https://doi.org/10.7910/DVN/9AIEIT respectively. Full data for this study would be made available on request to the KEMRI-WTRP Data Governance Committee (Data_Governance_Committee@kemri-wellcome.org). All individual data and summary information collected or generated in this study and shared does not have any personal identifiers.

3.9.5 Feedback information

The results of this study are presented to the University of Nairobi for the award of a PhD degree through this thesis. These study findings have also been communicated to the WHO, Gates Foundation, PATH, the scientific community within KWTRP, KEMRI-CGHR, CDC-Kenya and ministry of health (MOH) stakeholders through study feedback and scientific meetings. The findings have also been communicated through open access peer reviewed publications, reports and presentation at conferences. Plans are under way by WHO to adopt the methodologies used for ANC timing data collection in Kenya to other countries in Africa and Asia.

3.9.6 Community engagement strategy

A Community Advisory Study Team (CAST) comprising of the principal investigator and members of the community liaison group (who are KWTRP staff involved with engagement of local community and other stakeholders on ongoing research activities) was formed to oversee the community engagement plans for this study at KWTRP and in Siaya. Information about the study was given to Village reporters, KEMRI Community representatives and Chiefs/Assistant Chiefs from the KHDSS through open days organised by the community liaison group from November to December in 2018 (Kilifi) and in February 2021 (Siaya).

A Stakeholders meeting was convened on 17th September 2018 at KEMRI headquarters conference hall, Nairobi, Kenya. The aim of the meeting was to sensitize key experts from the Ministry of Health, Division of Vaccines and other professionals in Kenya about the study to seek their advice and plans for a way forward. The meeting discussion was relevant to planning possible future RSV vaccine intervention and identifying knowledge gaps. This meeting was attended by 18 participants who were representatives of the following organisations: KEMRI, CDC-Kenya/CGHR, Wellcome Trust Research Programme, Integrated Disease Surveillance and Response Unit (IDSRU), Kenya Expanded Program on Immunization (KEPI)-Division of vaccines, Kenya Pediatric Association, Pediatric group at KNH/UoN, Ministry of Health, National Vaccines Immunization Program, Paediatric Infectious Disease fellows/ Aga Khan University, National Influenza Centre and National Public Health Laboratories. The detailed stakeholders meeting report is available at https://wrap.warwick.ac.uk/124333/1/WRAP-RSV-Kenya-stakeholders-meeting-report-2018.pdf. A one-hour radio programme was set up through Baraka FM station in Mombasa in collaboration with KEMRI-WTRP on 14th March 2019 to sensitize the coastal community about the study and to discuss benefits and progress in maternal immunization.

CHAPTER FOUR

IMPLICATIONS OF GESTATIONAL AGE AT ANTENATAL ATTENDANCE ON THE SUCCESSFUL IMPLEMENTATION OF A MATERNAL RESPIRATORY SYNCYTIAL VIRUS (RSV) VACCINE PROGRAM IN WESTERN AND COASTAL KENYA

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Abstract

Maternal immunisation to boost respiratory syncytial virus (RSV) specific antibodies in pregnant women is being considered as a strategy to enhance infant protection. The timing of maternal vaccination during pregnancy may be critical for its effectiveness. However, Kenya has no documented published data on gestational age distribution of pregnant women attending antenatal care (ANC), or the proportion of women attending ANC during the proposed window period for vaccination, to inform appropriate timing for delivery or estimate potential uptake of this vaccine.

To address this gap, a cross-sectional survey was conducted within the Kilifi Health and Demographic Surveillance System (KHDSS), in coastal Kenya and Siaya HDSS in Western Kenya. A simple random sample of 1000 women was selected from each HDSS area census register. These women were registered as pregnant through enumeration rounds conducted in 2017 to 2018 for Kilifi and 2018 to 2020 in Siaya, and by the time of data collection had a birth outcome. The selected women were followed at their homes, and individually written informed consent was obtained. Records of their antenatal attendance during pregnancy were abstracted from their ANC booklet. The proportion of all pregnant women from KHDSS (55%) who attended for one or more ANC in 2018 was used to estimate vaccine coverage for Kilifi women.

Of the 1000 women selected from Kilifi HDSS, 935 were traced at home and 607/935

(64.9%) were available for interview. Of those interviewed, 470/607 (77.4%) had antenatal care booklets. In Siaya, 2000 women were uploaded into the computer tablet to account for refusals and missed visits. Of those selected, 1327 women were traced at home, 1029 consented to participate in the study from whom, 791/1029 (76.9%) had ANC booklets available. The median age during pregnancy and at the time of first ANC visit was 28.6 (23.4-33.6) years for Kilifi women and 28.4 (24.0-32.5) years among Siaya women. The median (interquartile range) gestational age in weeks at the first to fifth ANC attendance was 26 (21-28), 29 (26-32), 32 (28-34), 34 (32-36) and 36 (34-38), and 22 (18-26), 26 (21-30), 30 (25-34), 32 (28-36), 34 (30-36) among Kilifi and Siaya women, respectively. The proportion of women attending ANC during a gestational age window for vaccination of 28-32 weeks (recommended), 26-33 weeks and 24-36 weeks was 76.6% (360/470), 84.5% (397/470) and 96.2% (452/470) in Kilifi and 76.9% (608/791), 82.4% (652/791) and 90.4% (715/791) in Siaya, respectively. Estimated vaccine coverage from Kilifi HDSS women was 42.1%, 46.5% and 52.9% within the narrow, wide and wider gestational age windows, respectively. In a random sample of pregnant women from Kilifi and Siaya HDSS, with card-confirmed ANC clinic attendance, about 77% would be reached for maternal RSV vaccination within the gestational age window of 28-32 weeks. Widening the vaccination window (26-33 weeks) or (24-36 weeks) would not dramatically increase vaccine coverage and would require consideration of antibody kinetics data that could affect vaccine efficacy.

4.1 Introduction

Respiratory syncytial virus (RSV) is the main cause of severe lower respiratory tract infections among infants 0-5 months of age (Berkley et al., 2010; PERCH, 2019). Globally, RSV is estimated to have caused 1·4 million hospital admissions for acute lower respiratory infections and 27,300 in-hospital deaths of infants under 6 months of age in 2015, with 96% of these deaths occurring in developing countries (Shi et al., 2017b).

With the highest burden of RSV disease in early infancy, particularly in those under 3 months of life (Nokes et al., 2009), a vaccine to administer in the first few weeks of life would appear to be the most logical target for RSV disease prevention. However, development of such a vaccine has faced major difficulties such as poor immunological responses and reactogenicity to vaccines in this age group (Karron et al., 2013; H. W. Kim et al., 1969; Murphy, Alling, et al., 1986). A preventive strategy which involves providing prophylaxis to infants at birth and during RSV season is in advanced stages of clinical trials (NCT03959488). Trials involving intramuscularly administration of a long acting RSV Prefusion F-targeting monoclonal antibody (MEDI8897) in healthy preterm infants born between 32 and 34 weeks which was conducted in the United States, South Africa and Chile, showed the monoclonal antibody to be safe and protective against medically attended RSV (Domachowske et al., 2018; Griffin et al., 2020). However, to date there are no licenced childhood vaccines for RSV.

Maternal vaccination is currently considered the most plausible strategy for the near term to protect these infants (Englund et al., 1998; Glezen et al., 1981; Roca et al., 2002). There are several candidate maternal RSV vaccines in phase 2 and phase 3 of clinical trials (Higgins et al., 2016; PATH, 2022). These vaccines include, a RSV F subunit protein vaccine design which is progressing towards late stage trials (NCT04032093). A maternal RSV vaccine of nanoparticle design (NCT02624947) was the first candidate to complete phase 3 of clinical trials in early 2019. The results of the phase 3 trial, showed that the vaccine prevented RSV associated disease hospitalization in young infants 3 months of age, by 44.4% (95% CI; 19.6 - 61.5%) and vaccine efficacy was higher across all endpoints for mothers immunized <33 weeks of gestational age (Engmann et al., 2020; Novavax, 2019).

As maternal RSV vaccines development advance towards licensure, it is considered that these vaccines will be particularly beneficial in low income countries where the burden of RSV disease is disproportionately higher. A gap analysis report on advancing maternal

immunization which was coordinated by PATH and involved RSV experts from diverse background (https://www.path.org/resources/roadmap-advancing-rsv-maternal-immunization/), proposes introduction of maternal RSV vaccines in low- and middle-income countries (LMICs) through the ANC clinics. However, maternal characteristics including ANC attendance patterns among women in LMICs are different from those in high income countries where efficacy trials are conducted. Research conducted by Sande and colleagues, in coastal Kenya found that, the level of RSV specific antibodies rapidly wanes over time (Nyiro et al., 2015) and decline to pre-infection levels within 3 months (Sande, Mutunga, Okiro, et al., 2013). Therefore, timing of a maternal RSV vaccine delivery should be within a window of gestational age that will result in maximum benefit to the infant. Thus, accurate gestational age information from pregnant women attending for ANC screening in LMICs is required to inform the timing of vaccine delivery and expected vaccine coverage. Data on multiple ANC visits has been lacking in sub-Saharan Africa countries, since the National Demographic Health Surveys (DHS) focus on collecting data from the first ANC visit only (GOK, 2014).

This thesis Chapter aimed to describe the distribution of gestational age at each attendance for ANC care among pregnant women from the population of the Kilifi and Siaya Health and Demographic Surveillance System (KHDSS) area, in Coastal and Western Kenya. It also describes the proportions attending ANC during a proposed window for a maternal RSV vaccine delivery, provides estimates of the maternal RSV vaccine coverage and describes how this may influence the successful implementation of a maternal RSV vaccine program in this setting.

4.2 Methods

4.2.1 Study site and population

This study was conducted in Kilifi County, coastal Kenya at the KEMRI Wellcome Trust Research Programme (KWTRP). Collection of gestational age data was carried out

within the Kilifi and Siaya HDSS areas as described in Chapter Three. As previously described in methods section 3.1.1, the Kilifi HDSS area, was established in 2000 by KWTRP for the purposes of demographic surveillance and epidemiological research. The area under surveillance comprises, administratively, 15 locations and 40 sub-locations, covering an area of 890km2, extending 35 km north and south of Kilifi County Hospital (KCH) in Kilifi town (Scott et al., 2012). Currently, the system monitors a population of around 300,000 residents through enumeration rounds conducted three times a year. The number of pregnancies occurring per year is registered during these enumeration rounds. The crude birth rate is approximately 8,000 live births per year (Scott et al., 2012). A survey asking all pregnant women in the KHDSS, if they attended ANC was introduced into the pregnancy monitoring questionnaire in 2018.

The Siaya HDSS areas cover 33 villages in Asembo which are part of a longitudinal population-based infectious disease surveillance (PBIDS) since 2006 (Feikin et al., 2011). The PBIDS platform is managed by KEMRI-Centre for Global Health Research (KEMRI-CGHR) with support from the US Centers for Disease Control and Prevention (CDC) (Breiman et al., 2012). Pregnancies and their outcomes are regularly (2-3 times-a-year) recorded through active household visits by trained field staff.

In this study, Kilifi and Siaya HDSS census registers were used to select a random sample of women to participate. A standard method (Charan & Biswas, 2013) was used to determine the sample size by which to estimate the proportion of women attending each ANC visit with a precision of +/-5%. Through this method, a sample size of 384 women was determined using an assumption that the gestational age of each of the recommended four ANC individual visits by pregnant women from Kilifi and Siaya HDSS population would be representative of the general population of pregnant women in coastal and western Kenya. This relates to the median gestational age at first ANC visit obtained from the 2014 Kenya Demographic Health Survey of 24 weeks (GOK, 2014).

To overcome limitations of missing data due to missed ANC visits and unavailable ANC booklets from KHDSS women residents, initially estimated at 60% (unpublished data from KHDSS pregnancy monitoring survey), a total of 1000 women were randomly selected from the census register within the KWTRP integrated database. The study included women with a pregnancy registered in 2017 and 2018 (Kilifi) and 2018-2020 (Siaya) census rounds and who had a birth outcome by the time of data collection (October 2018 to February 2019 in Kilifi and February to May 2021in Siaya). These women were traced and visited at their homes by trained fieldworkers. Informed consent was sought and if a woman was willing to participate, she was requested to present her ANC booklet. Records on gestational age at attendance for ANC care, tetanus vaccine uptake, birth outcomes and other demographic details were extracted from the ANC booklets. Gestational age in the ANC booklets was estimated by fundal height. Additional information on socio-cultural factors and other obstetric history was collected using a standardized electronic questionnaire (Appendix IV) loaded in computer tablets. For women not found at home during the first visit, two more follow-ups were made and further attempt to locate them through other household members were conducted, after which they were confirmed to be not available for interview.

Sampling of women from Siaya for participation in this study delayed due to emergence of Covid-19 pandemic in March 2020. Following the experience from Kilifi HDSS in tracing participants and reaching out to only 47% of the women with complete data on gestational age at ANC attendance, adjustments were made to the Siaya sample. An additional sample of 1000 women was selected prior household visits for replacement of those who would not be found at home and both lists were uploaded to computer tablets and given to field workers for tracing. This was to ensure the target sample size of 384 women with complete data on gestational age and that of 1000 women with whom data for birth outcomes could be abstracted from MCH booklets.

4.2.2 Statistical analyses

Gestational age dating in weeks was measured by fundal height which is standard practice in all public health facilities in Kenya. Trimester was defined as 1 (1-12 weeks), trimester 2 (13-26 weeks) and trimester 3 (27-42 weeks). A further three gestational age categories were generated by which to assess ANC initiation, i.e. <16 weeks, early initiation as recommended by Kenya national guidelines for quality obstetric care (MOH., 2012), 16-28 weeks: mid initiation (timing of second visit), 29-42 week: late initiation which corresponds with timing of third and fourth ANC visits.

Gestational ages in weeks at ANC visits were presented as mean (Standard Deviation; (SD)), median and interquartile range (IQR). Proportions of women attending ANC during three potential gestational age windows for vaccine delivery (i.e. 28-32 weeks, 26-33 weeks and 24-36 weeks) were calculated. For each of the three defined vaccination windows, namely gestational age of pregnancy ranging from 28 to 32 weeks or 26 to 33 weeks or 24 to 36, the number of women attending ANC was computed and presented as a proportion of all women with ANC attendance records.

Vaccine coverage was estimated as a product of the proportion of women attending ANC during a potential gestational age window for maternal RSV vaccine delivery and the proportion of all women from the Kilifi or Siaya HDSS with birth outcomes registered in 2018 and who reported to have attended ANC. A chi-square test was performed to assess the association between gestational age at first ANC attendance and maternal characteristics. The characteristics of women within the KHDSS area with and without ANC booklets were compared using the chi-square test. Density curves for the distribution of gestational age at ANC attendance were generated. Data were analysed in STATA version 15.0 (Statacorp, College Station, Texas, USA).

4.3 Results

In this study, from the 1000 women selected from the Kilifi HDSS database, 935 were visited at their home of whom 607/935 (64.9%) were available for interview. Of those available, 594/607 (97.9%) consented to enroll into the study. Overall, 470/607

(77.4%) reported their ANC booklet was available, 119/607 (19.6%) reported it was lost and 5/607 (0.8%) said it was not issued. Figure 4 below illustrates study participants enrolment.

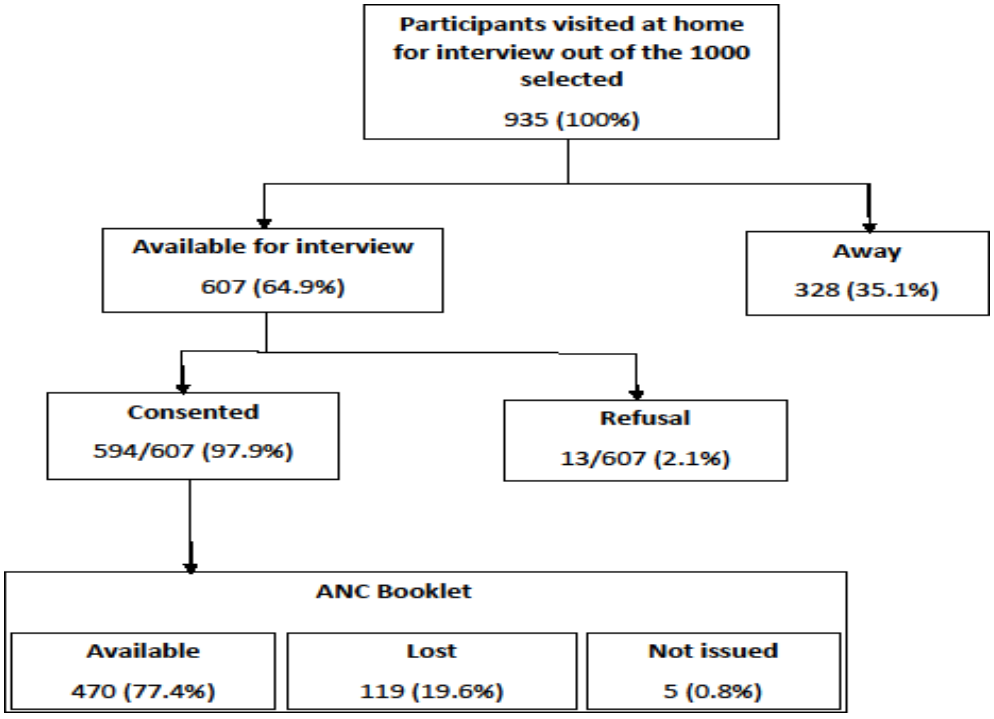

Figure 4. Flow chart showing sampling of women from Kilifi Health and Demographic Surveillance System area who participated in the study.

The median (interquartile, IQR) age for Kilifi women at the time of pregnancy was 28.6 years (23.4-33.6). The youngest was 14.5 years while the oldest was 48.3 years. About a third of these women, 228 (38.4%), were either in their first or second pregnancy. The highest parity was 13 pregnancies. From the 2000 women selected in Siaya HDSS area,, 1327 women were traced. Out of those traced, 1029 consented to participate in the study from whom, 1025/1029 (99.6%) had attended ANC during pregnancy and 791/1029 (76.9%) women had ANC booklets available. The median age of women from Siaya during pregnancy and at the time of first ANC visit was 28.4 (24.0-32.5) years, while the median gestational age at ANC initiation was 22 (IQR, 18-26) weeks.

Table 1*: Characteristics of women sampled in Kilifi and Siaya HDSS areas, Kenya*

Characteristics	Health Demographic Surveillance Sites	
	Kilifi	**Siaya**
	n%	**n%**
Women interviewed (N)	594	1029
Women attended ANC	594(0.0)	1025(99.6)
Women with ANC booklets	**470 (79.1)**	**791 (76.9)**
Median age (IQR) in years	28.6(23.4-33.6)	28.4(24.0-32.5)
Education level		
None	82 (17.5)	1(0.1)
Primary	326 (69.4)	530(67.0)
Secondary	48 (10.2)	231(29.2)
Tertiary-College/University	14 (3.0)	29(3.4)
Marital status		
Married	434 (92.3)	720(91.0)
Single	34 (7.2)	49(6.2)
Divorced/Sep/Widowed	2 (0.4)	22(2.8)
Delivery place		
Health facility	341 (72.6)	756(95.6)
Home	129 (27.5)	35(4.4)
Proportion at each ANC Visit attended		
ANC1	470 (100)	791(100)
ANC2	393 (83.6)	725(91.6)
ANC3	286 (60.8)	668(84.5)
ANC4	162 (34.5)	540(68.3)
ANC5	46 (9.8)	322(40.7)
Median Gest age at ANC Visit in weeks		
ANC1	26(21-28)	22(18-26)
ANC2	29(26-32)	26(21-30)
ANC3	32(28-35)	30(25-34)
ANC4	35(32-36)	32(28-36)
ANC5	36(34-38)	34(30-36)

4.2.3 Characteristics of pregnant women with and without ANC booklets from Kilifi HDSS

In Kilifi, there were no significant differences between pregnant women with (n=470) and without (n=124) ANC booklets in the following demographic characteristics: maternal age (χ^2, P= 0.431), education level ($\chi^{2,}$ P = 0.238), occupation (χ^2, P = 0.266), marital

status (χ^2, P= 0.288), religion (χ^2, P = 0.081) and number of pregnancies (χ^2, P = 0.189). Significant difference was identified for place of delivery (χ^2, P = 0.015). Women without ANC booklets were more likely to have delivered at home (38.7% vs 27.5%) (Table 2).

Table 2: Characteristics of women with and without ANC booklets sampled from the Kilifi Health Demographic Surveillance System (KHDSS) area, Coastal Kenya

Characteristics	With booklet n (%)	Without booklet n (%)	Total N (%)	Chi2 P value
N	470 (79.1)	124 (20.9)	594 (100)	
Age class				
15-19	24 (5.1)	5 (4.0)	29 (4.9)	
20-24	115 (24.5)	29 (23.4)	144 (24.2)	
25-29	110 (23.4)	21 (16.9)	131 (22.1)	0.431
30-34	116 (24.7)	35 (28.2)	151 (25.4)	
35-39	66 (14.0)	16 (12.9)	82 (13.8)	
40-44	34 (7.23)	15 (12.1)	49 (8.25)	
45-49	6 (1.3)	2 (1.6)	8 (1.4)	
Marital status				
Married	434 (92.3)	111 (89.5)	545 (91.8)	
Single	34 (7.2)	11 (8.9)	45 (7.5)	0.288
Divorced/Sep/Widowed	2 (0.4)	2 (1.6)	4 (0.7)	
Delivery place				
Health facility	341 (72.6)	76 (61.3)	417 (70.2)	0.015
Home	129 (27.5)	48 (38.7)	177 (29.8)	
Education level				
None	82 (17.5)	17 (13.7)	99 (16.7)	
Primary	326 (69.4)	97 (78.2)	423(71.2)	0.238
Secondary	48 (10.2)	7 (5.7)	55(9.3)	
Tertiary-College/University	14 (3.0)	3 (2.4)	17 (2.9)	
Gravida				
1-2	188 (39.8)	40 (32.3)	228 (38.4)	
3-5	159 (33.8)	43 (33.8)	201 (33.8)	0.189
6-9	110 (23.4)	39 (31.5)	149 (25.1)	
10-15	14 (3.0)	2 (1.6)	16 (2.7)	

Women in Siaya with booklets and those without booklets were significantly different in maternal age (χ^2, P= 0.001), marital status(χ^2, P= 0.001) , place of delivery(χ^2, P< 0.001) and number of pregnancies (χ^2, P< 0.001) (Table 3).

Table 3: ***Characteristics of women with and without ANC booklets sampled from the Siaya Health Demographic Surveillance System (HDSS) area, Western Kenya***

Characteristics	With ANC booklet n (%)	Without ANC Booklet n(%)	Total N (%)	Chi2 P value
N	791 (76.9)	238 (23.1)	1029 (100)	
Age class				
15-19	30 (3.8)	23 (9.7)	53 (5.2)	
20-24	164 (20.9)	62 (26.2)	226 (22.1)	
25-29	226 (28.8)	57 (24.1)	283 (27.7)	0.001
30-34	229 (29.2)	50 (21.1)	279 (27.3)	
35-39	107 (13.6)	33 (13.9)	140 (13.7)	
40-44	28 (3.6)	11 (4.6)	39 (3.8)	
45-49	1 (1.3)	1 (0.4)	2 (0.2)	
Marital status				
Married	720 (91.0)	192 (82.0)	912 (89.0)	
Single	49 (6.2)	29 (12.4)	78 (7.6)	0.001
Divorced/Sep/Widowed	22 (2.8)	13 (5.6)	35 (3.4)	
Delivery place				
Health facility	756 (94.0)	211 (88.7)	967 (94.0)	<0.001
Home	35 (4.4)	27 (11.3)	62 (6.0)	
Education level				
None	1 (0.1)	2 (0.9)	3 (0.3)	
Primary	530 (67.0)	144 (61.5)	674(65.8)	0.080
Secondary	231 (29.2)	82 (2.6)	35(3.4)	
Tertiary-College/University	29 (3.7)	6 (2.6)	35 (3.4)	
Gravida				
1-2	238 (30.1)	85 (35.7)	323 (31.4)	
3-5	469 (59.3)	109 (45.8)	578 (56.2)	<0.000
6-9	81 (10.2)	39 (16.4)	120 (11.7)	
10-15	3 (0.4)	5 (2.1)	(0.8)	

4.2.4 Distribution of gestational age at ANC visits among pregnant women in Kilifi and Siaya, Kenya

The distribution of how pregnant women in Kilifi and Siaya attended for ANC screening is shown using density curves in Figure 5 and Figure 6. Gestational age at first ANC visit varied widely and progressively diminished with increasing ANC visit number (Figure 5). Some women attended for first ANC screening at less than 10 weeks of gestation while others visited in their 40th week of pregnancy.

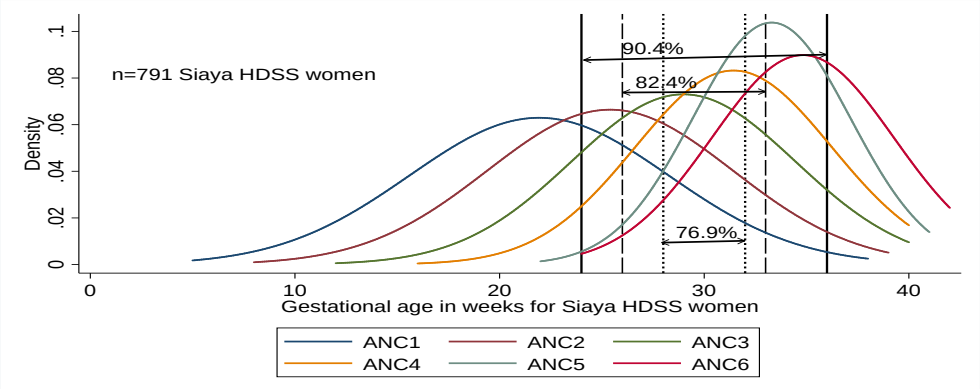

Figure 5. Density distribution curves of gestational age by ANC visit among women sampled from the Siaya HDSS area. Each curve represents participant's ANC visits i.e. visit one to fifth. The three gestational age windows (28-32 weeks), (26-33 weeks) and (24-36 weeks) for maternal RSV vaccination and the proportion of women attending within that gestational age window (76.9%, 82.4% and 90.4% respectively), are also shown.

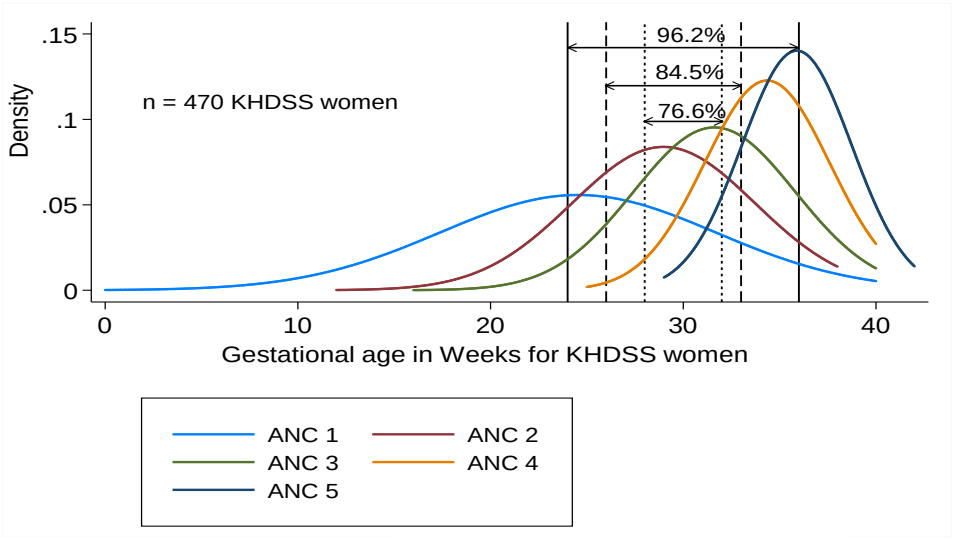

Figure 6. Density distribution curves of gestational age by ANC visit among women sampled from the Kilifi HDSS area. Each curve represents participant's ANC visits i.e. visit one to fifth. The three gestational age windows (28-32 weeks), (26-33 weeks) and (24-36 weeks) for maternal RSV vaccination and the proportion of women attending within that gestational age window (76.6%, 84.5% and 96.2% respectively), are also shown.

