The utility of mathematical modelling of serological data in assessing the impact of vaccination programmes in Kenya

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Declaration

Statement of Own Work

I, Caroline Mburu, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, this has been indicated in the thesis. I have read and understood the school's definition of plagiarism and cheating given in the Research Degrees Handbook.

Caroline Mburu,
May 2024

Abstract

The concept of employing serosurveillance to assess the presence of pathogen-specific antibodies within populations and define the infectious disease landscape is gaining rapid momentum. This approach is increasingly recognized as a potent tool that can complement conventional case-based disease surveillance and routine vaccination coverage estimates, supplying a substantial amount of information to shape and guide immunisation programs. Despite its prevalence in high-income countries (HICs), it is still underutilised in low- and middle-income countries (LMICs). In this thesis, I have utilised a series of case studies in an LMIC setting across different pathogens to critically assess the added value of serosurveillance beyond vaccine coverage estimates and case-based surveillance data in enhancing our understanding and ability to control vaccine-preventable diseases (VPDs).

Using age-stratified seroprevalence estimates spanning 2009 to 2021, encompassing measles, rubella, tetanus, diphtheria, pertussis, and Hepatitis B, I have demonstrated how serosurveys enhance situational awareness regarding the proportion of susceptible populations. I have also shown examples of how these estimates can inform revisions to existing programs like guiding targeted SIAs in the case of measles or assessing the need for booster doses in the cases of diphtheria and tetanus.

Next, I have illustrated the synergistic utility of combining serosurveillance data, case surveillance data, and routine coverage data in evaluating the trade-offs among various intervention programs. In addition to emphasizing the importance optimising routine coverage timing and uptake to reduce dependence on SIAs and measles susceptibility in our context, this analysis underscores the significant value derived from integrating these diverse datasets. Therefore, beyond the integration of serosurveys into disease surveillance, it is imperative to enhance routine vaccination coverage and case surveillance data for optimal disease control.

I have also demonstrated the enhanced utility of integrating seroprevalence data into modelling frameworks for outbreak risk prediction, particularly in situations relying on herd protection thresholds, such as in measles control programs. This approach is valuable for rapidly assessing the potential impacts of healthcare system disruptions and gauging progress toward measles elimination.

I have demonstrated the value of serosurveillance data in monitoring the effective coverage of immunisation programs. This approach offers additional advantages compared to crude vaccination coverage as it provides insights into the population protected against infection or disease. I consider this method as a valuable means to identify vaccination gaps, especially in communities with inadequate record-keeping, which can be addressed during immunisation campaigns. However, it is essential to carefully consider the cost implications and logistical challenges associated with serologic testing in relation to the potential benefits of incorporating immune markers for vaccination monitoring.

Finally, I have illustrated how these estimates can be utilised to assess the effectiveness of a vaccination program, either independently as demonstrated with rubella or through integration into a modelling framework as exemplified with Hepatitis B. This proves invaluable, especially when evidence is required for potential revisions to existing vaccination programs.

Collectively, the research in this thesis addresses the value of information added by serological surveys in control of VPDs in an LMIC setting.

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Acronyms

DALYs	Disability Adjusted Life Years
DHS	Demographic and Health Survey
DPT	Diphtheria, Pertussis and Tetanus
EPI	Expanded Programme on Immunisation
GAVI	Global Alliance for Vaccines and Immunisations
Hib	Haemophilus influenza type b
LMICs	Low- and Middle-Income Countries
HICs	High-Income Countries
MICS	Multiple Indicator Cluster Survey
NIP	National Immunisation Programme
Re	Effective reproductive number
Ro	Basic reproductive number
SIAs	Supplementary Immunisation Activities
RIs	Routine Immunisation programs
VC	Vaccination Coverage
VPDs	Vaccine Preventable Diseases
WHO	World Health Organisation
HBV	Hepatitis B Virus
HepB3	3rd dose of hepatitis B vaccine
HepB-BD	Birth dose of hepatitis B vaccine
MCV	Measles Conjugate vaccine
MR	Measles-Rubella vaccine

1. Chapter 1: Introduction

Background

Vaccination remains one of the most important and cost-effective public health interventions for the prevention and control of vaccine preventable diseases (VPDs). In addition to reducing the mortality and morbidity associated with infectious diseases, it has also contributed to the realization of sustainable development goals as countries can now allocate resources to economic development due to the reduced financial burden of these diseases [1].