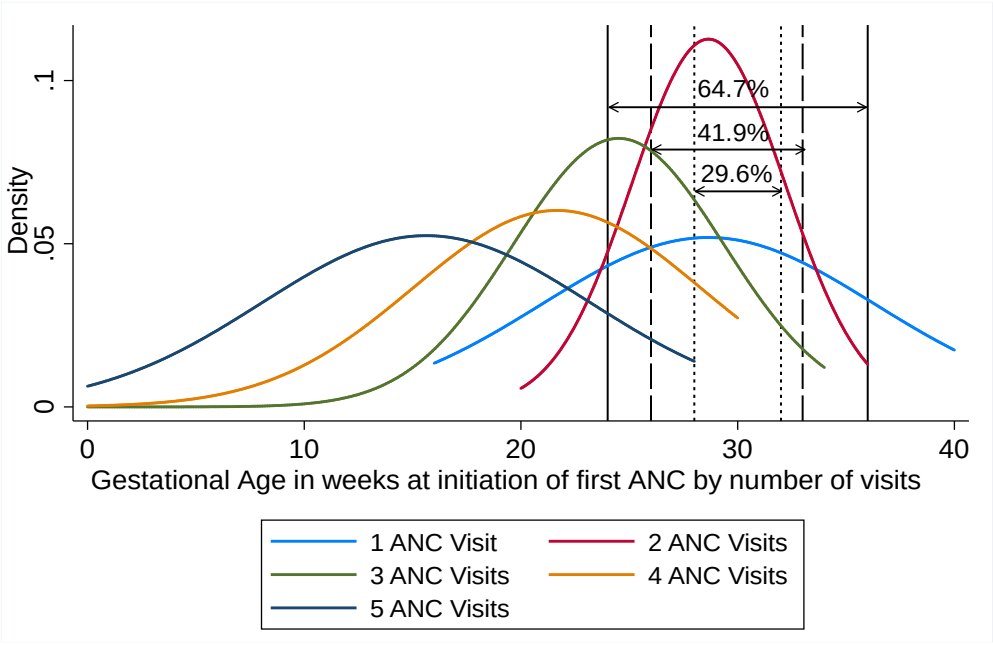

Figure 7. Density distribution curves of gestational age in weeks at initiation of first ANC visit by number of ANC visits attended among women sampled from the KHDSS area. Each curve represents participant's number of ANC visits i.e. one visit to five visits. Three gestational age windows (28-32 weeks), (26-33 weeks) and (24-36 weeks) for maternal RSV vaccination and the proportion of women attending within that gestational age window during the first ANC visit (29.6%, 41.9% and 64.7% respectively), are also shown.

The median gestational age among women at attendance for first to fifth ANC visit was 26 weeks (IQR: 21-28), 29 weeks (26-32), 32 weeks (28-35), 34 weeks (32-36) and 36 weeks (34-38) (Figure 8). and 22 (18-26), 26 (21-30), 30 (25-34), 32 (28-36), 34 (30-36) among Kilifi and Siaya women, respectively.

The proportion of women in Kilifi attending for first to fifth ANC visit is shown in Figure 8. A total of 83.6% (393/470) attended second ANC, 60.9% (286/470) attended third ANC, 34.5% (162/470) attended fourth ANC and 9.8% (46/470) attended a fifth ANC visit.

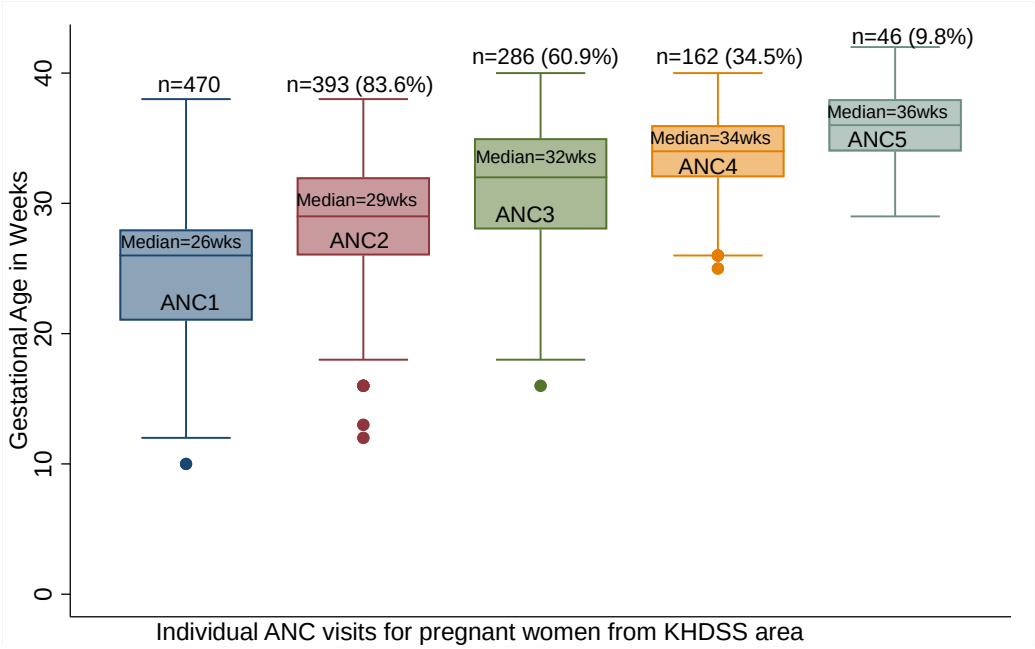

Figure 8. A box plot showing the gestational age in weeks against ANC visits among participants from the KHDSS area. Each box represents ANC visit from first to fifth (i.e. ANC1, ANC2, ANC3, ANC4 and ANC5). The median and proportion of women out of the 470 participants with ANC booklets attending ANC in each visit is also shown.

Further analysis for Kilifi data showed that, the mean (SD) gestational age in which women attended for their first to fifth ANC visit was 24.5 (7.2) weeks, 28.9 (5.0) weeks, 31.6 (4.2) weeks, 34.3 (3.2) weeks and 35.9 (2.8) weeks, respectively (Table 4). The median gestational age at first ANC visit among women who attended one, two, three, four or five visits was 30 weeks (IQR: 26-34), 28 weeks (26-31), 24 weeks (22-28), 23 weeks (20-26) and 18 weeks (12-20), respectively (Table 5). The proportion of women who attended one, two, three, four or five ANC visits was 16.4% (77/470), 22.8% (107/470), 26.4 % (124/470), 24.7 % (116/470) and 9.8 % (46/470), respectively. Results from pregnancy monitoring data collected during enumeration rounds showed that, the proportion of all pregnant women from the KHDSS area with birth outcomes registered in 2018 who reported to have attended at least one ANC was 55%.

4.2.5 Proportion of women attending ANC within a specific gestational age window for maternal RSV vaccine delivery

The proportion of pregnant women participants with ANC booklets, who attended for ANC during the narrow (28-32 weeks gestation period) and wide (26-33 weeks) or wider (24-36 weeks gestation period), was 76.6% (360/470), 84.5% (397/470) and 96.2% (452/470) in Kilifi HDSS and 76.9% (608/791), 82.4% (652/791) and 90.4% (715/791) in Siaya HDSS, respectively. More detailed analysis was carried out with data from KHDSS area since the patterns for ANC attendance and proportions attending within the proposed vaccine window were not different. Of the women attending an ANC clinic during weeks 28-32 gestational age, 29.6% were attending for their first visit, 39.8% for their second, 26.2%, for their third, 10% for their 4th and 1% for their 5th. For the 26 – 33 weeks gestational age window, close to half (48.9%) were attending for their second visit. For the wider vaccination window of 24-36 weeks gestation, 64.7% were in their first ANC visit, 72.3% in their second, 52.9% third and 6.6% in their fifth visit (Table 4).

Table 4: ***Summary of gestational age in weeks by ANC visit and proportion available for vaccination within a specific window among pregnant women interviewed from Kilifi Health Demographic Surveillance System area, Coastal Kenya***

ANC Visit	ticipant's n (%)	Mean Gest age (weeks)	95% Confidence Interval (CI)	Median Gest age (weeks)	IQR Gest age (weeks)	Proportion VAC-Window1 (28-32 weeks)	Proportion VAC-Window2 (26-33 weeks)	Proportion VAC-Window3 (24-36 weeks)
ANC1	470 (100)	24.5	23.9-25.2	26	21-28	29.6	41.9	64.7
ANC2	393 (83.6)	28.9	28.4-29.4	29	26-32	39.8	48.9	72.3
ANC3	286 (60.8)	31.6	31.1-32.1	32	28-35	26.2	31.9	52.9
ANC4	162 (34.5)	34.3	33.8-34.8	34	32-36	10	12.3	26.4
ANC5	46 (9.8)	36.9	35.0-36.7	36	34-38	1.1	1.3	6.6
Total	**470**					**76.6**	**84.5**	**96.2**

The proportion of pregnant women who attended only one ANC visit and would have been reached for vaccination at the gestational age window of 28-32 weeks, 26-33 weeks and 24-36 weeks was 6.0% (28/470), 7.5% (35/470) and 12.8% (60/470) respectively (Table 5).

Table 5*: Summary of gestational age in weeks at initiation of first ANC visit by number of visits attended and proportion available for vaccination within a specific window during the first visit among pregnant women interviewed from Kilifi Health Demographic Surveillance System area, Coastal Kenya*

Number of ANC Visits	Participants n (%)	Median Gest age (weeks) at First ANC visit	IQR Gest age (weeks) at first ANC visit	Proportion VAC-Window1 (28-32 weeks) at first ANC visit	Proportion VAC-Window2 (26-33 weeks) at first ANC visit	Proportion VAC-Window3 (24-36 weeks) at first ANC visit
1	77 (16.4)	30	26-34	6.0	7.5	12.8
2	107 (22.8)	28	26-31	13.6	17.2	21.5
3	124 (26.4)	24	22-28	6.6	10.2	18.1
4	116 (24.7)	23	20-26	3.2	6.8	11.7
5	46 (9.8)	18	12-20	0.2	0.2	0.6
Total	**470 (100)**			**29.6**	**41.9**	**64.7**

4.2.5 Tetanus vaccine coverage during the proposed maternal RSV vaccination window

Pregnant women received either a single or multiple doses of tetanus vaccine as they attended for ANC care. Out of the 470 women with ANC booklets, 18 (3.8%) received a tetanus in their first trimester, 206 (43.8%) in the second trimester and 298 (63.4%) during the third trimester (27-42 weeks gestation). A total of 257 (54.7%) women received tetanus vaccine within the potential gestational age window period for maternal RSV vaccination of 28 - 32 weeks and 284 (60.4%) during the wide window of 26 - 33 weeks. Overall, 316/470 (67.2%) of the women received a tetanus vaccine (either one or more doses) during ANC visits.

4.2.6 Factors influencing ANC initiation and uptake of health services among pregnant women in Kilifi

The proportion of women attending for first ANC visit while in their first, second or third trimester of pregnancy were 4.9% (23/470), 53.8% (253/470) and 41.3% (194/470) respectively. A very low proportion of the women (5.9%; 28/470) had an early initiation of first ANC visit of less than 16 weeks as recommended by the Kenya national guidelines for quality obstetric care (MOH., 2012). Delay on initiation of first ANC visit was significantly associated with being of older maternal age (i.e. 35 years and above) at the time of pregnancy (χ^2, P = 0.022), education below secondary level (χ^2, P = 0.021) and home births (χ^2, P < 0.001). We did not find any significant association between gestational age at first ANC visit and marital status (χ^2, P = 0.798), travel distance (in kilometres) from home of the participant to the ANC health facility (χ^2, P = 0.436), gravida (χ^2, P = 0.078) and religion (χ^2, P = 0.477) (Table 6).

When the 594 women were asked about the decision on timing for ANC screening when pregnant, 564 (94.9%) reported having made their own decision on when to attend for ANC screening. However, 30 (5%) reported they consulted either spouse or relative. The great majority of women interviewed (585, 98.5%) reported they attended ANC for the wellbeing of themselves and their unborn child. Six (1.0%) reported it was because of pregnancy complications while the remainder were following advice from friends and relatives.

Among those who delivered at home (177 women, 29.8 %), a total of 58 (32.8%) of the participants reported that this was due to the distance to the health facility, 47 (26.6%) reported it was as a result of doctors' and nurses' strikes, while 71 (40.1%) had other reasons.

Table 6*: Characteristics of participants by gestational age at first ANC visit in Kilifi Health Demographic Surveillance System area, Coastal Kenya*

Participants Gestational age at First ANC visit	0-15 weeks	16-28 weeks	29-42 weeks	Total n (%)	Chi2 P value
Total participants (%)	28 (5.9)	319 (67.9)	128 (26.2)	470 (100)	
Age class					
15-19	1 (4.2)	19(79.2)	4 (16.7)	24 (5.1)	
20-24	5 (4.4)	83(72.2)	27 (23.5)	115 (24.5)	
25-29	9 (8.2)	81 (73.6)	20 (18.2)	110 (23.4)	0.022
30-34	4 (3.5)	72 (62.6)	39 (33.9)	115 (24.5)	
35-39	6 (9.1)	42 (63.6)	18 (27.3)	66 (11.0)	
40-44	1 (2.9)	20 (58.8)	13 (38.2)	34 (7.23)	
45-49	2 (33.3)	2 (33.3)	2 (33.3)	6 (1.3)	
Gravida					
1-2	12 (6.4)	142 (75.5)	34 (18.1)	188 (39.8)	0.078
3-5	9 (5.7)	102 (64.6)	47 (29.8)	158 (33.8)	
6-9	6 (5.5)	67 (60.9)	37 (33.6)	110 (23.4)	
10-15	1 (7.1)	8 (57.2)	5 (35.7)	14 (3.0)	
Distance to ANC health facility (Kms)					
0-5	15 (5.2)	198 (68.8)	75 (26.0)	288 (66.5)	
6-10	3 (4.0)	52 (69.3)	20 (26.7)	75 (17.8)	0.436
11-20	3 (7.1)	33 (78.6)	6 (14.3)	42 (9.8)	
21-30	0 (0)	6 (54.6)	5 (45.4)	11 (2.6)	
31-40	1 (11.1)	4 (44.4)	4 (44.4)	9 (2.1)	
40-70	1 (16.7)	4 (66.7)	1 (16.7)	6 (1.4)	
Education level					
None	3 (3.7)	51 (62.2)	28 (34.1)	82 (17.5)	
Primary	19 (5.8)	220 (67.5)	87 (26.7)	326 (69.4)	0.021
Secondary	3 (6.2)	37 (77.1)	8 (16.7)	48 (10.2)	
Tertiary-College/University	3 (21.4)	11 (78.6)	0 (0)	14 (2.9)	
Delivery place					
Health facility	25 (7.3)	248 (72.7)	68 (19.9)	341 (72.6)	0.001
Home	3 (2.33)	71 (55.0)	55 (42.6)	129 (27.4)	
Marital status					
Married	26 (6.0)	296 (68.2)	112 (25.8)	434 (92.4)	
Single	2 (5.9)	21 (61.8)	11 (32.3)	34 (7.2)	0.798
Divorced/Sep/Widowed	0 (0)	2 (100.0)	0(0)	2(0.4)	

4.4 Discussion

Maternal immunization to boost RSV specific antibodies, is a strategy proposed to protect infants against RSV associated disease within the first few months of life (Hogan et al., 2017; Novavax, 2019). Implementation of a maternal RSV vaccine program will be influenced by several factors, one of them being the appropriate timing of vaccination. However, a major unknown is the distribution of gestational age at ANC visits that determines the proportion of women who attend at the ideal window for vaccination. In this chapter, a detailed analysis of the gestational age at attendance for ANC screening, the proportion attending ANC within a specific gestational age window for vaccine delivery and description of the related factors among pregnant women from Kilifi, coastal part of Kenya and in Siaya, Western Kenya is provided.

In this study, a random sample of women was selected from the registers of a demographic surveillance areas of Kilifi and Siaya. Not all women had an ANC booklet. Even though among Siaya women with or without ANC booklets differed in maternal age and marital status, in Kilifi, those with or those without ANC booklet did not differ in most characteristics (e.g. maternal age, occupation, education level, marital status, religion and number of pregnancies), but did differ in place of delivery. This was found to be an important observation to address potential bias in the results from Kilifi that would have been associated with sampling of the participants or missing data. We also found a high proportion of single women in Siaya did not have ANC booklets. It is likely that, these single women in Siaya might have had the ANC booklets but did not present them to the study team due to fear of disclosure of the spouse or partner's details.

Initiation of first ANC visit (median 26 weeks Kilifi and 22 weeks Siaya) among these women was found to be later than the WHO guidelines for a first visit of first 12 weeks' gestation (WHO, 2016) and as recommended by the Kenya national guidelines for quality obstetric care (MOH., 2012). Although, the delay in ANC initiation was found to be associated with other multiple factors, late presentation for first ANC screening limited

the number of visits a pregnant woman could attend. To date, Kenya still implements the basic ANC model of four ANC visits (MOH., 2012) which recommend first visit less than 16 weeks of gestation, second visit between 16 to 28 weeks, third visit between 28 to 32 weeks, and fourth visit between 32 to 40 weeks (MOH., 2012). The WHO too recommends pregnant women to have a total of 8 contacts with a health care provider (WHO, 2016). In this study, about 10% of the women with ANC booklets in Kilifi attended five ANC visits. Nevertheless, even at the 5th ANC visit, at least 1% of these women were attending within the proposed gestational age window for vaccination of 28-32 weeks. Whereas, among the 77 women who attended only one ANC visit in this study, 40 of them would have been missed for maternal RSV vaccination. To achieve a high vaccine coverage in this setting, will require majority of pregnant women to attend ANC within the proposed gestational age window period for vaccination. This observation emphasizes the need to encourage pregnant women to attend multiple ANC visits which will increase the opportunity of receiving all required health services, including vaccination.

This study also computed the estimates of the proportion of pregnant women that would be reached for vaccination if delivery is through ANC clinics. It was found that, about 76.6% of pregnant women (with at least one ANC visit) from the KHDSS area and 76.9% from Siaya HDSS area were within the gestational age window period targeted for vaccination of 28-32 weeks. Widening the vaccination window to 26-33 weeks and 24-36 weeks could see the proportion increase to 84.5% and 96.2% in Kilifi and 82.4% and 90.4% in Siaya, respectively. The current maternal RSV vaccines in clinical trials are antibody boosting vaccines (Higgins et al., 2016; PATH, 2022). Previous studies have shown that, maternal RSV antibodies from the KHDSS population decay rapidly at the rate of -0.58 (SD: 0.20) $\log^2$PRNT titre per month (Nyiro et al., 2015) and reach very low levels within a period of three months (Sande, Mutunga, Okiro, et al., 2013). The gestational age window for maternal RSV vaccination is therefore defined to occur within a time when delivery of the vaccine will ensure there is efficient maternal antibody

transfer, such that the level rising from boosting and the antibody decay kinetics combine to provide the infant maximum benefit. In a study to assess effect of timing of Tetanus-Diphtheria-acellular pertussis vaccine administration in pregnancy which was conducted among women in Melborne, Australia showed that, vaccination during 28-32 weeks gestation was associated with higher anti-pertussis IgG avidity, as compared with vaccination during 33-36 weeks gestation (Abu-Raya et al., 2019). Incorporation of antibody kinetics data to the wide and wider potential gestational age windows for maternal RSV vaccine delivery will therefore be necessary to confirm vaccine efficacy within this maternal population.

This study estimated maternal RSV vaccine coverage among Kilifi women using pregnancy data for ANC attendance collected during enumeration rounds from all KHDSS pregnant women. This is because there were no estimates of pregnant women who reported not to have attended ANC from the selected sample. A pilot survey within the KHDSS, asking all women with birth outcomes if they attended ANC, showed that 55% of the women who had birth outcomes in 2018 attended for at least one ANC visit. The KHDSS area records about 8000 pregnancies per year (Scott et al., 2012). Assuming 55% of the 8000 KHDSS pregnant women attended ANC and 76.6% are available for vaccination during the gestational age window of 28-32 weeks, the vaccine coverage among all pregnant women within KHDSS area would be 42.1%. A gestational age window of 26-33 weeks, with 84.5% visiting ANC in the vaccine window, would increase the overall vaccine coverage to 46.5%. A window of gestational age of between 24-26 weeks with 96.2% of women attending ANC, will have 52.9% maternal RSV vaccine coverage.

In this study, maternal tetanus vaccine was also used to assess the proportion of pregnant women who would be reached for vaccination if there was concomitant administration with the maternal RSV vaccine through ANC platform. It was approximated that, 54.7%

of the women in Kilifi attending ANC who received a tetanus vaccine would be reached for maternal RSV vaccination within the gestational age window period of 28-32 weeks. This implies, the estimated coverage for maternal RSV vaccine within KHDSS area would be 30.1% if it is co-administered with tetanus vaccine. However, these estimates were found to be lower than the maternal tetanus vaccine coverage reported by the ministry of health, District Health Information System (DHIS2) for Kilifi County of 45% for the year 2018. It was also noted that, co-administration of multiple vaccines during ANC attendance is possible though there might be challenges in obtaining accurate estimates of the vaccine coverage. This is likely if one of the vaccines is not influenced by the gestational age at the time of delivery and is limited in the number of recommended lifetime doses one should be eligible for, such as the tetanus vaccine.

Factors that influenced utilization of health care services were also found could potentially influence the level of success of a new intervention delivered through health facilities. In this study, women who had home births were found to report the main reasons as long distance to a health facility and health-care workers strikes. Notably, most of these women did not have education beyond primary school level. A study in Ghana reported socio-cultural perceived threats to pregnancy forced women to seek care during pregnancy from multiple sources including traditional herbalists (Dako-Gyeke et al., 2013). While, a study among Ethiopian women showed that education status at primary level was associated with home deliveries (Abeje et al., 2014). Analysis of data in this study, did not show travel distance to the health facility to be related to timing for initiation of ANC visit (P=0.436) or choice of a place for delivery. These results contradict earlier findings by Moindi and colleagues, who concluded that, the major reason why mothers still delivered at home in coastal Kenya, was the long distance from nearest health facility (Moindi, Ngari, Nyambati, & Mbakaya, 2016) . It was also noted that, the cost of accessing maternal care might not necessarily directly hinder utilization of health services among Kilifi women. This is because there are initiatives in Kenya

("Linda Mama" and "Beyond Zero") (Beyondzero, 2014) to provide free access to maternal and child health services for all pregnant women, but 30% of births in this study were reported to have occurred at home. The fact that some women declined to disclose reasons for not seeking obstetric care during pregnancy, shows there could be other underlying and unknown factors among these women that could negatively impact the delivery of any new maternal vaccine program. Multiple initiatives as a strategy to encourage and influence positive health care seeking practices might be useful for these women.

There are some limitations to this study. The first and major limitation is that, pregnancy dating by fundal height is not an accurate method for estimating gestational age. Fundal height is likely to under or overestimate gestational age in this setting. However, this is the method available and in use in all public hospitals in Kenya. Pregnancy dating by last menstrual period which is dependent on participant recall was missing for most women. Second, only 47% of the random sample of 1000 pregnant women had ANC booklets available. Therefore, the sample of 470 women with complete gestational age data might not be representative of the general KHDSS pregnant women population. Although assessment for this bias using demographic data from the census registers found that these women were similar in most characteristics. Furthermore, a large sample size of 781 women in Siaya did not show significant difference with that of Kilifi in the proportion of women that would have been reached for vaccination. Third, women without available ANC booklets or those who deliver at home may still have visited ANC clinics and could therefore potentially receive a vaccine. Fourth, the estimated vaccination window can only infer vaccine coverage and not necessarily vaccine effectiveness. Whether within this proposed window, the infant will have maximum benefit requires incorporation of data on antibody kinetics. In addition, vaccine effectiveness will require assessment of data on birth weight, gestational age at delivery, prematurity, as well as health factors like maternal malaria, anaemia, HIV infection, hypergammaglubinemia etc., which this part of the study could not address. The present

Chapter provides insights into the distribution of gestational age at attendance for ANC screening and proportion of pregnant women likely to be reached for maternal RSV vaccination. Based on the current knowledge, this is the first study from Kenya and sub-Saharan Africa to present data on timing for ANC visits which includes gestational age for subsequent visits and describes how this timing in ANC attendance is likely to affect the successful implementation of a maternal RSV vaccine program.

4.5 Conclusions

At least 77% of pregnant women from Kilifi and Siaya HDSS, attending ANC would be reached for maternal RSV vaccination delivery through the ANC clinics at the currently optimum gestational age window. Despite the diverse geographical region, the timing for ANC attendance and proportion of women who will be reached for vaccination is similar. Concomitant administration of tetanus and RSV vaccine in the same period suggests 55% of women in Kilifi attending ANC would be available for uptake of both vaccines. Widening the vaccination window leads to a potential modest increase in vaccine coverage and its effect requires taking account of antibody kinetics data. Improving ANC attendance is a high priority for the success of a maternal RSV vaccine.

CHAPTER FIVE

EFFICIENCY OF TRANSPLACENTAL TRANSFER OF RESPIRATORY SYNCYTIAL VIRUS (RSV) SPECIFIC ANTIBODIES AMONG PREGNANT WOMEN IN KENYA

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Abstract

Maternal immunisation to boost respiratory syncytial virus (RSV) antibodies in pregnant women, is a strategy being considered to enhance infant protection from severe RSV associated disease. However, little is known about the efficiency of transplacental transfer of RSV-specific antibodies in a setting with high burden of malaria and HIV, to guide implementation of such a vaccination program.

Four hundred pairs of cord and maternal serum specimens from pregnant women for RSV-specific antibodies were screened using a plaque reduction neutralization assay. Participants were pregnant women of two surveillance cohorts: 200 participants from a hospital cohort in Kilifi, Coastal Kenya and 200 participants from a surveillance cohort in Siaya, Western Kenya. Transplacental transfer efficiency was determined by cord to maternal titre ratio (CMTR). Logistic regression was used to determine independent predictors of impaired transplacental transfer of RSV-specific antibodies.

A total of 800 samples were screened from the 400 participants. The median age at enrollment was 25 years (Interquartile range (IQR): 21-31). Women from Kilifi cohort were younger (16% vs 2%; p<0.001), had more premature births (5% vs 2%; p=0.022), had more women with education up to primary (73.5% vs 55%; p<0.001) and had more babies with low birthweight (21.5% vs 6%; p<0.001) compared to Siaya cohort. Overall, transplacental transfer was efficient and did not differ between Kilifi and Siaya cohort (1.02 vs 1.02; p=0.946) but was significantly reduced among HIV-infected mothers compared to HIV-uninfected mothers (mean CMTR :0.98 vs 1.03; p=0.015). Prematurity <33weeks (Odds ratio (OR): 0.23, 95% confidence interval (CI)0.06-0.85; p=0.028), low

birth weight <2.5kgs (OR: 0.25, 95% CI:0.07-0.94; p=0.041) and HIV infection (OR: 0.47, 95% CI:0.23-0.98; p=0.045) reduced efficiency of transplacental transfer among these women.

Transplacental transfer of RSV-specific antibodies among pregnant women in Kenya is efficient. A consideration to integrate other preventive interventions with maternal RSV vaccination targeting infants born premature (<33 weeks gestation), with low birth weight<2.5kgs or HIV-infected mothers is highly recommended in this setting.

5.1 Introduction

Globally, respiratory syncytial virus (RSV) is a significant cause of acute lower respiratory tract infection (LRTI) among infants leading to hospital admissions and in-hospital deaths, with 99% of these deaths occurring in developing countries (Nair et al., 2010). In sub-Saharan Africa and Asia, RSV has been observed to be responsible for about 40% of all hospital admissions with severe or very severe pneumonia among infants under 1 year (PERCH, 2019). Severe RSV-associated LRTI is most common among infants under six months of age (Li et al., 2021; Nokes et al., 2009; Shi et al., 2017b), accounting to about 32% of hospitalised infants in rural coast of Kenya, during epidemics (Nokes et al., 2009).

Maternal immunisation is currently being considered as a strategy to protect infants from severe RSV associated disease because of the lack of licenced RSV vaccines targeting infants and frequency of cases aged less than the first scheduled infant immunisations (Englund et al., 1998; Glezen et al., 1981; Madhi et al., 2020; Roca et al., 2002). Efforts to advance maternal immunisation for RSV have shown promise and several candidate maternal RSV vaccines are in late stages of clinical trials (Higgins et al., 2016; Madhi et al., 2020; PATH, 2022). Despite the advancement in development of maternal RSV vaccines, the success of this program will depend on how efficiently vaccine-induced RSV-specific antibodies are transferred to the infant.

Previous studies have shown that transplacental transfer of RSV-specific antibody to the

infant is usually efficient with a cord blood to maternal blood antibody titre ratio of ≥ 1 (Cox, Azevedo, Cane, Massad, & Medley, 1998; R. O. Suara et al., 1996). Transplacental transfer of IgG antibodies begins during the 28th week of gestation which is coincident with the timing for expression of Fc gamma RII (FcgII) receptor responsible for materno-foetal transfer of antibodies (Kameda et al., 1991). Thus, infants born preterm, shortly after or before initiation of transplacental transfer of antibodies are less likely to benefit from a maternal RSV vaccine program. Placental malaria, hypergammaglobinaemia (total IgG >15g/L), HIV infection and possible illness episodes or infection occurring during the third trimester of pregnancy have been known to influence the level of antibodies transferred to the infant (Hartter et al., 2000; B. J. Okoko, L. H. Wesumperuma, M. O. Ota, et al., 2001; J. B. Okoko et al., 2001a). However, there is limited data on efficiency of transplacental transfer of RSV-specific antibodies in settings where the maternal population experiences comorbidities such as malaria, HIV and undernutrition as well as premature deliveries which could negatively impact the effectiveness of a maternal RSV vaccine.

This Chapter aimed to describe the efficiency of transplacental transfer of RSV-specific antibodies among pregnant women in Kenya using cord-maternal blood sample pairs collected from pregnant women in the counties of Kilifi (coast region) and Siaya (western region). It also describes background factors and illness episodes occurring during pregnancy that could influence transplacental transfer of RSV-specific antibodies, which is useful information required to support successful implementation of a maternal RSV vaccine program in Kenya.

5.2 Methods

5.2.1 Study sites and population

Data collection for this study was conducted at Kilifi County, Siaya and Bondo areas in Siaya County, Kenya. The study population were pregnant women who were participants in two separate cohort studies; a hospital-based surveillance investigating risk factors for

severe morbidity and mortality in mothers and their infants in Kilifi, coastal Kenya, and a second in Siaya County, Western Kenya, for surveillance of influenza disease.

The surveillance to investigate risk factors for severe morbidity and mortality in mothers and their infants was set up by KEMRI-Wellcome Trust Research Programme (KWTRP) at the maternity ward of Kilifi County Hospital (KCH) and the Kilifi Health and Demographic Surveillance System (KHDSS) area in Coastal Kenya in 2011 (Seale, Barsosio, Koech, Berkley, & group, 2015). This surveillance had proposed to observe 4600 births with approximately 2300 being residents of KHDSS. All mothers presenting for delivery at the maternity ward of KCH were invited to enroll. Routine clinical data were collected using a standardized questionnaire at admission to the maternity department and following delivery. In this surveillance, consent was sought from pregnant women presenting at KCH maternity ward to collect cord and maternal blood samples after delivery. These samples were securely stored at -80˚C at KEMRI-Wellcome Trust laboratories in Kilifi for molecular and serological testing for viral and bacterial pathogens.

From the Western part of Kenya, in Siaya County, a cohort of pregnant women was set up through a collaboration between KEMRI-Centre for Global Health Research and Centers for Disease Control and Prevention (CDC) Kenya in 2015. This surveillance included pregnant women who were recruited either from their homes or when they visited for antenatal care at Bondo sub-County or Siaya County Referral Hospital. Participants were enrolled at gestational age <20 weeks. These pregnant women were followed up weekly through a phone call or by home visit to record occurrence of influenza like illness episodes. Blood samples were collected at enrolment and a maternal and cord blood at birth. If a pregnant woman was identified with cough or fever during follow up, a respiratory specimen was collected and screened for Influenza virus type A and B and for RSV using molecular methods (Nyawanda et al., 2020). All participants were requested to

deliver their children in the hospital where birth outcomes were recorded; thereafter both the baby and the mother were followed up weekly for a period of up to 6 months post-delivery to assess infection from respiratory viruses by testing nasal and throat swabs from symptomatic cases by reverse transcription polymerase chain reaction (RT-PCR).

A sample of 400 participants (200 from each region) was randomly selected from the cohort registers based on availability of meta-data and paired cord and maternal blood samples for births (including preterm births) that occurred in 2018 and 2019 using methods earlier described in Chapter Three.

5.2.2 Laboratory Procedures

All blood samples were screened for RSV specific antibodies using an inhouse plaque reduction neutralization titre (PRNT) assay (Nyiro et al., 2019; Sande, Mutunga, Okiro, et al., 2013) at KWTRP laboratories, Kilifi, Kenya. Details of the procedure for this assay have been provided in Appendix VII. The PRNT procedure determines the concentration of functional antibodies from a human serum sample (or antibody preparation) required to induce 50% neutralization of a known titration of RSV virus using the Spearman Karber method (Sande, Mutunga, Medley, Cane, & Nokes, 2013). As previously described in Chapter Three, in this PRNT assay, micro-RSV plaques are stained brown by immunoperoxidase and counted with an ELISPOT reader.

A RSV group A human reference standard (RSV IS 16/284) (McDonald, Rigsby, Dougall, Engelhardt, & Study, 2018) obtained from National Institute for Biological Standards and Control (NIBSC), Potters bar, UK and an inhouse pooled adult sera were incorporated into each assay run to check for antibody deterioration, standardization of sample titres and for quality control.

5.2.3 Ethical considerations

Written informed consent to collect samples and data for storage and use in other studies was obtained from all participants through the parent studies i.e. the influenza cohort

surveillance (SERU #2880) and the surveillance for risk factors cohort (SERU #1778). Ethical approval to screen samples for RSV-specific antibodies and use of data from the parent studies for this study was granted by the KEMRI Scientific and Ethical Review Unit Committee (SERU #3716). All methods were carried out in accordance with relevant guidelines and regulations for conducting research in human subjects.

5.2.4 Statistical Analysis

Separate analysis was done for each cohort and with the combined data from both cohorts. The efficiency of transplacental transfer of RSV-specific antibodies was calculated for each mother-infant pair of blood samples. A CMTR (i.e. PRNT titre cord/PRNT titre maternal blood) of ≥1 was considered normal or efficient, CMTR <1 but ≥0.8 as moderately impaired and <0.8 as severely impaired or poor. Duration of transplacental transfer was calculated as gestational age at delivery minus 28; where 28th week was estimated as the gestational age when transplacental transfer of IgG antibodies begins during pregnancy. The difference in CMTR, cord or maternal RSV PRNT titres between HIV-infected versus HIV-uninfected mothers and RSV-infected infants vs uninfected infants were analysed using a two-sample paired t test. The Chi square test was used to compare characteristics of women between Kilifi and Siaya cohort; and was also applied to determine association between maternal/infant characteristics (HIV infection, malaria infection, RSV infection, anaemia, education level, occupation, gestational age at delivery and birth weight) and efficiency of transplacental transfer of RSV-specific antibodies. Logistic regression adjusted for each variable category (HIV infection, malaria infection, gestational age at delivery, gravida, birthweight and RSV infection during pregnancy) was used to determine independent predictors of an impaired transplacental transfer of RSV specific antibodies. Data analysis was conducted using STATA version 15.0 (Stata Corp, College Station, Texas, USA).

5.3 Results

5.3.1 Characteristics of study participants

A total of 800 samples of cord and maternal blood from 400 participants selected from the two cohorts were screened for RSV specific neutralizing antibodies. The median age of the women at enrollment was 25 years (Interquartile range (IQR): 21-31 years). About 95% of these women reported being married, 6% had no formal education, 57% were housewives and 21% had experienced more than 6 live births (Table 5). The overall mean (SD) birth weight (from both cohorts) of infants was 3.03kgs (0.56), and 55 (14%) of the infants were born with low birth weight <2.5 kilograms. Mean (SD) gestational age at delivery was 38.3 weeks (2.62). There were 11 infants out of 200 infants born from women sampled from Siaya cohort who got RSV infection under 6 months of age. Additionally, among women from Siaya cohort, 37 (19%) were HIV infected, 52 (26%) had malaria infection, 5 (3%) had RSV infection and 12 (6%) had severe anaemia during pregnancy. These additional data were not available for women from Kilifi cohort.

Analysis of the difference in characteristics of participants from the two cohorts showed these women were significantly different in most characteristics (Table 7). Compared to the Siaya sample, Kilifi pregnant women were younger i.e.15 to19 years (16% vs 2% ; P<0.001), had more premature births (5% vs 2% ; P=0.022), more babies were born with low birthweight <2.5kgs (21.5% vs 6%; P<0.001), more women had lower than secondary level of formal education (73.5% vs 55% ;P<0.001) and more women were housewives (68% vs 46%; P<0.001). However, these women were similar in proportion of those married (95.5% vs 95%; P= 0.814) and sex of the infant (47% vs 48%; P=0.841).

Table 7*: Participants Characteristics*

Characteristic	Kilifi (n)	%	Siaya (n)	%	Total (n)	%	P* value
	200	50	200	50	400	100	
Maternal age							
15-19	31	15.50	4	2.00	35	8.75	
20-24	65	32.50	81	40.50	146	36.50	
25-29	43	21.50	60	30.00	103	25.75	<0.001
30-34	30	15.00	32	16.00	62	15.50	
35-39	16	8.00	19	9.50	35	8.75	
40-44	15	7.50	2	1.00	17	4.25	
45-49	0	0.00	2	1.00	2	0.50	
Marital status							
Married	191	95.50	190	95.00	381	95.25	
Single	9	4.50	10	5.00	19	4.75	0.814
Gestational age at delivery							
<33 weeks	10	5.00	3	1.50	13	3.25	
33-37 weeks	59	29.50	44	22.00	103	25.75	0.022
38-42 weeks	131	65.50	153	76.50	284	71.00	
Education level							
None	23	11.50	2	1.00	25	6.25	
Primary	124	62.00	108	54.00	232	58.00	<0.000
Secondary	37	18.50	74	37.00	111	27.75	
Tertiary-College/University	16	8.00	16	8.00	32	8.00	
Gravida							
1-2	112	56.00	41	20.50	153	38.25	
3-5	65	32.50	127	63.50	192	48.00	<0.000
6-9	18	9.00	31	15.50	49	12.25	
10-15	5	2.50	1	0.50	6	1.50	
Number of ANC visits							
1	9	4.52	146	73.00	155	38.85	
2	22	11.06	45	22.50	67	16.79	
3	45	22.61	7	3.50	52	13.03	<0.000
4	57	28.64	2	1.00	59	14.79	
5	38	19.10	0	0.00	38	9.52	
6	28	14.07	0	0.00	28	7.02	
Sex of child							
Female	94	47.00	96	48.00	190	47.50	0.841
Birthweight							
Underweight (<2.5kgs)	43	21.50	12	6.00	55	13.75	<0.000
lacental transfer Efficiency							
Impaired	77	38.50	89	44.50	166	41.50	0.946

(*P-Chi squared P value)

5.3.2 Distribution of Cord and Maternal RSV-specific antibodies among pregnant women in Kenya

Overall, the mean cord PRNT titres from both cohorts was 10.69 log2PRNT (SD: 1.17), with a median titre of 10.85 log2PRNT (IQR 10.00-11.59), while the mean maternal log2PRNT RSV antibodies was 10.53 log2PRNT (SD:1.19), with a median of 10.62 log2PRNT (IQR 9.92-11.40). The mean titres of cord RSV-specific antibodies from Kilifi cohort was 10.64 log2PRNT (SD:1.25), with a median titre of 10.91 log2PRNT (Interquartile Range (IQR) 9.92 -11.62). In the Siaya cohort, the mean cord RSV antibody titre was 10.74 log2PRNT (SD:1.08), and median 10.79 log2PRNT (IQR 10.09-11.55) respectively (Figure 9). Both mean cord (10.64 vs 10.74; p=0.374) and mean maternal (10.47 vs 10.59; p=0.319) log2PRNT titres of RSV-specific antibodies between Kilifi and Siaya cohorts were not significantly different.

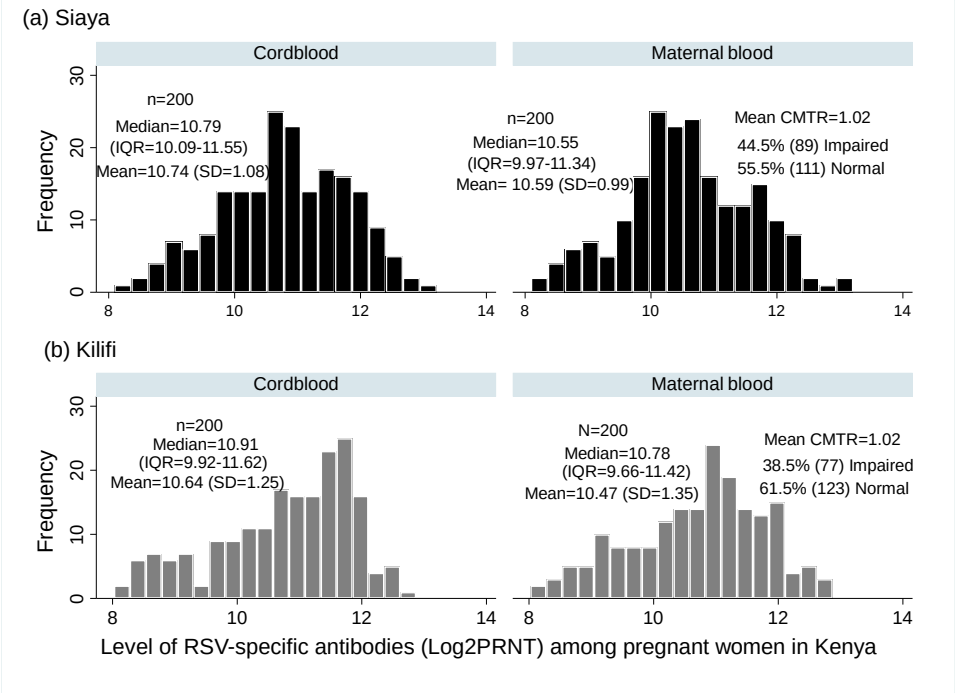

Figure 9. Frequency distribution of cord and maternal RSV specific antibodies (log2 ransformed PRNT titres) at birth from 400 women from Kilifi and Siaya, Kenya. The mean (STDDeV), median (Inter quartile range) and the mean cord to maternal transfer ratio for each study cohort is shown.

No difference was found in the mean log2PRNT titres in cord blood for infants with RSV infection compared to infants without RSV infection in the first 6 months of life (10.50 log2PRNT (SD: 0.99) vs. 10.74 (SD: 1.07) respectively, p=0.186). The mean (SD) cord PRNT titres transferred to infants of 37 (19%) HIV-infected mothers of 10.41 (SD: 1.14) log2PRNT was significantly lower (p=0.039) than that of infants from 163 HIV-uninfected mothers 10.81 (SD: 1.05) log2PRNT. The cord log2PRNT RSV antibody levels of infants from mothers with and without severe anaemia (10.87 versus 10.73: p=0.202) and mothers with and without malaria (10.90 vs 10.68: p=0.666) were not significantly different.

5.3.3 Efficiency of transplacental Transfer of RSV-specific antibodies

The transplacental transfer of RSV-specific antibodies among these women was efficient with a mean CMTR of 1.02 (SD=0.09) and median 1.01 (IQR 0.97-1.06). The mean CMTR values were similar for pregnant women from Kilifi and Siaya (1.02 vs 1.02; p=0.946; t=0.067).The transplacental transfer of RSV specific antibodies for women from Kilifi and Siaya cohort was; severely impaired in 1 (0.5%) and 6 (3.0%), moderately impaired in 76(38.0%) and 83 (41.5%), and normal in 123 (61.5%) and 111 (55.5%), respectively. The overall proportion of women from the two cohorts with impaired transplacental transfer was 41.5% (166/400; 77 Kilifi vs 89 Siaya).

Analysis of the trend in efficiency of transplacental transfer and characteristics of these women (Table 8), showed a significantly lower CMTR value among women who were HIV-infected (mean CMTR :0.98 vs 1.03; p=0.015) and women who reported their occupation as farming (mean CMTR :0.96 vs 1.02; p=0.012). The CMTR value among women who got RSV infection during pregnancy (mean CMTR :0.98 vs 1.02; p=0.416) and that of mothers whom infants got RSV disease under 6 months of age (1.01 vs 1.02; p=0.489) was not significantly different.

Table 8*: Transplacental transfer of RSV specific antibodies (CMTR) and characteristics of participants*

Efficiency of Transplacental Transfer of RSV specific antibodies									
Characteristic	**Kilifi**			**Siaya**			**All women**		
	n=200	**Mean CMTR**	**SD***	**n=200**	**Mean CMTR**	**SD***	**n =400**	**Mean CMTR**	**SD***
ıational age at delivery									
<33 weeks	10	1.00	0.08	2	0.90	0.06	12	0.98	0.09
34-37 weeks	59	1.02	0.06	44	1.02	0.08	103	1.02	0.07
38-42 weeks	131	1.02	0.07	154	1.02	0.11	285	1.02	0.10
Gravida									
1-2	112	1.02	0.07	41	1.05	0.10	153	1.03	0.08
3-5	65	1.01	0.08	127	1.02	0.11	192	1.02	0.10
6-9	18	1.00	0.07	31	0.98	0.09	49	0.99	0.08
10-15	5	0.97	0.11	1	0.99		6	0.98	0.10
Occupation									
Farmer	1	0.98		13	0.96	0.06	14	0.96	0.06
Businesswoman	23	1.03	0.07	59	1.02	0.09	82	1.02	0.09
Housewife	136	1.01	0.07	92	1.01	0.11	228	1.01	0.09
Salaried worker	16	1.03	0.07	18	1.03	0.14	34	1.04	0.11
Other	24	1.02	0.08	18	1.04	0.06	42	1.03	0.07
Marital status									
Single	9	1.01	0.06	10	0.99	0.11	19	1.00	0.08
Married	191	1.02	0.07	190	1.02	0.10	381	1.02	0.09
Maternal age (yrs)									
15-19	31	1.02	0.07	4	0.96	0.20	35	1.02	0.09
20-29	108	1.02	0.07	141	1.02	0.10	249	1.02	0.09
30-39	46	1.02	0.07	51	1.00	0.10	97	1.01	0.09
40-49	15	1.03	0.08	4	0.94	0.06	19	1.00	0.09
Education level									
None	23	1.01	0.08	2	1.06	0.04	25	1.01	0.08
Primary	124	1.01	0.07	108	1.02	0.09	232	1.02	0.08
Secondary	37	1.03	0.07	74	1.01	0.13	111	1.02	0.12
Tertiary	16	1.05	0.09	16	1.03	0.05	32	1.03	0.07
HIV status									
Negative				163	1.03	0.11			
Positive				37	0.98	0.08			
Severe anaemia									
No				188	1.02	0.11			
Yes				12	1.00	0.07			
Malaria									
No				148	1.01	0.11			
Yes				52	1.04	0.08			
Maternal RSV infection									
Negative				195	1.02	0.11			
Positive				5	0.98	0.86			

*SD- Standard deviation)

5.3.4 Illness episodes during pregnancy and transplacental transfer of RSV-specific antibodies

Assessment of illness episodes from the 200 women sampled from Siaya cohort showed 120 (60%) pregnant women had sick outpatient visits captured during weekly follow ups and 6 of them required hospitalisation. Data on illness episodes during pregnancy among Kilifi women was not available for this study.

The most common complaints for the outpatient visits among Siaya women were cough 67(55.8%), abdominal pain 67(55.8%), other acute respiratory illness (runny nose, shortness in breathing and chest pain while breathing 35(29.2%)), joint pain 27 (22.5%), vomiting or diarrhoea 22(18.3%), urinary tract infection 18(15.0%), fever 17(14.2%) and sore throat 12 (10.0%). One participant had premature labour. Experiencing cough episodes during pregnancy was found to be associated with impaired transplacental transfer of RSV-specific antibodies (67.3% vs 32.7%: Chi^2 p=0.027).

Multiple illness episodes during the third trimester of pregnancy occurred in 79/120 (66%) of the sick participants. There were none of the following illness episodes reported during pregnancy in this sample of women: gestational diabetes, hypertension or pre-eclampsia. No illness episode during the third trimester of pregnancy was found to be associated with efficiency of transplacental transfer of RSV-specific antibodies.

5.3.5 Gestational age at delivery and transplacental transfer of RSV- specific antibodies

The effect of gestational age in influencing transplacental transfer of RSV antibodies is demonstrated in a scatterplot of CMTR by duration in weeks of transplacental transfer in Figure 10. The scatter plot shows level of CMTR is less than 1 or impaired within 4 weeks after onset of transplacental transfer (onset period estimated at 28^{th} week of gestation), CMTR gradually increases above one in most participants in the next 8 weeks and starts to decline to low levels 12 weeks after onset of transplacental transfer.

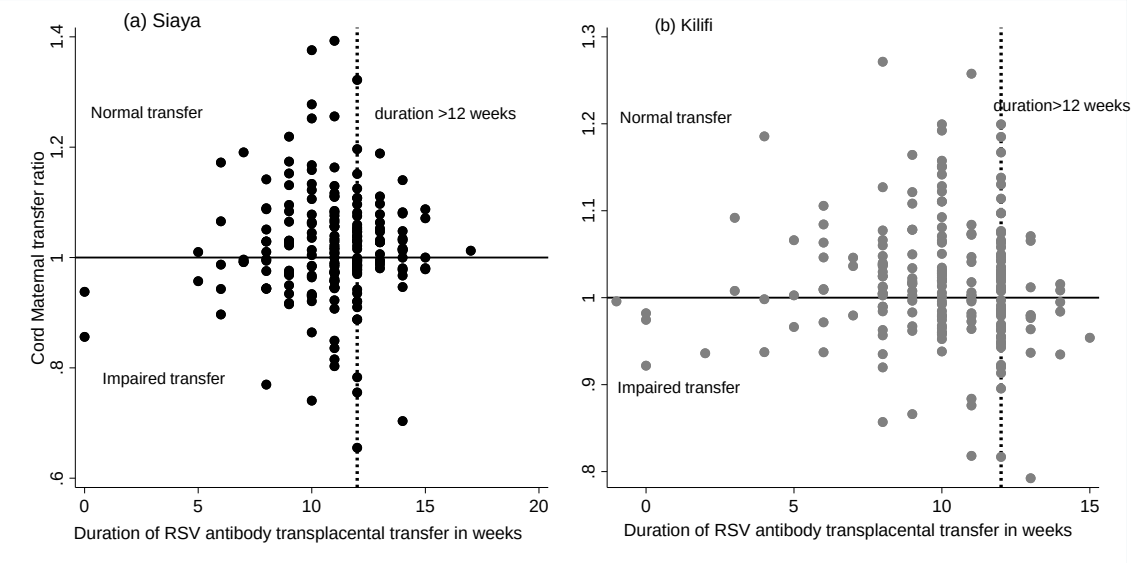

Figure 10. A scatter plot for cord to maternal transfer ratio by duration of transplacental transfer among pregnant women from Kilifi and Siaya, Kenya. The line for efficiency in each cohort is shown.

5.3.6 Factors influencing transplacental transfer of RSV-specific antibodies among pregnant women in Kenya

Among pregnant women in Kilifi, gestational age at delivery of <33 weeks was found to be significantly associated with reduced transplacental transfer of RSV-specific antibodies (p=0.034). In a univariate logistic analysis, transplacental transfer was likely to be increased 5.8 times more in births occurring between 34-37weeks compared to births in less than 33 weeks of gestation (Odds ratio (OR): 5.8, 95% confidence interval (CI)1.33-24.95) (Table 9).

Table 9: ***Predictors of an Impaired transplacental transfer among women from Kilifi cohort***

Efficiency of transplacental transfer (Kilifi)										
Characteristic	**Category**	**Normal**		**Impaired**		**Chi2 P value**	**OR**	**Odds Ratio (95% CI)**		**Odds ratio P value**
		n (123)	**%**	**n (77)**	**%**			**LCL**	**UCL**	
Gestational age delivery										
	<33 weeks	3	2.4	7	9.1		Ref			
	34-37 weeks	42	34.2	17	22.1	**0.034**	5.8	1.33	24.95	0.019
	38-42 weeks	78	63.4	53	68.8		3.4	0.84	13.88	0.083
Birthweight										
	nderweight (<2.5kgs)	25	20.3	18	23.4					
	Normal	98	79.7	59	76.6	0.609				
Maternal age(yrs)										
	15-19	18	14.6	13	16.9					
	20-29	65	52.9	43	55.8	0.589				
	30-39	32	26.0	14	18.8					
	40-49	8	6.5	7	9.1					
ucation level										
	None	13	10.57	10	12.99					
	Primary	74	60.16	50	64.94	0.718				
	Secondary	25	20.33	12	15.58					
	Tertiary	11	8.94	5	6.49					
Gravida										
	1-2	72	58.54	40	51.0					
	3-5	41	33.33	24	31.2	0.166				
	6-15	10	8.13	13	16.9					
On medication										
	No	13	10.6	6	7.8					
	Yes	110	89.4	71	92.2	0.515				

In Siaya cohort, transplacental transfer of RSV-specific antibodies was found to be significantly impaired among women with; gravida of more than 6 (OR: 0.56, 95% CI:0.35-0.91; p=0.02), occupation as farming (OR: 0.13, 95% CI:0.03-0.60; p=0.009), HIV infection (OR: 0.47, 95% CI:0.23-0.98; p=0.045) and infants with low birth weight <2.5 kilograms (kgs) (OR: 0.25, 95% CI:0.065-0.94; p=0.041).

Table 10*: Predictors of an Impaired transplacental transfer among pregnant women from Siaya cohort*

Efficiency of transplacental transfer (Siaya)						Chi2				Odds ratio
Characteristic	**Category**	**Normal**		**Impaired**		**P value**	**OR**	**Odds Ratio (95%CI)**		**P value**
		n (111)	**%**	**n (89)**	**%**			**LCL**	**UCL**	
ernal age (yrs)	15-19	3	2.7	1	1.12					
	20-29	83	74.77	58	65.17					
	30-39	25	22.52	26	29.21	0.068				
	40-49	0	0	4	4.49					
estational age at delivery	<33 weeks	0	0.9	2	2.25					
	34-37 weeks	22	19.82	22	24.72	0.186				
	38-42 weeks	88	80.18	65	73.03					
Birthweight										
	nderweight (<2.5kgs)	3	2.7	9	10.11					
	Normal	108	97.3	80	89.89	**0.028**	4.05	1.06	15.4	0.041
Gravida										
	1-2	27	24.32	14	15.73		Ref			
	3-5	72	64.86	55	61.8	**0.049**	0.67	0.33	1.42	0.301
	6-9	12	10.81	20	22.47		0.31	0.12	0.82	0.018
Occupation	Farmer	2	1.8	11	12.36		Ref			
	Business woman	37	33.33	22	24.72		9.25	1.87	45.65	0.006
	Housewife	50	45.05	42	47.19	**0.031**	6.55	1.37	31.21	0.018
	Salaried worker	10	9.01	8	8.99		6.87	1.17	40.38	0.033
	Other	12	10.81	6	6.74		11	1.82	66.37	0.009
Education level										
	Primary/None	57	51.35	53	59.55					
	Secondary	43	38.74	31	34.83	0.378				
	Tertiary	11	9.11	5	5.62					
HIV status										
	Negative	96	86.49	67	75.28		Ref			
	Positive	15	13.51	22	24.72	**0.043**	0.48	0.23	0.98	0.045
Severe Anaemia										
	No	106	95.5	82	92.13					
	Yes	5	4.5	7	7.87	0.32				
Sick_Cough										
	No	36	52.94	17	32.69		Ref			
	Yes	32	47.06	35	67.31	**0.027**	2.3	1.09	4.9	0.028
Malaria										
	No	76	68.47	72	80.9		Ref			
	Yes	35	31.53	17	19.1	**0.046**	1.95	1	3.78	0.048

Multivariate analysis of Siaya cohort data including gravida, HIV infection, occupation and birthweight to the model for Siaya women, only low birth weight <2.5kgs was strongly associated with reduced efficiency of transplacental transfer of RSV specific antibodies (OR: 0.21, 95% CI:0.05-0.85; p= 0.029). Malaria infection was significantly associated with increased transplacental transfer of RSV-specific antibodies (OR: 1.95, 95% CI:1.01-3.79; p=0.048) (Table 10). The 14/400 (3.5%) women who reported were farmers, were 25 years and older and had education up to primary level. Majority of pregnancies (76%) among Siaya women were delivered at term i.e. ≥37 weeks and therefore, no significant association was found between efficiency of transplacental transfer and gestational age at delivery.

In a multivariate logistic analysis of combined data from Kilifi and Siaya cohorts, occupation as a farmer (OR: 0.16, 95% CI:0.03-0.73; p=0.018), gravida>6 (OR: 0.70, 95% CI:0.52-0.94; p=0.023) and gestational age at delivery<33 weeks (OR: 0.22, 95% CI:0.06-0.84; p=0.027) were significantly associated with reduced transplacental transfer of RSV-specific antibodies. Adjusting for study site did not have any effect on these factors.

5.4 Discussion

In this Chapter, Kenyan women from the two geographical regions of Siaya and Kilifi were found to differ in many characteristics, but these differences did not affect the mean levels of RSV-specific antibodies transferred to infants or overall efficiency of transplacental transfer. Multiple factors including gestational age less than 33 weeks, having had multiple pregnancies and farming as an occupation were found to be associated with reduced transplacental transfer of RSV specific antibodies among pregnant women from the two cohorts.

The concentration of RSV antibody transferred to infants by HIV-infected mothers were significantly reduced. Similarly, the trend of cord to maternal antibody titre ratio showed

a decrease with HIV infection. These findings are in line with previous studies (Alonso et al., 2021) which together raise concerns involving the effectiveness of a maternal RSV vaccine introduction to low-and middle-income countries (LMICs) which is thought might be negatively impacted by the existing comorbidities. Furthermore, ongoing clinical trials (NCT04424316; NCT04605159) (Madhi et al., 2020) of maternal RSV vaccines are not taking into account HIV diverse population, or populations with high malaria prevalence. Therefore, investigating differences in transplacental transfer in these populations could be important in validating vaccine response in the future.

In this study, gestational age at delivery <33weeks showed reduced transplacental transfer of RSV-specific antibodies. Gestational age has been known to influence transplacental transfer of IgG antibodies in the Gambia (J. B. Okoko et al., 2001a) and in Sri Lanka (Wesumperuma et al., 1999) where materno-foetal transfer of RSV-specific antibodies was impaired in premature babies. Similarly, in estimating the duration of transplacental transfer by gestational age using the 28th week of pregnancy as the onset for transplacental transfer, babies born shortly (<4weeks) after the beginning of this transfer were found to have an impaired transplacental transfer. While, babies born more than 3 months after onset of transplacental transfer showed decreased CMTR which was as a result of antibody decay (Swamy & Garcia-Putnam, 2014). Studies have also shown that, before the 26th week of gestation, IgG transfer is blocked by a barrier of cytotrophoblasts under the syncytiotrophoblast layer (Kristoffersen, 2000) and Fc gamma RII (FcgII) receptors responsible for mediation of materno-foetal transfer of antibodies are not well expressed (Kameda et al., 1991). These results, therefore, demonstrate the phenomenon of accumulation of antibody concentration among infants with time of transfer, waning of RSV-specific antibodies occurring with wild type RSV infection and confirm the influence of gestational age on timing for vaccination, likely to be observed during the implementation of a maternal RSV vaccine in this setting. Having had more than 6 pregnancies, HIV infection, and low birth weight <2.5kgs was found to be associated with impaired transplacental transfer of RSV-specific antibodies. These results

are similar to findings from a study in the Gambia, where low birthweight <2.5kgs was found to influence transplacental transfer of RSV-specific antibodies (J. B. Okoko et al., 2001a). The role of HIV infection in impairing transplacental transfer of RSV antibodies has been found in studies conducted in Botswana (M. S. Patel et al., 2020) and Malawi (de Moraes-Pinto et al., 1998). HIV-infected pregnant women have shown reduced immunogenicity to vaccines and this is thought to be related to immune activation leading to production of inflammatory cytokines at the materno-foetal interface (Abu-Raya, Smolen, Willems, Kollmann, & Marchant, 2016; Wilcox, Holder, & Jones, 2017). Women with multiple births or pregnancies are usually older and due to repeated exposure to RSV infection, they are thought to have an accumulation of higher levels of antibodies which causes saturation of Fc transport receptors (Clements et al., 2020) leading to reduced transfer and thereby low levels of antibodies observed in neonates.

Women diagnosed with malaria during pregnancy had efficient transplacental transfer in this study contrary to what has been observed in other studies in Papua New Guinea and Malawi (Atwell et al., 2019; de Moraes-Pinto et al., 1998), where malaria was associated with a decrease in transplacental transfer of IgG antibodies of 81%. The process of materno-foetal transfer of pathogen specific antibodies is likely to vary between different populations due to impairment caused by saturation of these receptors with infection related hypergammaglobineamia (Wilcox et al., 2017). Since antimalarial prophylaxis uptake is mandatory to all pregnant women in Kenya during antenatal visits, it is likely that, this might have played an important role in reducing malaria related hypergammaglobineamia. Thus, 67% of the women diagnosed with malaria infection were found to have a normal transplacental transfer of RSV-specific antibodies. However, further screening of samples for evidence of placental malaria is warranted among this sample of women.

This study has some limitations. First, total immuno-globulin G levels were not screened and thus not able to confirm any infection related hypergammaglobulinemia to the impaired placental transfer of RSV antibody seen in HIV-infected women, women with illness episodes during pregnancy and those diagnosed with malaria in this study. Second, by the time this analysis was conducted, results for placental malaria among women from Siaya was not yet available. Therefore, could not ascertain the positive effect of transplacental transfer in the presence of malaria infection. In addition, data for HIV antiviral therapy, adherence or viral load among these women was not available by the time of analysis, although HIV infected mothers were all under comprehensive care programme. Additionally, the sample size was small for women with premature births, HIV infection, RSV infection leading to wider confidence intervals and not so strong positive effect on predictors. However, this study have provided important baseline data on efficiency of transplacental transfer of RSV-specific antibodies in a setting where the maternal population experiences a high prevalence of malaria and HIV infections and have outlined some of the factors which would require mitigation or use of alternative prevention strategies during introduction of a maternal RSV vaccine program for optimal vaccine outcome among infants in Kenya.

5.5 Conclusions

Transplacental transfer of RSV-specific antibodies among pregnant women in Kenya is efficient. Maternal characteristics differed between women from the two different geographical regions, but this did not have a significant effect on the overall transplacental transfer of RSV antibody. Maternal immunisation in the third trimester of pregnancy as a strategy to prevent infants from severe RSV disease should be considered with other interventions which will help protect infants born prematurely<33weeks gestation, infants with low birth weight and infants from HIV infected mothers.

CHAPTER SIX

PREVALENCE OF ADVERSE BIRTH OUTCOMES, THEIR PREDICTORS, AND IMPLICATIONS ON THE SUCCESSFUL IMPLEMENTATION OF A MATERNAL RESPIRATORY SYNCYTIAL VIRUS (RSV) VACCINE PROGRAM IN KENYA

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Abstract

Maternal immunization to prevent respiratory syncytial virus (RSV) associated disease among infants is in focus. However, little is known about adverse birth outcomes and associated factors occurring in a setting with high morbidities of malaria, HIV infection and undernutrition. Quantifying these ahead of a maternal vaccine introduction in Kenya would guide the interpretation of potential impact of the vaccination program on birth outcomes.

A cross-sectional survey was conducted to collect data on birth outcomes from women, residents of the health and demographic surveillance systems (HDSS) of Siaya and Kilifi, Kenya and from the maternity wards of Siaya County referral hospital and Bondo sub-county hospital. Participants of the HDSS sites had pregnancies registered in the years 2017 to 2020 through census rounds and were traced at home for interview. All women had a birth outcome by the time of data collection. Multiple logistic regression was used to determine independent predictors of adverse birth outcomes.

A total of 2219 women were interviewed. Median age during pregnancy was 27.7years (range: 22.7-32.4), 1857 (83.7%) attended antenatal care clinic (ANC), 1,979 (89.2%) delivered at a health facility and 2204 (99.3%) reported they would take up a new maternal vaccine. Adverse birth outcomes occurred in 781/2219 (35%) of pregnancies;

490 (22.1%) were preterm, 247 (11.1%) low birth weight, 189 (8.5%) macrosomia and 42 (1.9%) still births. Predictors of adverse birth outcomes were, gestational diabetes (Aor 3.01 (1.24-7.30; p=0.015) and home delivery (aOR 2.48 (1.20-5.13); p=0.014). Being multiparous (aOR 0.52 (0.33-0.81); p=0.004) was protective of an adverse birth outcome. Home delivery was significantly associated with older maternal age 40-49 years (p=0.001), multiparous >5 (p=0.001), level of formal education below primary (p=0.001) and Islamic religion (p=0.001).

In these maternal populations, about a third of pregnancies have adverse birth outcomes. Recognizing this baseline prevalence will be important in validating safety of a new maternal vaccine. Initiatives aimed to mitigate against factors affecting utilization of obstetric services and individual factors associated with adverse birth outcomes will be required for reliable evaluation of vaccine safety outcomes.

6.1 Introduction

Maternal immunization is one of the strategies to help achieve the third sustainable development goal of ensuring healthy lives and promoting well-being for all at all ages by 2030, through reduction of maternal and infant mortality (UN, 2021). Recent decades have seen an increase in development of maternal vaccines that may reduce infant mortality (Engmann et al., 2020). Maternal vaccines to prevent influenza and pertussis diseases are licensed (Abu-Raya et al., 2019; Mazzilli, Tavoschi, & Lopalco, 2018; Munoz, 2019; Romanin et al., 2020) and in use in high income countries, while, maternal tetanus toxoid vaccine has successfully reduced the burden of neonatal tetanus in Kenya (Ibinda et al., 2015; Vouking, Tadenfok, & Ekani, 2017). New maternal respiratory syncytial virus (RSV) vaccines are in the advanced stages of clinical evaluation and are prioritized for introduction in low and middle income countries (LMICs) where the burden of RSV associated disease is high among infants (Engmann et al., 2020; Madhi et al., 2020; Munoz & Jamieson, 2019; PATH, 2022). The maternal RSV vaccine would be

beneficial if implemented optimally and if the key factors likely to impact the vaccine effectiveness in LMICs are clearly understood. For instance, the associated risks or perception of risks of the vaccine to pregnancy outcomes would influence the vaccine implementation (Kharbanda et al., 2018; Kharbanda et al., 2017; Sancovski et al., 2019). Since vaccine safety data among pregnant women is limited, assessment of baseline rates of adverse birth outcomes and associated factors ahead of clinical trials, would benefit validation of risks and safety of the new maternal vaccines (Munoz, 2018; Sancovski et al., 2019).

A review of adverse events following immunization during pregnancy and a pharmacovigilance survey among pregnant women in Taiwan have reported that, maternal immunization may cause adverse outcomes to the mother or infant before or after delivery (Fulton et al., 2015; Lin et al., 2012). Settings with high rates of morbidities such as HIV, malaria and undernutrition are likely to experience the largest burden of adverse birth outcomes such as preterm births, low birth weight and still births (Adane et al., 2014), which can obscure outcomes in a maternal vaccine program (Heyderman et al., 2016). However, many of these birth outcomes are underreported if they do not occur within a health facility. Furthermore, many pregnant women do not complete all recommended antenatal care (ANC) visits where cost-effective interventions to help prevent adverse birth outcomes can be provided. In low resource settings such as Kenya, many births still occur at home (GOK, 2014) and little is known about these pregnancy outcomes, which undermines government efforts of providing free maternal health care services (Beyondzero, 2014; MOH, 2016) and hinders accurate evaluation of the effectiveness of new vaccine programs.

This Chapter aimed to quantify rates of adverse birth outcomes, where they occur, their predictors, factors that influence the choice of a place for delivery and the implications of these adverse birth outcomes in influencing the effectiveness of a new maternal vaccine program in Kenya with specific focus on RSV vaccine.

6.2 Methods

6.2.1 Study site

This study was conducted at Siaya county referral hospital and Bondo sub-county hospital and within the Kilifi and Siaya HDSS areas, in Kenya. The HDSS areas have been described in detail in Chapter Three.

The Kilifi HDSS area (**Error! Reference source not found.**) (Scott et al., 2012), is situated along the coastal part of Kenya, covering an area of 890km^2 and a population of ~300,000 residents as of 2019. Kilifi HDSS monitors population through census rounds, three times-a-year, and registers about 8000 pregnancies every year. (Scott et al., 2012). The Kilifi HDSS area is endemic for malaria which has a mortality rate among children aged 6 months to 4 years of 0.57 per 1000 person-years (95% CI 0.2, 1.2) (A. Kamau et al., 2020). However, in the recent years malaria incidence has declined, partly due to public health interventions that have reduced transmission (Njuguna et al., 2019). The KHDSS population is served by over 60 health facilities (both private and public) in which pregnant women attend for care and about 60% of the deliveries at Kilifi county referral hospital are from this HDSS area (Scott et al., 2012).

The Siaya HDSS is managed by KEMRI-Centre for Global Health Research (CGHR) with technical and financial support from US Centers for Disease Control and Prevention (CDC), Kenya (Odhiambo et al., 2012), and is situated in Siaya County, in the rural western part of Kenya (**Error! Reference source not found.**). This surveillance system covers an area of 700km^2 and monitors a population of about 260000 individuals and records approximately 6000 births per year (CGHR). The Siaya HDSS area has a high burden of malaria, pneumonia and diarrheal diseases (Breiman et al., 2014; Feikin et al., 2011) and is used to conduct longitudinal population based infectious disease surveillance (PBIDS). HIV prevalence in Siaya county is 21% according to 2018 Kenya

HIV estimates report, which is among the highest prevalence in Kenya (MOH, 2018). Routine home visits to collect morbidity data are conducted twice a year since 2015 and residents within villages where PBIDS occur are given free care at St. Elizabeth Lwak Mission hospital for all potentially infectious disease syndromes (Odhiambo et al., 2012).

6.1.2 Study Population

The study population comprised of women residents registered as pregnant during 2017-2020 census rounds in the HDSS areas of Kilifi and Siaya (Asembo). The study also included, pregnant women presenting at maternity wards of Bondo Sub-county Hospital and Siaya County referral hospital for delivery between February and April 2021. All women had a birth outcome by the time of the interview and data collection.

6.1.3 Study Design

This was a cross sectional survey to collect data on birth outcomes and gestational age at attendance for ANC among pregnant women.

The target sample size for women with data on birth outcomes was 1000 per HDSS area. These were randomly selected from census registers, with an equal number of women selected from each of the HDSS administrative locations as previously described in the methods section in Chapter 3. However, to replace women that would be missed during home visits, an additional random sample of 1000 women, matching the first sample set of 1000 women by geographical location, were selected from each of the census registers of Kilifi and Siaya HDSS areas, and the lists uploaded in the study databases and assigned to trained field interviewers for tracing. The interviewers visited homesteads of the selected women, consented them for participation into the study and electronically collected data on ANC attendance, birth outcomes and other obstetric or demographic details using a standardized questionnaire (Appendix IV) uploaded in computer tablets.

Pregnant women from Bondo and Siaya hospitals maternity wards were also interviewed to provide comparative data on timing for ANC attendance and birth outcomes occurring in referral hospitals within Siaya HDSS. All pregnant women were eligible for enrolment as they presented in the maternity wards of these hospitals. They were all approached after delivery, consented and those who accepted participation were interviewed and their records of birth outcomes collected using similar tools as those used in the households within the HDSS areas.

6.1.4 Ethical considerations

Written informed consent was obtained from all study participants. This study was approved by the KEMRI Scientific and Ethical Review Unit Committee (SERU #3716).

6.1.5 Statistical Analysis

All data collected from the community in Siaya and Kilifi HDSS areas were merged for specific analysis of the differences in participants' characteristics and factors influencing choice of place for delivery. Data collected from the hospitals of Bondo and Siaya was merged with datasets form Kilifi and Siaya HDSS sites for analyses of birth outcomes and predictors of adverse birth outcomes. These analyses focused on the following birth outcomes**:** normal live births**,** still births**,** preterm births, macrosomia, and low birthweight. A stillbirth was defined as a death or loss of a baby before or during delivery after 20 weeks of pregnancy (CDC., 2018). Preterm birth (PTB) was defined as baby born alive before 37 weeks of pregnancy are completed (WHO., 2018). Preterm births were further categorized into <32 weeks as very early PTB, 32-<34 weeks as early PTB and 34-36 weeks as late PTB. Low birthweight was defined as the weight of a newborn below 2500 grams (g) (Kozuki et al., 2015).

Proportions, mean (standard deviation: SD) and median (Interquartile range: IQR) were reported in the descriptive analysis. The characteristics of pregnant women from Kilifi

and Siaya HDSS sites were compared using a chi-square test. The chi square test was also applied to determine variables which influence choice of a place for delivery and to assess association between adverse birth outcomes and maternal characteristics.

Univariate and multivariable logistic regression models were used to determine predictors for adverse birth outcomes and factors for choice of place for delivery. Maternal age, place of delivery, marital status, maternal weight, level of education, occupation, religion, parity, gravida, gestational diabetes, malaria infection, eclampsia, gestational age at delivery, number of ANC visits, timing for ANC initiation and delivery mode were examined in the logistic regression. P-values of < 0.05 were considered statistically significant. All analysis was conducted in Stata version 15.0 (Stata Corp, College Station, USA).

6.2 Results

6.2.1 Characteristics of participants

A total of 2219 women were enrolled in this study: 1029 (46.4%) women from Siaya HDSS area, 594 (26.8%) from Kilifi HDSS area, 263(11.9%) from Siaya County Referral Hospital and 333(15.0%) from Bondo sub-County Hospital.

The median age of these women at the time of delivery was 27.7 years (IQR: 22.7-32.4). 218 (9.8%) women did not have data on gestational age at delivery, while 136 (6.1%) women were missing data on infant's birth weight. Of the 2219 women interviewed, only 4 (0.2%) did not attend antenatal care at all during pregnancy. Of those who reported to have attended ANC, 1,857 (83.7%) had booklets available to confirm attendance. The proportion of women initiating first ANC visit at less than 12 weeks significantly increased from 3% in 2017 to 14% in 2021 (p=0.001). All women with whom deliveries occurred in 2019, 2020 and 2021 reported to have attended at least one ANC visit and the proportion of women with more than four ANC visits was significantly higher (52.1%

(196) in 2019 but declined to 28.6% (8) and 31.0% (185)) in the subsequent years of 2020 and 2021 respectively (p=0.001) (Table 12).

The median birth weight of infants was 3.3 kilograms (kgs) (IQR: 2.9-3.5), while median gestational age at delivery was 37.8 weeks (IQR: 37-40). Overall, 1374 (62.0%) of the women had formal education at primary level, 1919 (86.6%) were married, 850 (34.4%) had no formal employment and 1,979 (89.2%) delivered at a health facility. Two hundred and forty (10.8%) delivered at home; 239 (99.6%) were interviewed from the community in HDSS sites, while one was enrolled from the maternity ward of Siaya hospital where she had attended due to retained placenta.

Women from Kilifi and Siaya HDSS sites were significantly different in most demographic characteristics. Compared to Siaya HDSS, the Kilifi site had fewer women who were divorced or separated (0.7% vs 3.4%; p=0.001), more women with no formal education at all (16.7% vs 0.29%; p=0.001), more women had more than 6 children (15.2% vs 6.0%; p=0.001), more babies were born underweight (10.1% vs 4.5%; p=0.001) and more deliveries occurred at home (29.8% vs 6.0%; p=0.001). However, women from Kilifi and Siaya HDSS areas were similar in gestational age at delivery of 38-40 weeks (73.0% vs 73.2%; p=0.358) and had a similar proportion of adverse birth outcomes (38.2% vs 38.0%; p=0.931). Table 11 below shows the characteristics of participating women from Siaya and Kilifi HDSS areas.

Table 11 ***: Characteristics of participating women from Kilifi and Siaya HDSS***

Characteristic	Both sites (n)	%	Kilifi (n)	%	Siaya (n)	%	P* value
	1616	100	594	36.8	1022	63.2	
Maternal age							
15-19	82	5.07	29	4.88	116	5.19	
20-29	509	49.80	275	46.30	873	49.80	
30-39	419	41.00	233	39.23	561	41.00	**<0.001**
40-49	41	4.01	57	9.60	57	4.01	
Marital status							
Married	1457	89.99	545	91.75	912	88.98	
Single	123	7.60	45	7.58	78	7.61	**0.002**
Divorced/separated	35	3.41	4	0.67	35	3.41	
Religion							
Christian	1521	93.95	502	84.51	1019	99.41	
Muslim	65	4.01	59	9.93	6	0.59	**<0.001**
Other	33	2.04	33	5.56	0	0.00	
Education level							
None	102	6.30	99	16.67	3	0.29	
Primary	1097	67.76	423	71.21	674	65.76	**<0.001**
Secondary	368	22.73	55	9.26	313	30.54	
Tertiary-College/University	52	3.21	17	2.86	35	3.41	
Parity							
0	212	13.06	98	16.50	114	11.08	
1-2	661	40.73	222	37.37	439	42.66	**<0.001**
3-5	598	36.85	184	30.98	414	40.23	
6-9	152	9.37	90	15.15	62	6.03	
Birthweight							
Underweight (<2.5kgs)	175	10.78	89	14.98	86	8.36	**<0.001**
38-42 weeks	1027	73.10	430	73.01	597	73.16	
Place of delivery							
Hospital	1384	85.27	417	70.02	967	93.03	
Home	239	14.73	177	29.80	62	6.03	**<0.001**

Table 12: ***Characteristics of study participants by Year of Delivery***

	Year of Delivery						
Characteristic	**2017**	**2018**	**2019**	**2020**	**2021**	**Total**	**Chi2 P-value**
	n (%)	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**	
# of Women	**508 (22.9)**	**711 (32.0)**	**376 (16.9)**	**28 (1.3)**	**596 (26.9)**	**2219 (100)**	
ANC Initiation							
0-12 weeks	13 (2.56)	39 (5.49)	53 (14.10)	4 (14.29)	85 (14.26)	**194 (8.74)**	
13-24 weeks	112 (22.05)	307 (43.18)	198 (52.66)	11 (39.29)	342 (57.38)	**970 (43.71)**	**0.000**
25-32 weeks	127 (25.00)	180 (25.32)	53 (14.10)	79 (32.14)	136 (22.82)	**505 (22.76)**	
33-42 weeks	51 (10.04)	46 (6.47)	18 (4.79)	1 (3.57)	29 (4.87)	**145 (6.53)**	
Data not available	205 (40.35)	139 (19.55)	54 (14.36)	3 (10.71)	4 (0.67)	**405 (18.25)**	
ANC visits							
0	2 (0.39)	2 (0.28)	0 (0.00)	0 (0.00)	0 (0.00)	**4 (0.18)**	
1	58 (11.42)	37 (5.20)	5 (1.33)	0 (0.00)	33 (5.54)	**133 (5.99)**	
2	71 (13.98)	74 (10.41)	16 (4.26)	0 (0.00)	90 (15.10)	**251 (11.31)**	
3	80 (15.75)	130 (18.28)	34 (9.04)	8 (28.57)	143 (23.99)	**395 (17.80)**	**0.000**
4	58 (11.42)	158 (22.22)	71 (18.88)	9 (32.14)	141 (23.66)	**437 (19.69)**	
>4	36 (7.09)	173 (24.33)	196 (52.13)	8 (28.57)	185 (31.04)	**598 (26.95)**	
Data not available	203 (39.96)	137 (19.27)	54 (14.36)	3 (10.71)	4 (0.67)	**401 (18.07)**	
Gestational age at delivery							
37-42 weeks	282 (68.95)	466 (73.85)	264 (77.19)	19 (79.17)	480 (80.67)	**1511 (75.51)**	
33-36 weeks	116 (28.36)	137 (21.71)	68 (19.88)	4 (16.67)	92 (15.46)	**417 (20.84)**	**0.001**
<33 weeks	11 (2.69)	28 (4.44)	10 (2.92)	1 (4.17)	23 (3.87)	**73 (3.65)**	
Data not available	99 (19.49)	80 (11.25)	34 (9.04)	4 (14.29)	1 (0.17)	**218 (9.82)**	
Adverse birth outcomes							
No	291 (57.28)	448 (63.01)	250 (66.49)	19 (67.86)	430 (72.15)	**1438 (64.80)**	**0.000**
Yes	217 (42.72)	263 (36.99)	126 (33.51)	9 (32.14)	166 (27.85)	**781 (35.20)**	
Place of delivery							
Hospital	374 (73.62)	618 (87.04)	364 (96.81)	21 (100)	7 (87.50)	**1384(85.27)**	
Home	134 (26.83)	92 (12.94)	12 (3.19)	0 (0.00)	1 (12.50)	**239 (14.73)**	**0.000**

6.3.2 Distribution of gestational age at delivery

Gestational age at delivery showed a near normal distribution, for births occurring at home, median 38 weeks (IQR: 37-40); mean 37.81 weeks (SD:2.38) and those occurring at the hospital, median: 38 weeks (IQR: 36-40); mean 37.73 weeks (SD:2.59) and between Kilifi median: 38 weeks (IQR: 36-40); mean 37.86 weeks (SD:2.30) and Siaya HDSS median: 38 weeks (IQR: 36-40); mean 37.78 weeks (SD:2.44) sites respectively. No significant difference was found in gestational age at delivery between hospital births and home births (p=0.619) or between Kilifi and Siaya HDSS (p=0.425) sites.

Table 13: ***Characteristics of participants selected from the maternity wards of Bondo and Siaya county referral hospital in Kenya***

Characteristics	Maternity Ward Surveillance Sites		
	Siaya	**Bondo**	**All**
	n%	**n%**	**n%**
Women interviewed (N)	263	333	596
Women attended ANC	263(100)	333(100)	596(100)
Women with ANC booklets	**263(100)**	**333(100)**	**596(100)**
Median age (IQR) in years	24.3(20.5-29.0)	21.3(24.0-29.8)	24.7(21.0-29.7)
Education level			
None	0 (0.0)	1(0.3)	1 (0.17)
Primary	146 (55.5)	131(39.3)	277 (46.5)
Secondary	97 (36.9)	145(43.5)	242 (40.6)
Tertiary-College/University	20 (7.6)	56(16.8)	76 (12.8)
Marital status			
Married	191(72.6)	271(81.4)	462 (77.5)
Single	68 (25.9)	61(18.3)	129 (21.6)
Divorced/Sep/Widowed	4 (1.5)	1(0.8)	5 (0.8)
ANC Visit attended			
ANC1	263 (100)	333(100)	596(100)
ANC2	248 (94.3)	312(93.7)	560(94.0)
ANC3	221 (84.0)	250(75.1)	471(79.0)
ANC4	159 (60.5)	166(49.9)	325(54.5)
ANC5	102 (38.8)	80(24.0)	182(30.5)
Median Gest age at ANC Visit in weeks			
ANC1	20(15-26)	24(20-27)	22(18-26)
ANC2	26(21-30)	27(23-31)	26(22-30)
ANC3	28(25-34)	32(26-35)	30(26-34)
ANC4	32(28-36)	34(30-36)	32(30-36)
ANC5	35(32-36)	36(32-38)	35(32-37)

6.3.3 Predictors of adverse birth outcomes

A total of 781 of the 2219 (35.2%) women interviewed had adverse birth outcomes, in whom, 490/2219 (22.1%) of infants were born preterm <37 weeks' gestation, while 50/2219 (2.3%) were very early preterm births <32weeks gestation, 247/2219 (11.1%) were born with low birth weight <2.5 kgs, 189/2219 (8.5%) were macrosomia and 42/2219 (1.9%) were still births. Proportions of adverse birth outcomes significantly declined from 217/508 (42.7%) in the year 2017 to 263/711 (37.0%), 126/376 (33.5%), 9/28 (32.1%) and 166/596 (27.9%) for births occurring in 2018, 2019, 2020 and 2021 respectively (p=0.001).

Proportion of specific adverse birth outcomes stratified by site were, preterm (27.0 vs 23.4%; p=0.092), low birth weight (15.2% vs 10.4%; p=0.003), macrosomia (2.4% vs 3.7; p=0.128) and still births (0.5% vs 2.4; p=0.004) in Kilifi and Siaya respectively. Overall, adverse birth outcomes were not significantly different between Kilifi and Siaya (38.2% vs 34.1%; p=0.072) but were significantly lower among women interviewed from the hospital than in the community (27.4% vs 38.1%; p=0.001). Proportions of adverse birth outcomes were also significantly higher for births occurring at home than those in hospital (43.8% vs 34.2 %; p=0.003) and for deliveries occurring in health centres than those in county referral hospital (36.1% vs 32.6%; p=0.005). Proportions of premature births <33 weeks gestation were significantly higher in the year 2018 and 2021 (p=0.001).

Multivariable logistic regression analysis focusing on specific adverse birth outcomes with maternal characteristics showed significant associations of low birth weight with gestational age at delivery <33 weeks' gestation (aOR 12.84 (7.81 -21.1); p=0.001), level of formal education above primary (aOR 0.53 (0.30-0.94); p=0.032), parity of more than 1 child (aOR 0.55 (0.38-0.79); p=0.002), maternal age >40years (aOR 0.29 (0.12-0.75); p=0.010) and attending only one or none ANC visits (aOR 1.96 (1.21-3.16); p=0.006). Still births were significantly associated with gestational age <33 weeks at delivery (aOR

12.5 (5.6-27.9); p=0.001) and cesarean section delivery (aOR 4.81 (2.42-9.59); p=0.001). Preterm births were significantly associated with gestational diabetes (aOR 3.34 (1.38-8.08); p=0.008) and one or none ANC visit (aOR 2.52 (1.72-3.70); p=0.001), while macrosomia was significantly associated with parity of more than 3 children (aOR 5.49 (1.31-25.1); p=0.020) and gestational age at delivery 33-36 weeks' gestation (aOR 0.35 (0.14 -0.88); p=0.025).

In a multivariable logistic regression analysis of all adverse birth outcomes with maternal characteristics, the predictors of adverse birth outcomes were, gestational diabetes (aOR 3.01 (1.24-7.30); p=0.015), home delivery (aOR 2.48 (1.20-5.13); p=0.014), while parity of 1 or more children (aOR 0.52 (0.33-0.81); p=0.004) had a protective effect on adverse birth outcomes (Table 14). Adjusting for HDSS site did not have any effect on the predictors for adverse birth outcomes.

Table 14*: Predictors of Adverse birth outcomes among pregnant women in Kenya*

Adverse birth Outcomes				Univariate logistic regression				Multivariate logistic regression			
Characteristic	**n**	**%**	**Chi2 P value**	**COR***	**Odds Ratio (95%CI)**		**P value**	**AOR****	**Odds Ratio (95%CI)**		**P value**
	781	35.10			**LCL**	**UCL**			**LCL**	**UCL**	
Maternal age											
15-19	60	7.73		**Ref**							
20-29	403	51.93	0.450	0.77	0.53	1.08	0.138				
30-39	274	35.31		0.75	0.52	1.07	0.113				
40-49	39	5.03		0.74	0.44	1.23	0.239				
Marital status											
Married	659	84.81		**Ref**				**Ref**			
Single	96	12.36	0.056	1.18	0.89	1.54	0.240	0.76	0.51	1.14	0.179
Divorced/separated	22	2.83		1.91	1.05	3.48	**0.034**	1.59	0.69	3.68	0.275
Education level											
None	42	5.41		**Ref**				**Ref**			
Primary	513	66.02	**0.002**	0.87	0.58	1.30	0.487	0.99	0.62	1.62	0.998
Secondary	191	24.58		0.66	0.43	1.02	0.059	0.77	0.45	1.30	0.327
Tertiary-College/University	31	3.99		0.46	0.26	0.82	**0.008**	0.56	0.29	1.08	0.084
Parity											
0	110	14.08		**Ref**				**Ref**			
1-2	325	41.61		0.67	0.51	0.89	**0.006**	0.52	0.33	0.81	**0.004**
3-5	274	35.08	**0.025**	0.68	0.51	0.90	**0.008**	0.45	0.28	0.74	**0.002**
6-9	72	9.22		0.83	0.57	1.22	0.346	0.60	0.30	1.18	0.141
ANC Initiation											
0-12 weeks	86	13.19		**Ref**				**Ref**			
13-24 weeks	250	38.34		0.82	0.6	1.11	0.208	0.84	0.56	1.23	0.394
25-32 weeks	181	27.76	**0.019**	0.93	0.67	1.30	0.684	0.73	0.46	1.16	0.184
33-42 weeks	135	20.71		0.64	0.45	0.89	**0.009**	0.80	0.53	1.22	0.303
Place of delivery											
Hospital	676	86.56		**Ref**				**Ref**			
Home	105	13.44	**0.003**	1.5	1.14	1.97	**0.003**	2.48	1.20	5.13	**0.014**
ANC visits											
0-1	68	8.70		2.13	1.49	3.06	**0.000**	1.53	0.85	2.74	0.152
2-3	239	30.60	**0.000**	1.23	1.00	1.52	**0.046**	1.36	1.03	1.80	**0.030**
4-5	328	42.00		**Ref**							
Data not available	146	18.69									
Gestational diabetes											
Yes	13	1.67	**0.013**	2.84	1.2	6.67	**0.017**	3.01	1.24	7.30	**0.015**
No	541	69.27		**Ref**							
Data not available	227	29.06									
Malaria											
Yes	71	9.09	0.154	1.25	0.92	1.73	0.155				
No	483	61.84		**Ref**							
Data not available	227	29.06									
Aneamia											
Severe	490	62.74		0.96	0.79	1.15	0.649				
Moderate	258	33.03	0.155	0.67	0.44	1.01	0.055				
None	33	4.23		**Ref**							

*Crude odds ratio **Adjusted odds ratio

6.3.4 Factors influencing choice of place for delivery

Among the 1623 women whom data was collected from the community in the Kilifi and Siaya HDSS areas, 186 (11.5%) had deliveries in county referral hospitals, 70 (4.3%) in sub-county hospitals, 566 (34.9%) in health centres, 95 (5.9%) in dispensaries, 467 (28.8%) in private hospitals and 239 (14.7%) occurred at home.

Of the 240 women who delivered at home, 104/240 (43.3%) reported had difficulty in accessing hospital due to lack of transport or long distance to hospital, 49/240 (20.4%) was due to doctor's strike in public hospitals, 43/240 (19.9%) was a result of spontaneous labor, 25/240 (10.4 %) reported labor started at night and could not reach the hospital in good time, 7/240 (2.9%) preferred home deliveries because they didn't have any history of pregnancy complications, 7/240 (2.9%) could not afford the cost of care, 4/240 (1.7%) did not disclose reasons while 1(0.4%) her religion did not allow hospital births. A total of 165/240 (68.8%) women who delivered at home had ANC booklets available.

Only 518 (26.2%) of the 1979 women who delivered in a health facility, responded to the question on reason for choosing facility as place for delivery, the rest refused to disclose. In 266/518 (51.4%), the facility was near their area of residence, 110/518 (21.2%) the facility offered good services, 80/518 (15.4%) were a referral by doctors or nurses due to pregnancy complications, 41/518 (7.9%) delivered in private hospitals/clinics due to a doctors' strike in public hospitals and 21/518 (4.1%) the facility was where they attended for ANC services. There was a significant decline in proportion of women who had home births from 26% (134) in the year 2017, 13% (92) in 2018, 3.2% (12) in 2019 to 0% in 2020 (p=0.001).

When all 2219 women were asked about the decision maker on the choice of a place for delivery, 1473 (66.4%) reported self, 325 (14.7%) doctors and other health care providers, 206 (9.3%) was both husband and wife, 116 (5.2%) was spouse, 91 (4.5%) was from friends and relatives, while 8 (0.3%) women reported was from village reporters on vital events.

Among all 2219 women interviewed in this study, the choice of place for delivery was significantly associated with the facility a pregnant woman attended for ANC screening (p=0.001), being of older maternal age 40-49 years (p=0.001), having had more than 5 live births (p=0.001), having level of formal education above primary (p=0.001), delayed ANC initiation (p=0.305) being of an Islamic religion (p=0.001), attending one or no ANC visit (p=0.001) and having no employment (p=0.001); but was not associated with marital status (p=0.134), or gestational age at delivery (p=0.085) (Table 15).

Pregnant women who attended ANC in dispensaries (aOR 3.3 (95%CI 1.8-6.1); p=0.000) were more likely to deliver at home than those who attended ANC in a county referral hospital. Pregnant women with formal education to tertiary level were less likely to deliver at home than those who did not have any formal education (aOR 0.02 (95%CI: 0.01-0.22; p=0.001). Muslim women were more likely to have home deliveries than Christians (aOR 3.8 (95%CI: 1.9-7.4; p=0.001), while women who were self-employed were less likely to deliver at home than those who reported not to have any employment at all (aOR: 0.4 (95%CI 0.2-0.5); p=0.001) (Table 16).

Table 15: ***Factors associated with choice for home delivery among pregnant women in Kenya***

Univariate logistic regression									
Characteristic	**Hospital (n)**	**%**	**Home (n)**	**%**	**Chi2 P value**	**COR***	**Odds Ratio (95%CI)**		**P value**
	1961	89.18	240	10.12			**LCL**	**UCL**	
Maternal age									
15-19	138	95.17	7	4.83		**Ref**			
20-29	1054	91.81	94	8.19	**0.000**	1.76	0.79	3.86	0.161
30-39	683	86.02	111	13.98		3.2	1.46	7	**0.004**
40-49	86	75.44	28	24.56		6.4	2.68	15.3	**0.000**
Marital status									
Married	1709	89.06	210	10.94		**Ref**			
Single	233	92.46	19	7.54	0.134	0.66	0.41	1.08	0.100
Divorced/separated	37	84.09	7	15.91		1.5	0.68	3.5	0.303
Religion									
Christian	1910	90.44	202	9.56		**Ref**			
Muslim	49	70.00	21	30.00	**0.000**	4.05	2.38	6.89	**<0.001**
Other	20	60.61	13	39.39		6.15	3.01	12.54	**<0.001**
Education level									
None	64	62.14	39	37.86		**Ref**			
Primary	1193	86.83	181	13.17		0.24	0.16	0.38	**<0.001**
Secondary	596	97.70	14	2.30	**0.000**	0.04	0.02	0.07	**<0.001**
Tertiary-College/University	126	98.44	2	1.56		0.02	0.01	0.11	**<0.001**
Parity									
0-<1	239	93.36	17	6.64		**Ref**			
1-2	890	92.13	76	7.87		1.2	0.69	2.07	0.511
3-5	712	87.90	98	12.10	**0.000**	1.93	1.13	3.31	**0.016**
6-9	138	73.80	49	26.20		4.99	2.77	9.01	**<0.001**
ANC Initiation									
0-12 weeks	196	90.74	20	9.26		**Ref**			
13-24 weeks	665	93.4	47	6.6		0.69	0.4	1.20	0.188
25-32 weeks	402	84.41	72	15.19	**0.000**	1.76	1.04	2.96	**0.035**
33-42 weeks	429	94.29	26	5.71		0.59	0.32	1.09	0.092
ational age at delivery									
<33 weeks	64	87.67	9	12.33		**Ref**			
33-36 weeks	360	86.33	57	13.67	0.085	1.13	0.53	2.39	0.757
37-42 weeks	1361	90.07	150	9.93		0.78	0.38	1.61	0.506
Pregnancy outcome									
Normal	1303	90.61	135	86.56		**Ref**			
Adverse birth outcome	676	9.39	105	13.44	**0.003**	1.5	1.14	1.97	**0.003**
NC Facility attended									
County referral hospital	172	92.47	14	7.53		**Ref**			
Sub-County hospital	194	99.49	1	0.51		0.06	0.01	0.49	**0.008**
health centre	805	90.76	82	9.24		1.25	0.69	2.26	0.456
Dispensary	347	78.51	95	21.49	**0.000**	3.36	1.86	6.07	**0.000**
Private hospital/clinic	461	191.29	44	8.71		1.17	0.63	2.19	0.618
ANC visits									
0-1	79	58.09	57	41.91		18.78	11.74	30.05	**0.000**
2-3	583	89.42	69	10.58	**0.000**	3.08	2.05	4.64	**0.000**
4-5	989	96.30	38	3.7		**Ref**			

*Crude Odds ratio

Table 16: ***Predictors for choice of home delivery among pregnant women in Kenya***

Characteristic	Hospital (n)	%	Home (n)	%	Chi2 P value	Multivariate logistic regression AOR**	Odds Ratio (95%CI) LCL	UCL	P value
	1961	89.18	240	10.12					
Maternal age									
15-19	138	95.17	7	4.83		Ref			
20-29	1054	91.81	94	8.19	**0.000**	2.91	0.94	9.00	0.063
30-39	683	86.02	111	13.98		4.53	1.38	14.94	**0.013**
40-49	86	75.44	28	24.56		4.41	1.11	17.41	**0.034**
Marital status									
Married	1709	89.06	210	10.94					
Single	233	92.46	19	7.54	0.134				
Divorced/separated	37	84.09	7	15.91					
Religion									
Christian	1910	90.44	202	9.56		**Ref**			
Muslim	49	70.00	21	30.00	**0.000**	3.76	1.92	7.40	**<0.001**
Other	20	60.61	13	39.39		2.50	1.03	6.07	**0.043**
Education level									
None	64	62.14	39	37.86		**Ref**			
Primary	1193	86.83	181	13.17		0.37	0.21	0.64	**0.001**
Secondary	596	97.70	14	2.30	**0.000**	0.07	0.03	0.17	**<0.001**
Tertiary-College/University	126	98.44	2	1.56		0.02	0.00	0.22	**0.001**
Parity									
0-<1	239	93.36	17	6.64		**Ref**			
1-2	890	92.13	76	7.87		1.43	0.69	2.98	0.338
3-5	712	87.90	98	12.10	**0.000**	1.26	0.57	2.84	0.562
6-9	138	73.80	49	26.20		1.48	0.56	3.92	0.433
ANC Initiation									
0-12 weeks	196	90.74	20	9.26		**Ref**			
13-24 weeks	665	93.4	47	6.6		0.59	0.32	1.07	0.08
25-32 weeks	402	84.41	72	15.19	**0.000**	1.23	0.69	2.18	0.488
33-42 weeks	429	94.29	26	5.71		0.74	0.38	1.43	0.375
Gestational age at delivery									
<33 weeks	64	87.67	9	12.33					
33-36 weeks	360	86.33	57	13.67	0.085				
37-42 weeks	1361	90.07	150	9.93					
ANC Facility attended									
County referral hospital	172	92.47	14	7.53					
Sub-County hospital	194	99.49	1	0.51		0.16	0.2	1.32	0.089
health centre	805	90.76	82	9.24		1.23	0.61	2.5	0.559
Dispensary	347	78.51	95	21.49	**0.000**	2.92	1.44	5.92	**0.003**
Private hospital/clinic	461	191.2 9	44	8.71		1.52	0.72	3.23	0.272
ANC visits									
0-1	79	58.09	57	41.91		10.2	5.84	17.83	**0.000**
2-3	583	89.42	69	10.58	**0.000**	2.17	1.37	3.45	**0.001**
4-5	989	96.30	38	3.7		**Ref**			

**Adjusted Odds ratio

6.3.5 Acceptance of a new maternal vaccine among pregnant women in Kenya

Majority of women 2204 (99.3%) interviewed reported they would accept a new maternal vaccine during ANC visits to prevent pneumonia among their infants. However, 803 (36.2%) reported, they would first consult before taking the vaccine. Of these, 499/803 (62.1%) would consult their spouse, 246 (30.6%) would consult their doctor or health care provider or other persons who received the vaccine to confirm it was safe and 58 (7.2%) would consult friends and relatives. The main reason of the 15 (0.7%) women who said would not accept the new maternal vaccine was fear that the vaccine might not be safe.

6.4 Discussion

Maternal vaccines to protect infants against severe RSV disease are in advanced stages of clinical evaluation in developed countries. Introduction of these vaccines to low-middle income countries will require knowledge on the maternal characteristics and factors that would influence successful implementation. Any factors likely to influence uptake of the vaccine such as safety and access may impact its effectiveness. This study assessed birth outcomes and other factors during delivery, likely to affect the effectiveness of the maternal RSV vaccine programme.

In this sample of women, two thirds of pregnancy outcomes were found to be normal live births. The proportion of adverse birth outcomes was significantly higher for births occurring at home than in hospital, but similar among all women from the two geographically diverse regions and who were different in demographic characteristics. Distribution of gestational age at delivery among these women was similar regardless of place of delivery or HDSS site. The difference in characteristics in this study provide a good comparison with the general population as these results are similar with findings of the 2014 Kenya demographic health survey (GOK, 2014). These results also imply

possibility of observing equal distribution of safety outcomes across maternal populations from the different geographical regions in Kenya on introduction of a new maternal vaccine.

Preterm births were found to be the most common adverse birth outcomes in this study accounting to nearly 22% (490/2219) of all infants. Additionally, 2% of the preterm births in this population, occurred within the gestational age period of <33 weeks and was high (4%) in 2018 and 2021. It's worth noting that, both 2018 and 2021 were preceded by years of restricted access to healthcare services which included the long health care workers' strike in 2017 and restricted movement due to covid-19 pandemic in 2020. Consequently, increase in proportion of adverse birth outcomes due to missed ANC services during pregnancy. A study conducted at the maternity ward of Kenyatta hospital found preterm births were significantly associated with Maternal age above 20 years, parity of greater than 4, twin pregnancy, maternal urinary tract infections, pregnancy induced hypertension, antepartum hemorrhage and prolonged prelabor rupture of membranes (Wagura et al., 2018). From the phenomenon discussed in Chapter Five, these preterm infants are likely not to have an optimum level of protective antibody transferred after maternal vaccination due to the short interval between onset of transplacental transfer of RSV specific antibodies and birth. Implying, this group of infants will be susceptible to severe RSV disease and might require use of other strategies such as prophylactic monoclonal antibodies for prevention (Domachowske et al., 2018; Griffin et al., 2017). To mitigate against preterm births, it is recommended that at-risk mothers should receive intensified antenatal care.

Tertiary level of education, delayed ANC initiation, number of ANC visits, being multiparous and home delivery were found to be significantly associated with adverse birth outcomes in this study. Women with low level of education might belong to a low socio-economic status group and could not afford the cost of care therefore, missing uptake of preventive services during pregnancy or skilled care during delivery (Moindi et

al., 2016). It has also been found that cultural practices during home delivery like massaging of the abdomen to align the baby, which is very common among indigenous populations along the coastal part of Kenya are associated with placenta praevia and abruption, asphyxiating the foetus and increased chances of trauma to the baby and premature delivery (Moindi et al., 2016). Introduction of the maternal RSV vaccines should therefore take into consideration both cultural and socio-economic factors likely to result into adverse birth outcomes to avoid misinterpretation of vaccine safety outcomes. Perhaps, a consideration to integrate strategies directed towards mitigating against causes of adverse birth outcomes such as proper management of high-risk pregnancies, educating traditional birth attendants on risks of some of the cultural practices during delivery might be worth an undertaking.

About 29.8% of births in Kilifi and 6.0% in Siaya were found to have occurred at home. However, these results show a decline in proportions of home births as observed prior to free care during the 2014 Kenya demographic health survey (47% in Kilifi vs 27% Siaya) (GOK, 2014). The decline in home births is also very significant across the years of observation in this study, from 29% in 2017 to 0% in 2020. The significant change in home deliveries could be attributed to current government initiatives focused to achieve universal access to maternal and child health services (MOH, 2016). These initiatives include, “Beyond Zero” (Beyondzero, 2014) which was launched in 2014, by the first lady in Kenya, and aims to prevent maternal and infant deaths by providing mobile clinics to provide care to pregnant women who have no access to hospitals during delivery. In addition, the government of Kenya through the Ministry of Health in 2016, also launched another initiative known as “Linda Mama” (MOH, 2016) which ensures pregnant women and infants have access to free, quality and affordable maternal and child health services by use of a public funded health insurance scheme. The impact of these initiatives seems evident through the observed reduction of home deliveries in this study, implying introduction of similar initiatives, which encourage pregnant women to

utilize health care services are likely to demystify concerns about healthcare interventions and increase their uptake.

Among the women who delivered at home, 165 (69%) attended ANC and had booklets available, indicating they received ANC services. Multiple ANC visits in this study were also found to be associated with less adverse birth outcomes. High rates of ANC attendance among pregnant women have been found to enhance uptake of interventions, ensure high vaccine coverage (Baral, Fleming, et al., 2020) which mitigates against poor outcomes during and after delivery. However, full use of ANC and services alone does not prevent all adverse birth outcomes even in the highest resource settings. This is because, most pregnancy complications occur during delivery and can result in poor pregnancy outcomes (Lawn, Cousens, Zupan, & Lancet Neonatal Survival Steering, 2005) and which can result into mis-interpretation of safety outcomes of a maternal vaccine. In a survey to find out birth preparedness and complication readiness among Kenyan women showed only 11.4% (59/519) were well prepared for births and its complications during pregnancy (Orwa et al., 2020). Initiatives such as provision of night transport services to pregnant women during labour, equipping lower levels health facilities with qualified staff, training, oversight and resources to handle emergencies which will encourage pregnant women utilize hospital services to reduce risks associated with home deliveries, will be reasonable, but should not be a barrier to vaccine rollout. Procedures to detect adverse outcomes should be put into place during trial or within a managed phase iv rollout setting, to provide a reliable system for validating effectiveness and safety of a new maternal vaccine.

The choice of place for delivery was found to be associated with maternal age, facility for ANC attendance, religion, parity and education level. Older women were more likely to deliver at home than in hospital and this is perhaps a result of experience in having previous successful deliveries or uncomplicated pregnancy (Moindi et al., 2016). Similarly, women who have had multiple pregnancies were more likely to deliver at

home than in hospital while higher education level was associated with less home delivery as observed in other studies in coastal Kenya (Chea et al., 2018; Moindi et al., 2016). A study in North Eastern Kenya, found male doctors attending to women in labour prevented pregnant women from delivering in hospital (N'Gbichi et al., 2019) because of religious beliefs and in this study, Muslim women were found to be more likely to deliver at home than in hospital. The role of influencers such as spouse, healthcare providers and relatives in determining place of delivery is also of much importance and might require empowering of pregnant women in decision making. Introduction of new interventions among these pregnant women may also need consideration of the socio-cultural factors such as religion, individual perceptions on births or cultural beliefs to ensure maximum uptake.

Majority (99%) of pregnant women in this study reported they would accept the maternal RSV vaccine despite a few having concerns that the vaccine might not be safe. Perceptions about risks associated with a vaccine might result to high rates of vaccine refusals which is likely to affect the overall effectiveness. For instance, a study in Quebec found a belief that a H1N1 influenza vaccine was not adequately tested resulted in its low uptake among pregnant women (Fabry et al., 2011). Most pregnant women appear to have more trust on their health care providers regarding information on interventions available and their uptake within health facilities (Mohammed, Clarke, Koehler, Watson, & Marshall, 2018) . For a successful implementation of the maternal RSV vaccine program in this setting, it is recommended to integrate sensitization and education sessions between health care providers and pregnant women perhaps through health talks and information brochures within ANC platforms to create awareness about the safety and efficacy of the new maternal vaccine, resolve doubts and increase confidence before its introduction.

There are some limitations in this study. HIV status was not collected among these women and, proportions of adverse birth outcomes attributed to HIV infection and how

HIV would likely have altered the observed associations of other variables in multivariable analyses. The data was drawn from a sample of women from two out of the 47 counties and may not be representative of all Kenyan women. Stillbirths that occur in the community often go unreported and could have also been missed in this study. Hospital enrolment of some of the participants may represent a bias. However, this study provides important baseline data on birth outcomes which has often been missed by studies involving a small sample size of women and gives a detailed description of the baseline proportions of adverse birth outcomes and associated factors in this setting likely to influence the effectiveness of maternal RSV vaccine and which can guide validation and monitoring of the safety outcome of a new maternal vaccine program.

6.5 Conclusions

In this sample of women, about a third of pregnancies had adverse birth outcomes most of which occurred at home. Recognizing this baseline prevalence will be important in validating safety of a new maternal vaccine. Births occurring at home might hinder evaluation of maternal vaccine safety. Acceptance of the maternal RSV vaccine is likely to be high with 99% of women reporting willingness for uptake during pregnancy. However, successful implementation of the maternal RSV vaccine program will require integrated initiatives to mitigate against individual factors associated with adverse birth outcomes and factors affecting utilization of maternal healthcare services such as educating women on the benefits of ANC services during pregnancy and attending early enough to get benefit and introduction of platforms to create awareness about the safety and efficacy of the new maternal vaccines.

CHAPTER SEVEN

GENERAL DISCUSSION, CONCLUSSIONS AND RECOMMENDATIONS

7.1 Summary of key findings

The main objective of the work presented in this thesis was to assess factors likely to influence the potential effectiveness of a maternal RSV vaccine program in Kenya. The specific objectives focused on three major factors critical in the successful implementation of a maternal RSV vaccine for LMICs some of which are outlined in the gap analysis report for advancing maternal immunization (PATH, 2018). These factors are:

1. Timing for ANC attendance: The gestational age at presentation for ANC screening and proportion attending within the required gestational age window suitable for maternal RSV vaccination delivery.
2. The efficiency of transplacental transfer of RSV-specific antibodies and the factors affecting this efficiency of transfer among pregnant women in Kenya.
3. Baseline rates of birth outcomes: Factors associated with adverse birth outcomes and the factors that influence the choice of a place for delivery among pregnant women in Kenya.

The interest in conducting this work arose from the knowledge that, maternal immunization is being considered as the most realistic approach to prevent severe RSV- associated disease among infants (Engmann et al., 2020; Madhi et al., 2020). The introduction of bacterial vaccines against *Streptococcus pneumoniae* and *Haemophillus influenzae type* b in Kenya (Ahmed et al., 2012; Cowgill et al., 2006), left a niche for viruses to become the leading cause of ARI seen among hospitalized

children under 5 years of age (Berkley et al., 2010; PERCH, 2019). At present, RSV is the leading virus known to cause lower respiratory tract infection among hospitalized infants less than 6 months of age globally (Li et al., 2021; PERCH, 2019). Despite the high RSV disease health burden, conventional strategies for prevention such as development of effective infant RSV vaccines has been difficult. This has been due to adverse reactions experienced among vaccinees during early clinical trials for infant vaccines and challenges with the immature immune system of infants, making it difficult to mount a protective immune response during the neonatal period.

Delivery of the maternal RSV vaccine is proposed to be integrated within ANC clinics (PATH, 2018) and will require administration within a gestational age window period which will allow optimum time for transfer of antibodies. Hence, understanding the proportion of women that can be reached for vaccination will inform vaccine coverage in a setting. This thesis has presented the proportions of women attending ANC from Kilifi and Siaya HDSS. It has also provided proportions of pregnant women attending ANC within specific gestational age windows for maternal RSV vaccination and proportions attending ANC in each visit for up to five visits from Kilifi HDSS area. In addition, emphasis on how factors affecting ANC initiation and utilization of health care services are likely to impact delivery of the maternal RSV vaccine have been provided. To the best of our knowledge, this is the first time ANC timing data is being made available and presented to guide successful delivery of a maternal vaccine program.

Pregnant women from some LMICs are thought to experience comorbidities of malaria and HIV infection and this has raised concerns that characteristics of maternal populations in LMIC's might affect effectiveness of the maternal RSV vaccine (PATH, 2018). To address this concern, this study assessed the characteristics among Kenyan women from two counties of Kilifi and Siaya which are areas with high

comorbidities of malaria and HIV infection respectively and has shown how each characteristic (maternal age, gravida, parity, occupation, HIV infection, malaria infection) is likely to influence transplacental transfer of RSV specific antibodies. The transplacental transfer of RSV- specific antibodies among Kenyan women shown through this work is generally efficient, with a cord blood to maternal blood transfer ratio of 1.02. This work has also shown the possibility of HIV infection impairing transplacental transfer of RSV antibodies, but other studies have suggested, with proper interventions like antiviral therapy, this effect might be prevented (S. M. Patel et al., 2020). Besides, this study has also demonstrated how the resultant antibody concentration transferred to infants is dependent on antibody dynamics which include boosting and waning (Sande, Mutunga, Okiro, et al., 2013).

Furthermore, this study has also shown why it is important to deliver the maternal RSV vaccine within a specific gestational age window and provided an explanation as to why infants born preterm <33 weeks will require alternative preventive strategy for RSV disease.

The role of adverse birth outcomes and how they can influence the successful implementation of the maternal RSV vaccine program if the baseline proportions are not known has been described. Additionally, the study has highlighted the significance of factors associated with adverse birth outcomes such as home delivery and how this can influence uptake of an intervention. This study has shown that, baseline proportions of adverse birth outcomes are almost at a third of all births which is relatively high and might obscure safety evaluations of the maternal RSV vaccine. However, this work has also shown that, there might be no variation in vaccine safety outcomes by geographical region even with the existing differences in maternal characteristics. It is possible that if other individual factors associated with adverse birth outcomes are addressed, there may be an increased uptake and utilization of

health care services which in turn would increase maternal RSV vaccine effectiveness when available and rolled out in Kenya.

7.2 Significance of findings

7.2.1 Timing for antenatal care attendance

One key factor which is thought might influence the effectiveness of a maternal RSV vaccine program in Kenya is ANC attendance. This is what will determine the proportion of women reached for vaccination within the required gestational age window period for maternal RSV vaccine delivery. It will also determine vaccine coverage, which will in turn have implications on the overall vaccine effectiveness (Baral, Fleming, et al., 2020). Currently, there are two proposed gestational age windows for a maternal RSV vaccine delivery under investigation. These are 28-32 weeks and 24-36 weeks (Baral, Fleming, et al., 2020). The gestational age window period of 28-32 weeks was found to be associated with higher anti-pertussis IgG avidity in a clinical trial investigating the effect of timing of Tetanus-Diphtheria-acellular pertussis vaccine administration in pregnancy (Abu-Raya et al., 2019), and has also been proposed by WHO for delivery of the maternal RSV vaccine. This thesis has explored how ANC attendance within three gestational age windows for maternal RSV vaccine delivery will influence vaccine coverage and consequently the overall effectiveness of the maternal RSV vaccine.

The results in Chapter Four where a sample of 594 women from Kilifi HDSS were interviewed and gestational age data abstracted from ANC booklets, have shown that, about 76.6%, 84.5% and 96.2%, of women (with at least one ANC visit) attended ANC within the gestational age window period of 28-32weeks, 26-33weeks and 24-36 weeks respectively. These would suggest an estimated vaccine coverage based on these proportions of, 42.1%, 46.5% and 52.9% if vaccine delivery was within the gestational age window period of 28-32weeks, 26-33weeks and 24-26 weeks

respectively. In Siaya HDSS, out of the 791 women with ANC booklets, the proportion that would have been reached for vaccination was 76.9%, 82.4% and 90.4%, within the gestational age window period of 28-32weeks, 26-33weeks and 24-26 weeks respectively. These results have shown that, despite the differences in geographical location and maternal characteristics, the patterns for ANC attendance is similar among pregnant women in Kenya, implying the possibility of observing similar vaccine coverage and outcomes across all counties. It is noted that, the restricted timing and use of ANC clinics only, for delivery of the maternal RSV vaccine in Kenya is likely to have a challenge in reaching out to all pregnant women for vaccination which might lead to low vaccine coverage. This is because not all pregnant women attend ANC and even if they attended, it is not always that the visits will coincide with the gestational age window period required for vaccine delivery. Additionally, as presented in this thesis, only 35% of women from Kilifi HDSS with evidence of ANC attendance completed all the four required visits (MOH., 2012), while, 52% (40/77) of women who attended only one ANC visit were not in any of the gestational age windows for vaccination and would have missed the opportunity to receive a vaccine. Mitigating against these effects will require individual initiatives to attend all recommended multiple ANC visits.

To enhance optimal vaccine uptake and to reduce cost of vaccine delivery, there has been the suggestion to have concomitant administration of both maternal RSV and tetanus vaccine (PATH, 2018). However, concomitant administration of both maternal RSV and tetanus vaccine might greatly impact vaccine coverage as seen in this study. Maternal tetanus vaccine does not require a restricted window for vaccination and is limited to the number of doses for uptake by a pregnant woman. If Kenya for instance, decides to administer the maternal RSV vaccine during the time a pregnant woman is getting a tetanus vaccine, then vaccine coverage will be very low and as presented in this study, it is found to be about 30% in settings like Kilifi

County for a gestational age window of 28- 32 weeks. However, this scenario is likely to change for other counties where there is a high proportion of women attending ANC and the overall vaccine coverage might be higher (Baral, Fleming, et al., 2020) than that seen in Kilifi.

The delay in initiation of first ANC visit (median 26 weeks), from the recommended 12 weeks (MOH., 2012) among Kenyan women as shown in this study is of much concern. Compared to results of ANC initiation of 24 weeks during the 2014 Kenya demographic health survey (GOK, 2014), in this study it is later by 2 weeks. This study has highlighted multiple factors such as older maternal age, education at primary level or below which are associated with the delayed ANC initiation. This study has also shown factors affecting ANC initiation including health care workers strikes and cost also influence the general utilization of health care services. Together, these factors would influence uptake of a vaccine offered through the ANC clinics and therefore the government and relevant stakeholders must take cognizant of the same and make sure most (if not all) women receive all required health care services during pregnancy.

7.2.2 *Efficiency of transplacental transfer of RSV-specific antibodies*

As described in section 7.2.1 above, successful implementation of the maternal RSV vaccine program would require delivery of the vaccine within a gestational age window period which will provide optimal timing for the transfer of RSV-specific antibody. However, for the infant to have maximum benefit from the maternal RSV vaccine, transplacental transfer of antibodies must be efficient. Underlying medical or obstetric conditions during pregnancy and other individual characteristics are likely to impair transplacental transfer thus reducing the concentration of protective immunity transferred to infants (Hartter et al., 2000; B. J. Okoko, L. H. Wesumperuma, M. O. Ota, et al., 2001).

Although, Kenya has a maternal population of high diversity in characteristics as seen in previous national health surveys (GOK, 2014), this study has shown that, these differences would not have any significant effect on transplacental transfer of RSV-specific antibodies. As a result, the majority of women in this study were found to have efficient transplacental transfer of RSV antibodies. This finding suggests the possibility of observing similar vaccine outcomes across maternal populations across the different geographical regions in Kenya. Furthermore, this finding alleviates the concern that, efficacy of a maternal RSV vaccine in LMICs might be different due to the existing comorbidities experienced by this maternal population.

Some of the important factors found to influence transplacental transfer of RSV-specific antibodies from the sub-set sample of 400 women with paired cord and maternal blood samples from Kilifi and Siaya included, gestational age at delivery of <33 weeks, HIV infection, being multiparous and low birth weight. Infants born from HIV infected mothers were found to have an impaired transplacental transfer and reduced concentration of RSV-specific antibodies which makes them more susceptible to severe RSV associated disease (Alonso et al., 2021). The effect of HIV infection in impairing transplacental transfer of RSV antibodies was also found among Botswana women (S. M. Patel et al., 2020). Furthermore, HIV-infected pregnant women have shown reduced immunogenicity to vaccines. The role of HIV infection in impairing transplacental transfer is thought to be related to immune activation leading to production of inflammatory cytokines at the materno-foetal interface (Abu-Raya et al., 2016; Wilcox et al., 2017). This result is of much concern as regions with high HIV prevalence such as counties within the lake basin region in Western Kenya are likely to experience reduced vaccine efficacy. However, it is thought that continued integration of strategies to mitigate against effects of HIV infection among pregnant women and their infants will ensure optimum benefit of the infant from the maternal RSV immunization program in Kenya.

Besides, this study has demonstrated the significance of delivery of the vaccine within a specific gestational age window period. This restricted timing for maternal RSV vaccination arises from the nature of RSV antibody dynamics (Sande, Mutunga, Okiro, et al., 2013; Swamy & Garcia-Putnam, 2014). Unlike other viruses such as measles, where vaccination or natural infection elicits long term protective immunity (Shinefield et al., 2002), RSV antibodies wane and reach unprotective levels within a period of 3 months after infection (Sande et al., 2014). Transplacental transfer of IgG antibodies are known to begin during the third trimester of pregnancy (Kristoffersen, 2000) from 28 weeks, which means, infants born preterm <33 weeks, will have both an impaired transplacental transfer and low concentration of antibodies because the duration of transplacental transfer is too short. This study found about 3% of infants are likely not to benefit from the maternal RSV immunization program in Kenya and might require inclusion of other RSV disease prevention strategies like use of prophylactic RSV monoclonal antibody (Domachowske et al., 2018). The rates of preterm births seem to vary by geographical region as found in this study (5% in Kilifi and 1% in Siaya). This implies distribution of resources for alternative preventive strategies might also require regional assessment of the existing proportions of preterm births.

7.2.3 Baseline rates of adverse birth outcomes

In order to have a successful routine vaccine delivery to pregnant women, there has to be a robust tracking system for adverse birth outcomes (PATH, 2018). However, this will only be possible if there is a clear understanding of the baseline rates of adverse birth outcomes before introduction of the vaccine in the Kenyan setting. In an evaluation of birth outcomes involving 2219 women (594 from Kilifi and 1625 from Siaya), this study has shown that, a total of 781 (35%) pregnancies had adverse birth outcomes, most of which (16%) occurred during home deliveries. The most common

adverse birth outcomes were preterm births <37 weeks' gestation, 490 (62.7%); low birth weight <2.5 kgs, 247 (31.6%); macrosomia, 189 (24.2%) and still births 42 (5.4%). Recognizing these baseline proportions of adverse birth outcomes will be very critical in monitoring vaccine safety.

As earlier described in Chapter Six of this thesis, despite significant differences in the characteristics of pregnant women between Kilifi and Siaya, the proportions of adverse birth outcomes were similar in both sites. This again ascertains a possibility of observing similar safety outcomes across the different counties in Kenya despite the diverse maternal population.

The results in Chapter Six of this thesis have also shown that, there was a significant reduction in adverse birth outcomes and home deliveries from the year 2017 to 2021. Kenya reported the first case of SARS-CoV-2 (the virus that causes Covid-19 disease) infection on 13th March 2020. The government immediately implemented control measures to prevent further spread of the virus which included staying at home and calling a helpline before accessing care in hospitals among others. Of much interest is that the effect of covid-19 pandemic on birth outcomes in the year 2020 when there was implementation of strict control measures is not significant. This is because, the county departments of health, gave priority to pregnant women to access delivery services in all public hospitals and implementation of the "Linda Mama" programme continued throughout the period. However, it is found that antenatal care was significantly disrupted and few women in 2020 attended more than 4 ANC visits compared to 2019 (28% vs 52%; p=0.001), possibly due to fear of contracting Covid-19 in the hospital while attending ANC clinic. This study has therefore shown that, even amidst natural disasters, if the government and other stakeholders implement strategies improving access to healthcare services, there is possibility of increasing utilization of these services including vaccination.

This study has also shown some of the factors that influence ANC attendance are similar with those influencing choice of place for delivery. This suggests that successful implementation of the maternal RSV vaccine will involve strategies which will enhance utilization of obstetric services starting from ANC initiation up to delivery. Individual characteristics such as tertiary level of education, gestational diabetes, delayed ANC initiation, number of ANC visits, being multiparous and home delivery were shown to be associated with adverse birth outcomes. Individual medical condition during pregnancy such as gestational diabetes, HIV infection and other illnesses are danger signs and are likely to define pregnant women's patterns for attending ANC or choice of place for delivery (Moindi et al., 2016). Such illness episodes if not well managed may result into poor pregnancy outcomes which might result in misinterpretation of the outcome of a maternal vaccine. As earlier suggested, during vaccine roll out, it will be worth putting in place procedures for proper management of high-risk pregnancies to mitigate against occurrence of some of these adverse birth outcomes and provide a reliable platform for monitoring vaccine safety and enhance effectiveness.

The majority of women (99%) interviewed in this study were optimistic about a new maternal vaccine to prevent pneumonia among their infants and would be willing to accept it if and when it is available. It is worth noting that, perceptions or beliefs that an intervention is likely to have an adverse outcome can lead to vaccine hesitancy and refusals which are likely to affect its uptake by the targeted population (Fabry et al., 2011). Vaccine refusals often lead to low uptake and therefore impacting the overall vaccine effectiveness. This study has shown that, the concern for vaccine hesitancy or refusal of the maternal RSV vaccine might be minimal in Kenya. However, considering previous experiences with delivery of human papillomavirus (HPV) vaccine in Africa (Abdullahi, Hussey, Wiysonge, & Kagina, 2020), programmes that create awareness of this vaccine should involve all relevant stakeholders and should

also be made available in ANC clinics to enhance uptake, build confidence and attain wide vaccine coverage.

Most women in this study were found to be aware of the importance of receiving a vaccine during pregnancy but lack resources to enable them to have unrestricted access to the recommended health care services. This and other factors often lead to home deliveries or few ANC visits. It was also clear that the level of making decisions regarding utilization of health care services for delivery is higher among women who have at least secondary level of education. Women with no formal education at all tend to adhere to the traditional norms and beliefs associated with pregnancy and birthing - such as giving birth at home. This is because they are largely economically less empowered (Moindi et al., 2016), are dependent on other family members when it comes to decision making and are often difficult in embracing change which is likely to impact the uptake of a new vaccine. It will therefore be important to have strategies such as mass campaigns which can reach out to all pregnant women for vaccination regardless of their socio- economic status to achieve a wider vaccine coverage and effectiveness.

7.3 Study Limitations

- Pregnancy dating assessed in this study was measured by fundal height which is not an accurate method for estimating gestational age. Although, fundal height is likely to under or overestimate gestational age, this is the method available and in use in all public hospitals in Kenya.
- This study could not screen for total immuno-globulin G levels and therefore not able to confirm any infection related hypergammaglobulinemia to the impaired placental transfer of RSV antibody seen in HIV-infected women, women with illness episodes during pregnancy and those diagnosed with malaria in this study.

- The results for malaria infection used in this study were from venous blood and not from placental infection. Results for placental malaria among these women was not available by the time of data analysis to confirm whether malaria parasites had also infected the placenta or not, for clear interpretation of the observed positive effect of transplacental transfer of RSV antibodies in the presence of malaria infection.
- Women with HIV-infection were missing data for HIV antiviral therapy, adherence or viral load. Among the KHDSS participants, HIV status was not collected among these women and, proportions of adverse birth outcomes attributed to HIV infection and how HIV would likely have altered the observed associations of other variables in multivariable analyses for birth outcomes.
- The sample size was small for women with premature births, HIV infection, RSV infection leading to wider confidence intervals and not so strong positive effect on predictors.
- This study did not characterize the IgG sub-classes of RSV specific antibodies being transferred to infants and their cord to maternal transfer ratios, but assessed the total IgG transferred. However, previous studies have detected IgG1 as the most RSV-specific antibody transferred to infants and thus its critical significance in mediating antibody responses against viral pathogens.
- The data was drawn from a sample of women from two out of the 47 counties and might not be representative of all Kenyan women.
- Hospital enrolment of some of the participants may represent a bias. However, these women were all from the general population and didn't differ in characteristics with those sampled from the community.

7.4 Conclusions

This thesis has detailed findings among pregnant women interlinking their patterns of

attendance for ANC screening, RSV antibody dynamics during pregnancy and the factors and resultant outcome at birth and how these together would influence the degree to which an infant will benefit from a maternal RSV immunization program in Kenya. From this study, it is evident that vaccine coverage which is dependent on the proportion of women attending ANC, will play a critical role in the effectiveness of a maternal RSV vaccine program.

The success of a maternal RSV vaccine implementation in Kenya if conducted through an ANC clinic will largely depend on individual's willingness to present for ANC screening at the appropriate time and the acceptance of utilizing health care services starting from early pregnancy to delivery. Therefore, initiatives which improve ANC services uptake are likely to increase vaccine coverage by reaching a high proportion of women within the required gestational age window for vaccine delivery.

The differences in characteristics within the diverse maternal populations in Kenya, might not have a significant effect in the efficiency of transplacental transfer of RSV specific antibodies or vaccine safety outcomes. Pregnant women with comorbidities such as HIV infection will require integration with existing HIV care programs to improve maternal RSV vaccine outcomes. Vaccine uptake is likely to be higher since the majority (99%) of women interviewed were looking forward to accepting a maternal RSV vaccine when it becomes available in ANC clinics. The findings in this study are useful in guiding policy development towards implementation of a maternal RSV vaccine program through the ANC platform in Kenya and can also be relevant to guide implementation of other maternal RSV vaccines in a developing country setting.

7.5 Recommendations

This study found that effective implementation of the maternal RSV vaccine program in Kenya, will be influenced by timing for ANC attendance and general willingness to utilize health care services among pregnant women. In order to attain high vaccine coverage, this vaccination program will require an integrated approach to mitigate against

factors affecting utilization of maternal healthcare services from both the individual and the healthcare system perspective which can be achieved by implementing the following recommendations:

1. Strategies to get women to come to ANC early and stay in care till delivery. Similar initiatives to “Linda Mama” and “Beyond Zero” which provide free ANC and delivery and post-natal services, if made available to all pregnant women are likely to improve utilization of healthcare services.
2. Having a good system of monitoring birth outcomes which can also monitor vaccine safety when implemented.
3. Encourage facility deliveries and find mechanisms to negate the economic challenges women face.
4. Consider integration of other preventive interventions with maternal RSV vaccination targeting infants born premature, with low birth weight<2.5kgs, or HIV-infected mothers to improve vaccine outcome.
5. Introduction of an electronic database for ANC attendance screening in health facilities to monitor vaccine uptake when rolled out.

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APPENDICES

Appendix I: Consent Form for Pregnant women from HDSS Sites

Study Title: Comprehensive assessment of factors influencing the potential effectiveness of a maternal RSV vaccine program in Kenya.

Lay Title: A study to investigate factors that would influence the success of a maternal RSV vaccine program in Kenya.

Institution	Investigators
KEMRI-WTRP, Kilifi	Ms. Joyce Nyiro, Prof. James Nokes, Dr. Charles Sande, Ms.Hope Mwangudzah, Prof. Jay Berkley, Dr. Amek Nyaguara and Mr. David Walumbe
KEMRI-CDC, Nairobi	Dr. Sandra Chaves, Dr. Mac-Allain Widdowson, Dr. Patrick Munywoki, Dr. Jennifer Verani
KEMRI-CMR, Nairobi	Prof. Elizabeth Bukusi
KEMRI-CGHR,Kisumu	Godfrey Bigogo, Ms. Nancy Otieno and Mr. Bryan Nyawanda
UNITID, Nairobi	Dr. Dufton Mwaengo
Manchester, University, UK	Dr. Timothy Kinyanjui

Who is carrying out this study?

- This study is being carried out by KEMRI, Kilifi in collaboration with KEMRI-CGHR and CDC Nairobi Kenya. KEMRI is a government organization that carries out medical research to find better ways of preventing and treating illness in the future for everybody's benefit.

What is this study about?

- One of the research problem that KEMRI currently seeks to understand are the factors that would influence the success of a maternal Respiratory Syncytial Virus (RSV) vaccine program in Kenya. RSV are germs which cause severe respiratory illness in very young babies under 6 months old. These germs spread through close contacts via large nasal droplets among people from infected persons. Commonly, people (especially older children and adults) have these germs in their noses without any symptoms at all. However, when they pass it on to someone else, especially a young child they may develop symptoms and have severe respiratory illness.
- One of the ways to prevent RSV respiratory illness is by vaccinating the mother. Maternal RSV vaccines are currently in final stages of testing in the developed countries to find out how well they work in preventing severe respiratory illness among babies. There are plans to do some of the final tests in Kenya, but this is difficult because we still do not know how well the new vaccine program can be successfully implemented. One requirement for this vaccine to work best is to have the mother vaccinated during the last three months of pregnancy. Now, we do not know the timing during pregnancy when majority of the pregnant women attend for antenatal care (ANC), where they can easily be reached for vaccination. Getting this information, will help us understand the best time within the last 3 months of pregnancy suitable to give the vaccine. That is why we are conducting this study to collect data of the weeks during pregnancy you attended for ANC screening and other factors that could possibly affect the maternal RSV vaccine program.

- This study will involve 1000 women from the area where KEMRI conducts census in Kilifi, Nairobi Kibera and Lwak in Siaya.
- We approached you because you have been selected randomly from registers of pregnant mothers per our 2017-2020 KEMRI census data.
- We are asking if you can participate in this study to help us learn important factors to be considered if the maternal RSV program is to be implemented in Kenya. We will request to see and collect records of your pregnancy size in weeks as you attended for antenatal screening. This information could be used to help us in setting the guidelines that can help to have these vaccines used in our country.

What will it involve for me?

- If you agree to participate in this research, we will ask to see the ANC booklet.
- We will collect information about your ANC attendance and pregnancy size, history of the pregnancy and other personal details as recorded in the booklet using a questionnaire.
- We will also request to ask you some questions related to the previous pregnancy if that information is missing from the ANC booklet or if you cannot find your ANC booklet.

Are there any risks or disadvantages to me in taking part?

- There are no anticipated risks involved when you take part in this study.
- We will only collect information from your ANC booklet and ask you a few questions. We will make sure we spend very little time of not more than 30minutes so that we do not cause any inconvenience for your time in any way.

Are there any advantages to me of taking part?

- There will be no direct benefits to you for participating in this study. However, the results of this study may have the potential to guide in policy development towards

implementing a maternal RSV vaccine in this country and provide important baseline data for trials of maternal vaccines in Kenya. If through the results of this study, vaccine trials for RSV become successful in Kenya, this will benefit the people of this community in future and you may also be part of them.

What happens if I refuse to participate?

All participation in research is voluntary. You are free to decide if you want to take part. If you do agree you can change your mind at any time and withdraw from the research. This will not affect you now or in the future.

Who will have access to information about me/my child in this research?

All our research records are stored securely in locked cabinets and password protected computers. In future, information collected or generated during this study may be used to support new research by other researchers in Kenya and other countries on other health problems. In all cases, we will only share information with other researchers in ways that do not reveal individual participant identities. For example, we will remove information that could identify people, such as their names and where they live, and replace this information with number codes. Any future research using information from this study must first be approved by a local or national expert committee to make sure that the interests of participants and their communities are protected.

- We will share anonymized individual and summary information we collect or generate with our collaborators in CDC in ways that do not reveal individual participants' identities.

Who has allowed this research to take place?

All research at KEMRI has to be approved before it begins by national committees in Kilifi and Nairobi who look carefully at planned work. They must agree that the research

is important, relevant to Kenya and follows nationally and internationally agreed research guidelines. This includes ensuring that all participants' safety and rights are respected.

What if I have any questions?

You are free to ask me or any of our staff any question about this research. You can also contact the research team using the contacts below:

Ms. _Joyce Nyiro or Prof. James Nokes, KEMRI Wellcome Trust Research Programme, P.O. Box 230, Kilifi. Telephone: 0722 203417, 0733 522063, 041 7522063

If you want to ask someone independent anything about this research please contact:

Community Liaison Manager, KEMRI Wellcome Trust Research Programme, P.O. Box 230, Kilifi. Telephone: 041 7522 063, Mobile 0723 342 780 or 0705 154 386

And

The Head, KEMRI Scientific and Ethics Review Unit, P. O. Box 54840-00200, Nairobi; Telephone numbers: 0717 719477; 0776 399979 Email address: seru@kemri.org

KEMRI-Wellcome Trust Research Programme Consent form for for investigating factors that would influence the potential effectiveness of a maternal RSV vaccine program in Kenya.

I_____ (name)], have had the research explained to me. I have understood all that has been read/explained and had my questions answered satisfactorily.

Please tick the boxes below where relevant:

□ **I agree to take part in this research**

I understand that I can change my mind at any stage, and it will not affect me in any way.

Subject signature: _____ **Date** _____

Subject name: _____**Time** _____

(Please print name)

Where participant cannot read, ensure a witness* observes consent process and signs below:

I attest that the information concerning this research was accurately explained to and apparently understood by the subject and that informed consent was freely given by the subject.

Witness' signature:_____ Date :_____

Witness' name: **_____ Time _____**

**A witness is a person who is independent from the trial or a member of staff who was not involved in gaining the consent.*

Thumbprint of the subject as named above if they cannot write: ----------
---

I have followed the study SOP to obtain consent from the participant. S/he apparently understood the nature and the purpose of the study and consents to the participation in the study. She has been given opportunity to ask questions which have been answered satisfactorily.

Designee/investigator's signature: _____ Date _____

Designee/investigator's name: _____Time _____

(Please print name)

THE PARTICIPANT SHOULD NOW BE GIVEN A SIGNED COPY TO KEEP

Appendix II. Consent Form for Siaya MATFLU participants

Study ID Number: ___ ___ ___ ___ ___

Name: _____

Title of the study: **Cohort study of influenza-associated illness in pregnant women in Western Kenya**

Investigators: Sandra S Chaves (CDC), Nancy Otieno (KEMRI/CGHR), Joshua Mott (CDC-Kenya), Meredith McMorrow (CDC), Gideon Emukule, (CDC-Kenya), Marc-Alain Widdowson (CDC), Joseph Bresee (CDC), Jazmin Duque (CDC), Bryan Nyawanda (KEMRI/CGHR), Daniel Omollo (KEMRI/CGHR), Meghna Desai (CDC-Kenya), Clayton Onyango (CDC-Kenya).

CDC- U.S. Centers for Disease Control and Prevention
KEMRI- Kenya Medical Research Institute CGHR- Center for Global Health Research

Introduction

Influenza (commonly known as the flu) is a virus that can cause mild or severe illness, often with cough and fever. Some people, including pregnant women and young children, may be at higher risk of severe illness from flu, or even dying. In Kenya, flu is found year-round, especially during and after the rainy season.

KEMRI and CDC are doing a study to determine the burden of flu in pregnant women in Kenya. You are being asked to join this study because you are pregnant and you live within the study area. Before you decide if you wish to join this study, you will be given more details about the study. You will also learn about the different things you will be asked to do if you decide to join.

Purpose of the study

The purpose of this study is to measure the burden of flu in pregnancy and its impact on mothers and babies in Kenya. This can help the Kenyan Government decide who the most important people to vaccinate for influenza may be.

Study population and sample size

We are asking women who are early in pregnancy to join this study. There will be 2250 pregnant women recruited from Bondo, Kombewa, and Siaya District Hospitals and they will be followed throughout pregnancy and for about 24 weeks after delivery.

Study procedures

On the day that you join this study, study staff will review your medical history and examine you. The exam will be performed by trained nurses and clinical officers. They will assess the stage of your pregnancy based on the last day you saw your monthly periods and they will examine your abdomen (tummy). An ultrasound examination may also be done to determine the size and the condition of your baby. They will also ask you for blood and urine samples. These measures will be part of your routine antenatal visit. **At study enrollment and again later in pregnancy (near delivery), the study staff will ask for a vaginal-rectal swab. The swab is a cotton bud, but smaller and rounder, that is wiped over the vagina and rectum to check for a bacteria that women commonly have in their vagina or rectum**. **We will use some of the urine collected for antenatal laboratory tests to also test for these bacteria.** You will also receive testing and counseling for HIV. Women found to be HIV-infected will be referred to the patient support center (PSC) for treatment.

After joining the study, study staff will contact you every week either by telephone, Village Reporter/Community Health Worker home visit, or home visit by study staff to ask if you have flu symptoms – fever and cough. How we contact you is your choice. If we are unable to contact you by phone or Village Reporter/Community Health Worker visit, a member of the study team will visit you at home. If you are sick, we will ask you

to come to the hospital so the study staff can assess you. If you have fever and cough, we will ask to put a small swab in your nose and mouth to take a sample to test for flu. We will ask for your permission to keep the samples stored**. We may also collect blood and urine** to test for other things that may have made you sick. Flu tests **and other tests** will be done in Kisumu or Nairobi. Sometimes we may need to send your sample**s (blood, urine, or swabs)** to a laboratory outside of Kenya for further testing **if we cannot have that done in Kenya**. You can still join the study if you don't want your samples stored or shipped outside of Kenya.

When you go into labor, you will be asked to come here to deliver. If your labor starts at night and you cannot arrange transport to the hospital, you can contact the study team for help with transport. At the time of delivery, you will be asked to present your study ID card to the healthcare worker. By showing this ID card the healthcare worker will be alerted to the need to collect study data and notify the study team of your delivery. If you require a caesarian section and for any reason it is unavailable at the study hospital, you will be transferred to the Jaramogi Oginga Odinga Referral and Teaching Hospital (JOORTH) in Kisumu.

After delivery, we will collect blood from you and from the umbilical cord to test for flu. We will also collect a sample of the placenta to look at how healthy the tissue is and investigate the presence of malaria parasites. After discharge we will continue to contact you to ask about fever and cough for up to 24 weeks. We will also ask you about your baby and whether they are sick. If you or your baby is sick, we will ask you to come to the hospital so study staff can assess you and/or your baby. If you or your baby is sick, we will put a small swab in yours and in the nose and mouth of the baby to test for flu.

We will also collect 10ml (1 tablespoon) of blood from you and/or your baby when either of you is sick with fever. This will help us understand the cause of the illness and will help us to assist other women and babies who get sick with fever during their pregnancy but don't have malaria. If we cannot contact you by phone or in person for more than 3

weeks during your pregnancy or during the 24 weeks after delivery, you will no longer be in the study and you will no longer get study benefits including transport reimbursement and sick care.

Participation is voluntary

It is up to you to decide if you want to join the study. You may decide not to join or to leave the study at any time. This will not affect the care you receive at this clinic. You may leave the study at any time.

If you choose not to join in this study or leave the study later, you are encouraged to come to the antenatal clinic in this hospital. This clinic can provide for your routine care and help answer any questions or concerns you may have related to your pregnancy and your baby. You will receive the same standard of care as before.

Risk or discomfort

The nose and mouth swabs may hurt a little for just a second. Rarely, they may cause nosebleed.

Benefits

By joining this study, you will get free treatment if you are sick. You and your baby will be regularly seen by study staff. You will also receive 300 Ksh transport reimbursement for each study visit. If you come to the hospital to deliver your baby you will also receive an infant care package containing a baby shawl/towel, wash basin, and nappies. If you deliver your baby at home or somewhere else, you will not receive this benefit. If you do not join this study, you will still get routine care and treatment as before.

Costs to you

There is no cost to you for joining this study. You will not have to pay for any of the clinic visits or medicines given to you by the study staff.

Your records will be private

All the information you provide regarding your health and the health of your baby will be kept private and only accessible by study staff. The study team is not allowed to share this information without your permission. Results of this study will be presented all together and you will not be named as a participant.

Contacts

If you have any questions about this study, please contact Ms. Nancy Otieno at 0720661245.

If you have any questions about your rights in the study or you feel you have been harmed in any way or you would like to talk to someone who is not part of the study team, please contact The Secretary, KEMRI Ethics Review Committee, PO Box 54840-00200, Nairobi, Telephone numbers: 0717719477, 02 2722541, 0722205901, 0733400003; E-mail address: ERCadmin@kemri.org.

CONSENT FOR PARTICIPATING IN THE STUDY

The above study has been explained to me and I agree to join.

I have been told about the risks and benefits of joining this study.

I have been able to ask questions about it and my questions have been answered.

I have been told that it is up to me if I want to join this study. I know that I can leave the study at any time without any consequences to me and my baby.

I agree to have study staff visit me at home if I am not able to be reached by phone or if I am unable to come to the hospital.

I understand that flu testing will be done in Kisumu or Nairobi and I may not get the results.

If you agree for yourself and your baby to join this study, please put your thumbprint on the proper lines (as you do when you seek an identification card) or sign your name below.

NOTE: You are not giving up any of your legal rights by signing this informed consent document.

If you agree, please circle "yes". YES

_____ _____

Participant's name Participant's signature or thumbprint Date

(Please print)

_____ _____

Witness's name Witness's signature Date

(If participant is illiterate. Please print)

I have explained the purpose of this study to the study participant. To the best of my knowledge, she understands the purpose, procedures, risks, and benefits of this study.

_____ _____

Investigator/Designee name Investigator/Designee signature Date

(Please print)

CONSENT FOR LINKING TO HEALTH AND DEMOGRAPHIC SURVEILLANCE DATA (Siaya and Kombewa only)

We would like to compare the data we collect to data collected in the health and demographic surveillance system (HDSS). You will not be required to answer additional questions to do this; we will only require permission to search the database for your household.

Please check one of the boxes below to indicate whether you allow us to compare study data to the HDSS.

☐ YES, I give consent to have data from this study compared to the HDSS

☐ NO, I DO NOT give consent to have data from this study compared to the HDSS

CONSENT TO SHIP SAMPLES OUTSIDE KENYA

We would like your permission to send your/your baby's samples to a laboratory outside Kenya for us to test for other causes of illness in pregnant women and babies. Your name will not be on the samples.

Please check one of the boxes below to indicate whether you allow study staff to save your samples and sometimes ship them to America.

☐ **YES, I give consent to send my nose and mouth swabs, vaginal-rectal swabs, urine, or blood, and/or my baby's nose and mouth swabs or blood for testing outside Kenya**

☐ **NO, I DO NOT give consent to send my nose and mouth swabs, vaginal-rectal swabs, urine, or blood, and/or my baby's nose and mouth swabs or blood for testing outside Kenya**

CONSENT TO STORE SAMPLES

We would like your permission to save your/your baby's samples for us to test for other causes of illness in pregnant women and babies. Rarely, we may want to ship a sample to Atlanta, Georgia in America for testing that can't be done in Kenya. Your name will not be on the samples and we would store the samples for no longer than 5 years.

Please check one of the boxes below to indicate whether you allow study staff to save your samples and sometimes ship them to America.

☐ **YES, I give consent to save my nose and mouth swabs, vaginal-rectal swabs, urine, or blood, and/or my baby's nose and mouth swabs or blood for testing outside Kenya**

☐ **NO, I DO NOT give consent to save my nose and mouth swabs, vaginal-rectal swabs, urine, or blood, and/or my baby's nose and mouth swabs or blood for testing outside Kenya**

NOTE: This consent form with original signatures must be retained on file by the principal investigator. A copy must be given to the study participants.

If the woman refuses to take her copy of the consent form with her, she states so below and signs and dates her decline statement. Provide the woman with contact information in case of a medical emergency whether she takes the consent form.

No, I do not wish to receive a copy of this signed consent form.

_____ _____ _____

Participant's name Participant's signature or thumbprint Date

(Please print)

_____ _____

_____

Witness's name Witness's signature Date

(If participant is illiterate. Please print)

Appendix III: Consent Form for KIPMAT research participants

Joint KEMRI / MoH Research into Maternal and Infant Risk Factors at Kilifi County Hospital

Institution	**Individuals**
KEMRI CGMR-C, Kilifi	James Berkley, Anna Seale, Michael Mwaniki, Charles Newton, Anthony Scott, Evasius Bauni, Greg Fegan, Margaret Mackinnon, Eunice Nduati, Susan Morpeth, Benjamin Tsofa, **Hellen Barsosio**
Kilifi County Hospital	Maureen Owiti
Department of Obstetrics & Gynaecology, University of Oxford. UK.	Stephen Kennedy

You are being admitted to the maternity unit where care is available to have your baby as safely as possible. There will be an admission blood sample for check for malaria and low blood levels, this is the normal procedure. You will receive the best treatment available in Kilifi.

What is KEMRI and what is this study about?

Kilifi County Hospital and KEMRI work together. KEMRI is a national research organization whose work is to find better ways of preventing and treating illness for the benefit of everyone in the future. To do this, we are finding out more about what causes mothers problems, how these problems affect their children, how their bodies fight

illness, what affects health and growth of babies in the womb, and why some mothers have problems having babies or become ill, while others do not. We are therefore asking your permission to be a part of understanding these problems.

What will it involve for me and my baby if I agree?

When you are admitted and you have your baby, we are asking:

1. To save some of the blood left over from the test for low blood levels (that you will normally have) to check how you and your baby's body fights infections. The amount will be between 1 and 2 teaspoons (5 to 10ml).
2. To perform a vaginal swab before you have your baby to check for infection.
3. To take a small amount of blood from the umbilical cord and after it has been cut and separated from the baby.
4. To take a small sample of your afterbirth (placenta) after delivery to look to see if you had infections during pregnancy such as malaria and to look at parasites that are found.

Everything else that is done during your stay in hospital will be part of normal tests and treatment requested by doctors in KCH. If there are any other research activities that KEMRI staff would like you/your child to be involved in, staff shall explain and ask you first.

Are there any risks or disadvantages to me taking part?

KEMRI's priority for every patient is her care. The blood sample from you will be taken with the routine check for low blood levels, so will not involve any additional needles. The cord blood and afterbirth (placental) samples taken for research will not affect you or your new-born's health as they are taken after delivery and the cord being cut and would normally be thrown away.

Are there any benefits to me/my child of taking part?

If low blood levels, malaria or other infections are detected you will be treated; there is no other direct benefit to you in participating, but you will also be helping us to improve care for mothers and babies in the future.

What happens if I refuse to participate?

It is up to you if you want to be a part of the research. If you do not want to take part, no afterbirth (placenta), or cord blood samples will be taken. You will have the same care whether you take part or not. If you do agree now, you can change your mind at any time and not take part in the research. This will not affect your care now or in the future.

What happens to the samples?

All the information and samples collected will be held confidentially. Individual names are removed from all samples and replaced by codes, so that samples can only be linked to mothers by people closely concerned with the research. Most of the research tests that will be done on the blood will be done here in Kilifi. However, for some tests that cannot be done in Kenya, part of the samples may be sent to laboratories overseas to better identify any bacteria or viruses found, and the body's response to infections. After the research, a small portion of the blood and placental samples will be stored. We would like to store them for up to ten years. In this time, new research about maternal and infant health may be done on these samples. This will involve using new ways of looking for infection and how your body fights infection and what may have affected your baby's growth in the womb. All such research must first be approved by a national independent expert committee to ensure your safety, rights and privacy are respected.

Who will have access to information about me/my child in this research?

All our research records are stored securely in locked cabinets and password protected computers. Only a few people who are closely concerned with the research will be able to view information from mothers.

Who has allowed this research to take place?

An independent national committee and a committee in Kilifi have looked carefully at this work and agreed that the research is important, that it will be conducted properly, and mothers' safety and rights have been respected.

What if I have any questions?

You may ask any of our staff questions at any time. You can also contact those who are responsible for the care of you and your child and this research:

PI's name(s) and contacts

Dr. James Berkley KEMRI- Wellcome Trust [**Kilifi County Hospital**,], P.O.Box. 230, Kenya. Telephone: 0720867011 or 041 7522063

If you want to ask someone independent anything about this research, please contact

Community Liaison Manager, KEMRI – Wellcome Trust P.O. Box 230, Kilifi. Telephone: 0723342780 or 041 7522063,

Or, The Secretary - KEMRI/National Ethics Review Committee, P. O. BOX 54840-00200, Nairobi, Tel number: 020 272 2541 Mobile: 0722205901 or 0733400003

Joint KEMRI / MoH Research into Maternal and Infant Risk Factors at Kilifi County Hospital

I, _____ (name), have had the research explained to me. I have understood all that has been read and had my questions answered satisfactorily.

Please insert the boxes below where relevant:

□ **I agree to take part in this research**

□ **I agree to samples being stored**

□ **I agree to samples being exported**

I understand that I can change my mind at any stage and it will not affect me or my baby in any way.

Subject's signature: **_____ Date _____**

Subject's name: _____Time _____

(Please print name)

I certify that I have followed the study SOP to obtain consent from the [participant]. She apparently understood the nature and the purpose of the study and consents to participation in the study. She has been given opportunity to ask questions which have been answered satisfactorily.

Designee/investigator's signature: _____ Date _____

Designee/investigator's name: _____ Time _____

(Please print name)

Only necessary if the participant cannot read:

I *attest that the information concerning this research was accurately explained to and apparently understood by the subject and that informed consent was freely given by the participant.

Witness' signature: _____ Date _____

Witness' name: **_____ Time _____**

(Please print name)

***A witness is a person who is independent from the trial or a member of staff who was not involved in gaining the consent.**

Thumbprint of the subject as named above if they cannot write:

THE PARTICIPANT SHOULD NOW BE GIVEN A SIGNED COPY TO KEEP.

Appendix IV. Pregnancy Assessment Form for HDSS participants

<u>Study lay Title:</u> A study to investigate factors that would influence the success of a maternal RSV vaccine program in Kenya.

Date today (DD/MM/YYYY) __ __ /__ __/ 20__ _ Time: ___:___hrs

Section I: Personal details (Extracted from HDSS database)

1. Study Person id: __ __ __ __ ___ __ DSS PID: __ __ __ __ __ __ Residence (Village): _____ HM Name: __ __ __ __ __ __ ____ ____ __ __ __ __ Location:_ ___ __ __ _

Section II: Antenatal Attendance (Fieldworker to fill)

2. Mother attended ANC? [ ] Y/N

3. If No give reasons for not attending ANC: [ ] Reason1__ __ __ [ ] Reason2__ __ __ [ ] Reason3__ __ __ __ **Proceed to fill section V.**

4. If attended ANC is **YES**, is ANC booklet available? [ ] Y/N

5. If ANC booklet available is **NO** give reason: [ ] Lost or misplaced [ ] not issued

And proceed to fill in **Q7-Q9, Q11-Q15, Q17-Q21 and Q24-Q31** by asking the participant.

6. If ANC booklet available is **YES**, proceed to fill in the sections below using the booklet and ask the participant for information not in the booklet for **Q17-Q21** and **Q24-Q31**.

Section III: Maternal Profile

7. Facility ANC attended: __ __ __ __ ___ __

8. Participant Names: 1_____ 2_____ 3_____

9. Age: __ __ __ __ ___ __ Gravida: __ __ __ __ ___ __ Parity: __ __ __ __ ___ __

10. LMP: __ __ __ __ ___ __ EDD: __ __ __ __ ___ __

11. Marital status: 1. Married [ ] 2. Single [ ] 3. Divorced/Separated [ ] 4. Widowed [ ]

12. Education level: 1. Some primary[ ] 2. Primary completed[ ] 3. Some Secondary[ ] 4. Secondary completed [ ] 5. Tertiary [ ] (Specify if College or University) 6. None [ ]

13. Occupation: 1. None [ ] 2. Employed (specify) [ ] 3. Farmer[ ] 4. Self employed /business woman [ ] 5. Casual labourer [ ] 6. Other [ ]

14. Ethnicity: __ __ __ ___ __ (Use DSS existing categories)

15. Religion: 1. Christian [ ] 2. Muslim [ ] 3. Other [ ]

Section IV: Antenatal profile for pregnancy being assessed

16**.** Antenatal profile table **(Extract information from ANC booklet)**

ANC visit	**ANC visit Date**	**Weight**	**Blood pressure**	**HB**	**Fundal Height**	**Adverse Events**	**Tetanus vaccine given**
1							
2							
3							

4							
5							
6							
7							
8							
9							
10							

*For each ANC visit, have a breakdown of the following categories for adverse events and the fieldworker should tick those appearing in the booklet.

1. pre-eclampsia
2. eclampsia,
3. Malaria
4. gestational diabetes,
5. respiratory illness
6. reproductive tract infection
7. Other infections.

17. Do you know the reason as to why you were given a tetanus vaccine during pregnancy? [ ] Y/N

18. If asked to take an injection during your pregnancy for a new vaccine to protect your child from pneumonia in the early months after birth, would you accept? [ ] Y/N

19. If answer is **NO**, give reasons: [ ] Reason1__ __ __ [ ] Reason2__ __ __ [ ] Reason3__ __ __ __

20. Would you need to consult anyone before taking a new vaccine injection during pregnancy? [ ] Y/N

21. If answer is YES, Who would you consult: 1. Spouse [ ] 2. Friends [ ] 3. Relatives [ ] 4. Other (specify)

Section V: Delivery details

22. Date of delivery: __ __ __ __ _____ ___ Duration of pregnancy: __ __ __ __ _____ _(Weeks)

23. Mode of delivery: [ ] Normal Vaginal Delivery [ ] CS Baby's weight at birth: __ __ __ __ __(Kgs)

24. Place of delivery: [ ] Health facility [ ] Home [ ] Other (specify)

25. If delivery occurred at home, give reasons: 1. Doctors strike [ ] 2. Distance to health facility [ ] 3. Cost [ ] 4. Quality of care / services [ ] 5. Other (specify) [ ]

26. If delivery occurred in health facility specify name of facility: __ __ __ __ __

29. Pregnancy outcome: [ ] Normal [ ] low birthweight [ ] premature [ ] Still birth

30. Adverse events at birth

*Have a breakdown of the following categories for adverse events at birth.

1. pre-eclampsia
2. eclampsia,
3. Malaria

4. Maternal Hemorrhage
5. other condition (specify).

31. What reasons made you attend for ANC services during pregnancy? 1. Previous pregnancy history [ ] 2. Free services [ ] 3. Pregnancy complications [ ] 4. Friends [ ] 5. Relatives [ ] 6. Wellbeing of mother and child [ ] 7. Other [ ] (specify) __ __ __

32. What reasons made you choose the place to deliver your baby? 1. Previous pregnancy history [ ] 2. Free services [ ] 3. Pregnancy complications [ ] 4. Friends [ ] 5. Relatives [ ] 6. Wellbeing of mother and child [ ] 7. Other [ ] (specify) __ __ ___ __

33. Who made the decision for you to attend for ANC services during pregnancy? 1. Self [ ] 2. Spouse [ ] 3. Both husband and wife [ ] 4. Community health worker [ ] 5. Relatives [ ] 6. Other [ ] (specify) __ __ __ __ __

34. Who made the decision for you on the choice of the place to deliver your baby? 1. Self [ ] 2. Spouse [ ] 3. Both husband and wife [ ] 4. Community health worker [ ] 5. Relatives [ ] 6. Other [ ] (specify) __ __ __ __ __

Initials of the Field worker collecting the data [ ] [ ]

Appendix V. Enrollment Form for participants of Influenza surveillance

Title of the study: **Cohort study of influenza-associated illness in pregnant women in Western Kenya**

LAST Name: _____

FIRST Name: _____

Age: __ __ years

Date of birth: DD/MMM/YYYY *(Allow dates between 1/1/1965 and 1/1/2003)*

Date of interview: DD/MMM/YYYY *(Auto fill today's date but allow changes)*

Health Facility: Bondo Kombewa Siaya

A. Initial assessment

1. Height: __ __ __ cm
2. Weight: __ __. __ kg
3. Vital signs: i. Temperature__ __. __C ❑Axillary ❑ Oral ❑ Tympanic_____

ii. Blood pressure __ __ __ / __ __ __ mm Hg

4. Reported Last Menstrual Period (LMP): DD/MMM/YYYY ❑Unknown
5. Fundal height: __ __ cm
6. If 4 weeks or more discrepancy in reported LMP and fundal height, and fundal height is at or above umbilicus perform an ultrasound for gestational age. Estimated gestational age by ultrasound: ___ ___ weeks

7. Study staff's best estimate of gestational age given data above: ___ ___ weeks

   Netbook will then give calculated LMP and EDD.

B. Inclusion Criteria (ALL must be 'Yes' to be eligible)

1. Resident of village within 10 km of study health facility ❑Yes ❑ No
2. Pregnancy confirmed by urine test or ultrasound ❑Yes ❑ No
3. Gestation ≤20 weeks by fundal height, ultrasound or within 4 weeks of quickening ❑Yes ❑ No
4. Does not plan to relocate out of the study area in the next 12 months and agrees to all follow-up visits/contact by phone ❑Yes ❑ No
5. Not currently enrolled in another intervention study ❑Yes ❑ No
6. Provides informed consent by signature or thumb print ❑Yes ❑ No
7. Consents to HIV testing and counseling ❑Yes ❑ No
8. Willing to deliver in the labor ward of the study hospital ❑Yes ❑ No

C. Exclusion Criteria (ALL must be 'No' or 'Don't Know' to be eligible)

1. Multiple pregnancy (twins, triplets, etc.) ❑Yes ❑ No ❑Don't Know
2. History of Fistula Repair or Leg/spinal deformity ❑Yes ❑ No ❑Don't Know
3. Unable to give informed consent (for example due to mental disability) ❑Yes ❑ No

D. Demographics

1. Marital status: ❑Single ❑Married ❑Widowed ❑Other

   If married, husband's name: _____

2. Village of residence: _____

3. Subject's Education level: ❑ Never attended school

   ❑ Primary

   ❑ Secondary

   ❑ University/College

E. Obstetric History

1. Is this the subject's first pregnancy? ❑Yes ❑ No ❑Don't Know

2. If yes to 1, SKIP to question 6. If no, how many previous pregnancies has she had – regardless of outcome? __ __

3. How many previous pregnancies resulted in a live birth? __ __

4. How many still births (death after 28 weeks, 0 days gestation) has she had? __ __

5. How many miscarriages (fetal loss before 28 weeks gestation) has she had? __ __

SUM of live births, still births, and miscarriages should equal total pregnancies – UNLESS woman has had twins, triplets, etc. Add data check here when SUM does not equal number in B.2.

"The number of live births, still births, and miscarriages does NOT equal the number of previous pregnancies. Please review this section with the participant again or verify that she has had multiples (e.g. twins, triplets)."

Go back to 2. If SUM of live births, still births, and miscarriages does not equal the number of previous pregnancies:

"Has this woman had multiples (e.g. twins or triplets)?" ❑Yes ❑ No

If no, "Ask supervisor to review obstetric history". If Yes, continue to 6.

From ANC Card:

7. Total number of antenatal visits made during this pregnancy including this visit? __ __

8. Date of first ANC visit for this pregnancy? DD/MMM/YYYY

Prior Pregnancies:

10. Has the patient ever been told by a clinician that they have had any of the following pregnancy-related complications (for any prior pregnancy or during this pregnancy)?

a. Previous cesarean delivery? ❑Yes ❑ No ❑Don't Know

b. Eclampsia or Pre-eclampsia? ❑Yes ❑ No ❑Don't Know

c. Pregnancy-induced hypertension? ❑Yes ❑ No ❑Don't Know

d. Ectopic pregnancy? ❑Yes ❑ No ❑Don't Know e. Gestational Diabetes? ❑Yes ❑ No ❑Don't Know

f. Premature labor? ❑Yes ❑ No ❑Don't Know

g. Other?_____ ❑Yes ❑ No ❑Don't Know

F. Medical History

1. Has the patient been told by a clinician that they currently have any of the following medical conditions?

a. Tuberculosis or on TB treatment ❑Yes ❑ No ❑Don't Know

b. Asthma ❑Yes ❑ No ❑Don't Know

c. Chronic obstructive pulmonary disease/Chronic bronchitis❑Yes❑ No ❑Don't Know

d. Diabetes (high blood sugar) ❑Yes ❑ No ❑Don't Know

e. Hypertension (high blood pressure) ❑Yes ❑ No ❑Don't Know

f. Epilepsy ❑Yes ❑ No ❑Don't Know

g. Other chronic illness Specify: _____

2. Is the patient living with someone infected with TB or on TB treatment?

❑Yes ❑ No ❑Don't Know

3. What is the woman's HIV status? ❑ HIV-infected ❑ NOT HIV-infected ❑ Unknown

Source of HIV status? ❑ ANC Booklet ❑ Rapid Test

If NOT HIV-infected or Unknown, SKIP to 5.

If HIV-infected, is the patient on HAART? ❑Yes ❑ No ❑Don't Know

If yes, when did the patient start HAART? DD/MMM/YYYY

4. If HIV-infected, is the patient on daily septrin/co-trimoxazole? ❑Yes ❑No ❑Don't Know

5. Did the woman receive a bednet from the ANC clinic? ❑Yes ❑ No ❑Don't Know

If no, please distribute net now. Net provided to woman: ❑Yes

6. Did you take prenatal vitamins one month before or during this pregnancy?

❑Yes❑ No❑Don't Know

If yes to 6, when did you start taking them? DD/MMM/YYYY

7a. Did you take medications during this pregnancy? ❑Yes ❑ No ❑Don't Know

If yes to 7a, what medication did you take? _____Approximate date DD/MMM/YYYY

7b. Did you take medicine for malaria during this pregnancy? ❑Yes❑ No ❑Don't Know

If yes to 7b, what medication did you take? _____Approximate date DD/MMM/YYYY

8. Did you smoke cigarettes one month before or during this pregnancy? ❑Yes ❑ No ❑Don't Know

If yes to 8, when did you start smoking? DD/MMM/YYYY Stop smoking? DD/MMM/YYYY

9. Have you ever been admitted to the hospital for something other than childbirth?❑Yes ❑ No

If yes to 9, please specify reason for admission: _____

10. Have you been ill in the last 24 hours? ❑Yes ❑ No ❑Don't Know

If yes to 10, what symptoms have you experienced? _____

11. Have you had fever in the last 24 hours? ❑Yes ❑ No ❑Don't Know

12. Have you been ill during this pregnancy? ❑Yes ❑ No ❑Don't Know

If no to 12, SKIP to section H.

If yes to 12, what was the diagnosis? _____

If yes to 12, when were you ill? DD/MMM/YYYY

If yes to 12, what medications were given? _____

If multiple illness episodes go back to 12 and repeat above up to 3 times. Otherwise continue. If more than 3 illness episodes STOP and discuss with supervisor.

G. Physical examination

1. Height__ __ cm
2. Weight__ __. __ kg
3. MUAC: __ __ cm
4. Vital signs:
   i. Temperature__ __. __C ❑Axillary ❑ Oral ❑ Tympanic
   ii. Respiratory rate__ __ breaths per minute
   iii. Pulse rate__ __ __ beats per minute
   iv. Blood pressure __ __ __ / __ __ __ mm Hg
   v. Oxygen saturation on initial evaluation (room air)___ ___%

5. Fundal height: __ __ cm

6. Fetal heart tones documented? ❑Yes ❑ No

7. Does the subject feel the fetus moving? ❑Yes ❑ No ❑Don't Know

8. Other abnormal physical exam findings? ❑Yes ❑ No

If yes to 8, specify finding: _____

H. Baseline laboratory tests

1. HIV status: ❑ Reactive (HIV-infected) ❑ Not reactive (uninfected) ❑ Not done
2. RDT for malaria: ❑ Positive ❑ Negative ❑ Invalid ❑ Not done
3. Urinalysis: Glucose: ❑ Positive ❑ Negative

   Protein: ❑ Positive ❑ Negative

   Nitrite: ❑ Positive ❑ Negative

4. Hemoglobin result: __ __ . __ g/dL ❑ Not done
5. Blood smear collected: ❑ Yes ❑ No
6. Other laboratory test ordered: ❑ Yes ❑ No

   If yes, specify lab test: _____

I. Study Eligibility

1. Subject meets all inclusion criteria and none of the exclusion criteria? ❑Yes ❑ No

   If no, reason for exclusion: _____
2. If yes, did the woman consent to participate in the study? ❑Yes ❑ No

   If enrolled:
3. Does the woman have regular access to a cell phone? ❑ Yes ❑No

   If yes, record the phone number here: _____
4. What is the woman's preferred method of follow-up? ❑ Phone call ❑ VR/CHW visit ❑ Study visit

5. If unable to contact her by the preferred method of follow-up, what is the second choice for follow-up? ❑ Phone call ❑ VR/CHW visit ❑ Study visit

7. If the woman has access to a phone can she receive text messages as well as phone calls? ❑ Yes ❑No

8. Assign Study ID Number: ___ ___ ___ ___ ___ ___

J. Completion and verification

Staff code: ___ ___ Signature: _____ Date: DD/MMM/YYYY

Electronic signature by password *Auto fill today's date when signed*

Supervisor code: ___ ___ Signature: _____ Date: DD/MMM/YYYY

Electronic signature by password *Auto fill today's date when signed*

QC code: ___ ___ Signature: _____ Date: DD/MMM/YYYY

Electronic signature by password *Auto fill today's date when signed*

Appendix VI. Delivery Form For Participants of influenza surveillance

Title of the study: **Cohort study of influenza-associated illness in pregnant women in Western Kenya**

Study ID Number: ___ ___ ___ ___ ___ Staff/Interviewer Code: ___ ___

Subject's LAST Name / SURNAME: _____

Subject's FIRST Name: _____

Date of interview: DD/MMM/YYYY *(Auto fill today's date but allow changes)*

A. Physical Examination

1. Mother's weight: __ __ . __ kg (post-partum)

2. Vital signs: i. Temperature: __ __ . __ ◦C❑Axillary ❑ Oral ❑ Tympanic

ii. Respiratory rate__ __ breaths per minute

iii. Pulse rate__ __ __ beats per minute

iv. Blood pressure __ __ __ / __ __ __ mm Hg

v. Oxygen saturation ___ ___% ❑ On room air ❑ With supplemental oxygen

3. Abnormality on physical exam of mother? ❑Yes ❑No

If yes to Q.3, specify: _____

B. Specimen collection

1. Rapid HIV test repeated (HIV-uninfected women only)? ❑Yes ❑No

If yes, what was result? ❑ Reactive (HIV-infected) ❑ Non-reactive (Uninfected)

2. Malaria RDT performed on mother? ❑Yes ❑No

If yes, what was result? ❑Positive ❑Negative ❑Invalid

3. Maternal hemoglobin measured? ❑Yes ❑No

If yes, what was hemoglobin level? __ __. __ g/dL

C. Delivery History

1. Date of Delivery: DD/MMM/YYYY (Auto fill today's date but allow changes)

2. Time of Delivery: __ __ : __ __ (24 hour)

3. Where was the infant delivered?

❑ Bondo ❑ Kombewa ❑ Siaya ❑Other hospital/clinic, specify: _____

❑ Home ❑ Other location, specify: _____

4. Who delivered the child?

❑ Doctor ❑ Clinical Officer ❑ Nurse ❑ Midwife ❑ Traditional Birth Attendant

❑ Village Health Worker ❑ Other, specify: _____❑ Don't know

5. Mode of delivery?

❑ Spontaneous vaginal delivery (Normal) ❑ Episiotomy ❑ Vacuum ❑ Forceps

❑ C-section ❑ Other, specify: _____

If answer to 5 is C-section, what was the indication? ELSE SKIP to 6.

❑ Prolonged labor ❑ Fetal distress ❑Meconium-stained amniotic fluid

❑ Antepartum hemorrhage ❑ Pre-eclamptic toxemia ❑Cephalopelvic disproportion

❑ Malpresentation ❑ Elective C-section ❑Pregnancy-induced hypertension

❑ Other, specify: _____ ❑ Don't know

6. Was the mother given anesthesia? ❑Yes ❑No

If yes to 6, what anesthesia was used? ELSE SKIP to C.7.

❑ Local ❑ Spinal ❑ General ❑ Don't know

7. Were there any complications at the time of delivery? ❑Yes ❑No

If yes to 7, what complications? ELSE SKIP to 8.

❑ Pre-eclamptic toxemia ❑ Prolonged rupture of membranes

❑Antepartum hemorrhage ❑Postpartum hemorrhage ❑Meconium-stained amniotic fluid

❑Pregnancy induced hypertension ❑Brachial plexus injury to infant

❑Other injury to mother, specify: _____

❑Other injury to infant, specify:_____

❑ Maternal death

❑ Still born

D. Infant Evaluation

1. APGAR scores

   a. 1 minute: __❑Not measured

   b. 5 minutes: __ ❑Not measured

2. Birth weight: __ __ __ __ grams

3. Birth length: __ __ cm

4. Head circumference: __ __ cm

5. Vital signs: i. Temperature: __ __ . __ C ❑Axillary ❑ Rectal ❑ Tympanic

ii. Respiratory rate__ __ breaths per minute

iii. Pulse rate__ __ __ beats per minute

iv. Oxygen saturation ___ ___% ❑ On room air ❑ With supplemental oxygen

6. Sex of the baby: ❑Male ❑Female ❑Indeterminate

7. Ballard Exam results:

a. Neuromuscular Maturity Score __ __ (cannot be performed on stillborn)

b. Physical Maturity Score __ __ (evaluate on live birth and stillborn)

c. Total __ __

8. Physical examination findings:

a. General: ❑Normal ❑Low tone ❑ Central cyanosis ❑Acrocyanosis ❑Jaundiced ❑Pale

b. Head: ❑Normal ❑Fused sutures/craniosynostosis ❑Anencephaly ❑Bulging fontanelle

❑Sunken fontanelle ❑Cephalohematoma ❑ Molding ❑Other

c. Eyes: ❑Normal ❑Congenital cataracts ❑Eyelids fused ❑ Choloboma (hole in iris, retina) ❑Other

d. Ears: ❑Normal ❑Ear tag ❑Ear pit ❑Microtia (too small) ❑Other

e. Nose: ❑Normal ❑Choanal atresia ❑Other

f. Mouth: ❑Normal ❑Micrognathia (small chin) ❑Cleft Lip ❑Cleft Palate ❑ Smooth philtrum ❑ Other

g. Cardiovascular: ❑Normal ❑Murmur ❑Gallop ❑Decreased pulses in lower extremities ❑Other

h. Respiratory: ❑Normal ❑Chest wall indrawing ❑Retractions ❑Grunting ❑Nasal flaring ❑Other

i. Abdominal: ❑Normal ❑Diastasis recti ❑Umbilical hernia ❑Omphalocele ❑Gastroschisis ❑ 2-vessel cord

j. Genitourinary: ❑Normal ❑Hypospadias ❑Inguinal hernia ❑Hydrocele ❑Fused labia ❑Other

k. Extremities: ❑Normal ❑ Extra digit ❑Polydactyly ❑Syndactyly ❑Club foot ❑Erb's palsy (plexus injury) ❑Other

l. Neuromuscular/Spine: ❑Normal ❑Imperforate anus ❑Sacral dimple ❑Sacral skin tag ❑Spina bifida ❑Sacrococcygeal teratoma ❑Other

m. Dermatologic/Skin: ❑Normal ❑Milia ❑Erythema toxicum ❑Mongolian Spot ❑ Pustular rash

❑ Vesicular rash ❑Petechiae ❑ Stork's bite (capillary hemangioma) ❑ Other

n. Describe findings of "other" in D.8a-m: _____

9. If stillborn in C.7, SKIP to section E.

Did the baby require respiratory support after the first 5 minutes? ❑Yes ❑ No

If yes to D.9, what type of support? Else SKIP to D. 10

❑Blow-by oxygen ❑Oxygen by nasal cannula ❑Oxygen by simple mask

❑Oxygen by non-rebreather mask ❑Positive pressure ventilation ❑Intubation and suction

❑Intubation and continued ventilation ❑ CPAP ❑Other, specify: _____

❑Don't know

10. Did the baby require other care beyond routine newborn care? ❑Yes ❑No

If yes to D.10, what type of care? Select all that apply. Else SKIP to D.11.

❑IV/IM antibiotics ❑IV fluids ❑Phototherapy

❑NG/OG feeding ❑Surgical intervention ❑Other,specify: _____

❑Don’t know

11. Did baby survive to discharge? ❑Yes ❑No

E. Completion and verification

Next appointment date: DD/MMM/YYYY

Staff code: ___ ___ Signature: _____ Date: DD/MMM/YYYY

Electronic signature by password *Auto fill today’s date when signed*

Supervisor code: ___ ___ Signature: _____ Date: DD/MMM/YYYY

Electronic signature by password *Auto fill today’s date when signed*

QC code: ___ ___ Signature: _____ Date: DD/MMM/YYYY

Electronic signature by password *Auto fill today’s date when signed*

Appendix VII: Plaque Reduction Neutralisation Test Procedure

KEMRI | Wellcome Trust

Standard Operating Procedure	**SOP No:** LEC-SSP-001 **Version: 2** **Supercedes:** 2

SOP TITLE:	**RSV NEUTRALISATION ASSAY PROCEDURE**	
AUTHOR:	**Name**	**Title**
	Joyce Nyiro	**Research Officer**

	Name	**Title**
APPROVED BY:	**Charles Sande**	**Post Doc. Scientist**

1.0 INTRODUCTION/ PURPOSE:

1.1 The serum virus neutralization (SVN) assay is a serological test to detect the presence and magnitude of functional systemic antibodies that prevent infectivity of a virus. The SVN assay is a highly sensitive and specific test that may be applied to viruses to measure the titer of neutralizing antibodies post- infection or after vaccination.

2.0 SCOPE / RESPONSIBILITY

2.1 VEC lab personnel

3.0 SAFETY/RISK ASSESSMENT

3.1 All samples are potentially infectious, ensure all proper use of PPE and observe all additional safety measures.

3.2 Avoid all loose electrical connections likely to cause shock.

3.3 Comply with all other health and other safety procedures and regulations in the laboratory.

4.0 DEFINITIONS & ABBREVIATIONS

4.1 RSV- Respiratory Syncytial Virus

4.2 PBS – Phosphate buffered saline

4.3 MEM – Minimum Essential Media

4.4 HRP – Horseradish peroxidase

4.5 H2O2- hydrogen peroxide

4.6 FCS – Fetal calf serum

4.7 DMSO- Dimethyl Sulphoxide

4.8 PFU- Plaque Forming Units

4.9 PPE- Personal Protective Equipment

5.0 SPECIMEN

5.1 Serum

5.2 Plasma

6.0 EQUIPMENT/MATERIALS/REAGENTS

6.1 Carbon dioxide incubator.

6.2 96 -Well plates.

6.3 Vortex Machine.

6.4 Working PBS solution (1 PBS tablet in100 mls of de-ionized water)

6.5 Antigen (RSV virus stock)

6.6 Standard/control sera.

6.7 MEM

6.8 FCS

6.9 Methanol

6.10 Acetone

6.11 Hydrogen peroxide

6.12 RSV mouse monoclonal antibody

6.13 HRP- conjugated antibody

6.14 DMSO

6.15 Multichannel pipette & tips

6.16 Detection reagent

6.17 Microscope

6.18 Elispot reader

6.19 Safety cabinet

6.20 Water bath

7.0 METHODOLOGY

7.1 Principle

RSV neutralization assay is used to determine the concentration of a human serum sample (or antibody preparation) required to induce 50% neutralization of a known titration of RSV. The assay has two stages, the neutralization step and a detection step. RSV is detected using a micro-plaque assay technique in which micro RSV plaques are stained brown by immunoperoxidase staining which can then be counted under a microscope or an automated reader. The assay is designed to show neutralization of a known stock of RSV. As this assay is conducted in a 96 well plate format, RSV stock virus concentration of 1000 PFU/ml is added to each appropriate well which gives a concentration of 50PFU/well. To ensure reproducibility between assays, a large number of 'single use' RSV aliquots prepared from a single titrated RSV stock is strongly recommended. Once aliquoted the titer of the identical single-use aliquots needs to be re-determined by performing a plaque assay titration on one of them, this will then allow calculation of the dilution required to give a working stock titer of 1000 PFU/ml. As a new single-use aliquot should be used each time the assay is performed, then each aliquot will be diluted by the same factor and the titer of RSV used in each neutralization experiment should be identical.

7.2 Seeding of 96 wells culture plates

7.2.1 Dilute Hep-2 stock cells in cell culture growth media (DMEM+10% FCS) to a concentration of 10000 cells per well.

7.2.2 Seed 96-well cell culture plates by dispensing 100µl of the diluted Hep-2 cells per well.

7.2.3 Incubate plates at 37˚C overnight.

7.3 **Preparing Samples**

Three or four test samples can be examined on a single 96-well plate. At least 1 plate in each batch must include a reference control serum. Each plate must also have control wells containing only the virus and Hep-2 cells (with no serum to ensure that the virus stock is of the correct titer and that no non-specific neutralization occurs).

Each 1/10 diluted test sample is repeatedly diluted 2-fold over 10 consecutive dilutions. Therefore, each test sample is analyzed for RSV neutralization activity at 1/10, 1/20, 1/40, 1/80, 1/160, 1/320, 1/640, 1/1280, 1/2560 and 1/5120 dilutions. Each sample dilution is set up in triplicate (Figure 1 below shows samples done in duplicate as conducted in our routine assays).

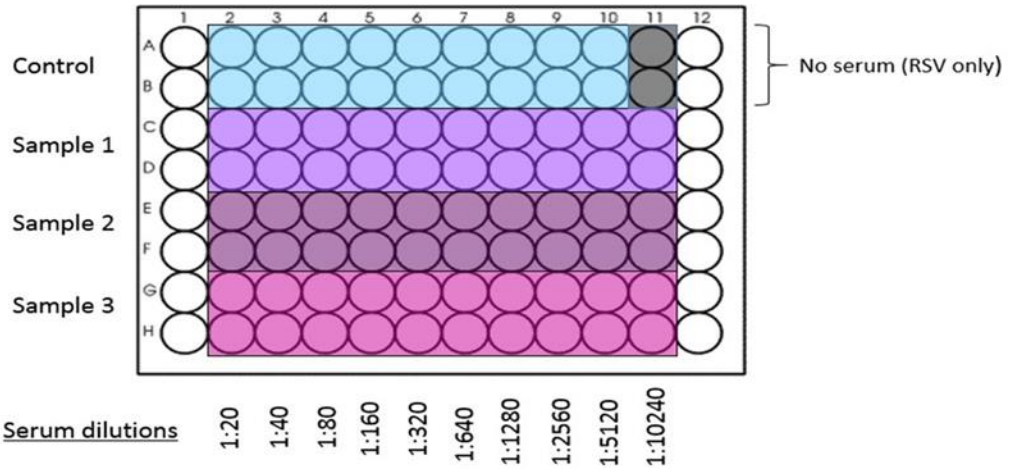

Figure 1: Plate layout. Do not add samples in the first and last columns.

For the serum dilution, keep the same plate layout as the one planned for the plaque assay. (Figure 1)

7.3.1 Incubate the test samples at 56˚C in a water bath for 30 minutes to inactivate complement cascade proteins.

7.3.2 Dilute test serum samples 1/10 in M10 (Cell culture media with 10% FCS). For example, add 6 µl of serum in 54 µl of M10 in the first well and use 30 µl to do 2-fold dilutions (9 times). A final volume of 30µl/well of serum is required for each test well.

7.3.3 A reference control should be included ideally on each plate (or alternatively in each batch) to control inter-plate variation. This control will either be a stock of pooled adult sera, NIBSC RSV standard or BEI control serum (detailed in section 10.1). Dilute the reference control at 1/10 in the first well (6 µl in 54 µl of M10). Each control sample is repeatedly diluted 2-fold over 8 consecutive dilutions.

7.3.4 Seal the plates and store overnight at 4˚C

7.3.5 If the neutralization assay will incorporate a complement serum, follow section 7.5 of the procedure. If assay does not include complement, skip section 7.5 and proceed to steps in section 7.6.

7.4 **Quality Control**

7.4.1 Positive Control

To ensure that the virus stock is of the correct titer and that no non-specific neutralization occurs, a positive control consisting of RSV-only is employed. A titer identical to the RSV stock virus used in the assay should be present on this control which is usually put in the last row of the plate.

Assay controls

To monitor consistency in the assay, serum controls with a fixed concentration of antibodies are included in each run (BEI controls and pooled adult sera). These should give approximately the same number of plaques between runs.

7.5 Use of complement in the neutralization reaction

7.5.1 Use complement sera from Guinea pig (Cat no. S1639-5ML, Sigma-Aldrich).

7.5.2 Dilute lyophilized sera in 5ml of distilled water.

7.5.3 Prepare 10% of complement sera in M10 (v/v)

7.5.4 Add 6µl of the 10% guinea pig complement to each of the 30 µl of diluted serum.

7.5.5 Carry out this procedure in an ice bath and in a safety cabinet.

7.6 Preparing the neutralization reaction

Use filter tips for all the steps of the neutralization reaction.

7.6.1 Thaw aliquot of RSV- B860 (Sweden, 1960) (Stock 21/06/2013) and transfer 30 µl of RSV-B in 30ml of M10 (This gives a virus concentration of 50pfu/well from the virus stock). From this step, the rest of the neutralization reaction should be carried out in 30min maximum.

7.6.2 Add 50pfu of RSV per well in 30 µl to achieve a total volume of 60 µl/well (30 µl serum + 30 µl virus). This addition will lead to a doubling of each of the original serum

dilutions, resulting in a final operational serum dilution range of 1/20, 1/40, 1/80, 1/160, 1/320, 1/640, 1/1280, 1/2560, 1/512 and 1/10240.

7.6.3 Mix the reaction by repeated pipetting with filter tips.

7.6.4 Remove confluent Hep-2 cells from the incubator and flick out the media.

7.6.5 Add 50 µl of the serum-virus mix onto the Hep-2 cell plates.

7.6.6 Place the plates in a 37˚C incubator for 1h.

7.6.7 Rotate the plates to make sure the virus is in contact with cells. Repeat this cycle 4times over a 4-hour period then incubate the plates for 24h in a 37˚C incubator.

7.6.8 Wash the plates with 100 µl of M10.

7.6.9 Dilute CMC 1% at ½ in M10. Add 200 µl per well on the plate and incubate at 37˚C for 48h.

7.7 **Plaque fixing**

7.7.1 Carefully remove medium from wells with a multichannel pipette and wash 2 times with 200 µl of 1xPBS. (DO NOT FLICK OUT THE 1xPBS)

7.7.2 Fix the cells on the 96-well plate by the addition of 100µl of fixing reagent (80% acetone + 20% methanol). The fixing procedure should be carried out in a fume cupboard. All the manipulations in the fume cupboard should be done within a plastic container in case of spillage of the fixative.

7.7.3 Incubate the plate for 10 minutes within the fume cupboard to allow fixation to occur.

7.7.4 To remove the fixative, invert the plates over a paper towel placed within the plastic box. Discard the used paper towels separately from other lab waste to reduce the risk of fire during autoclaving.

7.7.5 Wash 3 times with 1xPBS and allow the plate to dry at room temperature. After fixation, the plate can be stored at 4°C until staining.

7.8 **Plaque staining**

7.8.1 Add 100 µl/well of a primary mouse anti-RSV antibody (RSV Fusion Protein Antibody|RSV3216 (B016) MCA490 – BIO-RAD) diluted 1:500 in PBS.

7.8.2 Incubate the plate for 2 hours at 37°C

7.8.3 Wash the plate 3 times with 200 µl/well of PBS

7.8.4 Add 100 µl/well of a goat anti-mouse HRP-conjugated secondary antibody (Sigma- Aldrich, Cat number A3682) solution diluted 1:1000 in PBS

7.8.5 Incubate the plate for 1h at room temperature

7.8.6 Wash the plate 3 times with 200 µl/well of PBS

7.8.7 Make up the AEC substrate as follows.

- To 10ml of Sodium Acetate buffer (PH 5.0-5.5)
- Add 600 µl of 3.3mg/ml of AEC solution (20mg AEC tablet dissolved in 6mls DMSO)
- 16 µl of hydrogen peroxide - mix

7.8.8 Add 50µl/well and incubate plate for 15 minutes for staining to occur. If the plaques are clearly visible by eye, wash once with water.

7.8.9 Store the plate at room temperature or proceed directly to reading plaques on ELISpot reader. If plates are to be read manually, 70 µl of 30% glycerol in PBS in each well will help enhance the differential between the plaques and the background under low power microscopy

7.8.10 Count the plaques in the sample and control wells. The positive RSV control wells should contain approximately 25 plaques/well

7.8.11 To calculate the amount of serum required to neutralize 50% of the input RSV titer, use the Spearman-Kerber formula.

7.9 **Calculation of the neutralizing antibody titer**

The neutralizing antibody titer is calculated as an ND50 value using the Spearman-Karber formula as follows:

$$\log10ND50 = m - \Delta (\Sigma p - 0.5)$$

Where m is the log10 dilution of the highest dilution of serum (i.e. log10 (1/10,240) = -4.01) Δ is the constant interval between dilutions expressed as log10 (i.e. log10 (2) = 0.3010)

$$\Sigma p = x1/y + x2/y + x3/y + x4/y + x5/y + x6/y + x7/y + x8/y + x9/y + x10/y$$

Where x1 is the number of plaques for the first well, x2 for the second well and so on, and y is the mean number of plaques for the virus (no-serum) control wells.

The final neutralizing antibody titer is the reciprocal.

7.10 **Analysis of the neutralising antibody titre**

Copy plaque counts from the excel sheet generated by the ELISpot reader and paste onto the analysis template containing Spearman-Karber formula to generate the final PRNT titer for each sample. See Neut Analysis Template in figure 2.

8.0 RELATED DOCUMENTS

None

9.0 PRNT ASSAY ANALYSIS TEMPLATE

Sample dilution	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	1:5120	1:10240	
Replicate 1											
Replicate 2											
mean plaque cnt	**#DIV/0!**	**#DIV/0!**	**#DIV/0!**	**#DIV/0!**	**#DIV/0!**	**#DIV/0!**	**#DIV/0!**	**#DIV/0!**	**#DIV/0!**	**#DIV/0!**	
Dilutions	20	40	80	160	320	640	1280	2560	5120	10240	
Σp	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
Neg control mean											50
m											-4.0103
Δ											0.3
Log10ND50	#DIV/0!										
Exponent	#DIV/0!										
Neut Titre	**#DIV/0!**										

10.0 REFERENCES

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