Currently about 3 million deaths are averted annually while 1.5 million deaths stand to be avoided if global vaccination coverage (VC) is improved [2]. A comprehensive modelling study in 194 countries projected that between 2021 and 2030, vaccination could prevent 18.8 million measles-related deaths (95% CI: 17.8–20), 0.5 million congenital rubella syndrome(CRS)-related deaths (95% CI: 0.4–0.6), 14 million hepatitis B virus (HBV)-related deaths (95% CI: 11.5–16.9), 0.4 million tetanus-related deaths (95% CI: 0.1–0.8), 0.2 million diphtheria-related deaths (95% CI: 0.1–0.3), and 5 million pertussis-related deaths (95% CI: 2.3–8.6)[3].

Disease surveillance, which typically involves counting cases of infection, disease or mortality, is used to monitor the dynamics of infections, particularly in response to interventions. It plays a vital role in assessing the reach and impact of vaccination programs. This is of particular importance for Low- and Middle-Income Countries (LMICs) that have introduced numerous vaccines at minimal costs. As these countries transition from GAVI, the Vaccine Alliance support, and assume full financial responsibility, they will need to significantly increase their healthcare expenditure on vaccines[4]. Surveillance becomes instrumental in justifying this expenditure by quantifying the benefits of vaccination.

Nonetheless, while surveillance is highly advantageous, it has several limitations. Typical disease surveillance is syndrome based and thus only catches those with clinically relevant symptoms and

with sufficient access to care. Consequently, it masks the vast majority of infections for many diseases with frequent subclinical outcomes making it inadequate for assessing the overall infection rate. Additionally, syndromic surveillance may suffer from other biases including non-specific diagnoses based on non-lab confirmed syndromic data and preferential reporting of diseases in certain age groups[5].Moreover, it may not address specific aspects, such as identifying immunity gaps for predicting outbreak risks or providing insights into protection against infection and/or disease which depend on the existence of reliable correlates of protection.

Serosurveillance is the use of data on the prevalence of biomarkers of infection or vaccination to gain insights into the natural history and epidemiology of infectious diseases [5, 6]. It involves testing serum samples from individuals to detect the presence of specific antibodies or antigens related to a particular disease. If good biomarkers exist, serological surveys can offer the most direct method for characterizing the immunity landscape by assessing the proportion of the population protected against a specific pathogen for various infectious diseases. These immunity estimates are crucial as they have a substantial impact on the timing, magnitude, and pace of outbreaks[7]. Moreover, serosurveillance plays a pivotal role in identifying high-risk groups that may require targeted interventions, such as large-scale vaccination campaigns, assessing the duration of vaccine-induced protection, and shaping evidence-based policies related to vaccine introductions. Consequently, it is unsurprising that many High-Income countries(HICs) have established national serosurveillance programs that underpin many of their evidence-based vaccine policy decisions[8-10].

Despite their advantages, serosurveys are resource intensive and require technical expertise which might explain why they are underexploited in LMICs. The limited use of serosurveys in African LMICs suggests that vaccine policy is not always driven by context-specific serological evidence. There is also very limited description of recent population immunity as a measure of the impact of vaccination programmes which is a major data gap. Many of the serosurveys where done often have limited representativeness, as they rely on convenience samples and they have reduced statistical power due to small sample sizes [11].

1.1 Case study pathogens

1.1.1 Measles

Measles is a highly contagious disease caused by the morbillivirus, belonging to the paramyxovirus family, which first infects the respiratory tract before spreading throughout the body. Common in young children, symptoms include high fever, cough, runny nose, and a distinctive red rash that starts on the face and spreads down the body. The illness typically lasts for about 7-10 days from the onset of symptoms, with the rash appearing around day 3-5 and gradually fading over several days[12]. Recovery typically follows the rash's appearance, though complications, more prevalent with age, malnutrition, or other underlying conditions, can affect 10-40% of cases[13].The virus which is transmitted from person to person is highly infectious causing about 14 to 18 secondary cases in a fully susceptible population[14].

Prior to the introduction of the monovalent measles vaccine in 1960s, the disease triggered frequent global epidemics, occurring approximately every 2-3 years and resulting in an estimated 2.6 million deaths annually[15]. Despite a substantial reduction in measles incidence following the introduction of vaccines through the Expanded Programme on Immunizations (EPI) in the 1980s,it still remains an important cause of global mortality and morbidity particularly in regions of Africa accounting for about 100,00 deaths annually despite availability of effective vaccines[16].

In addition to 1st dose of measles containing vaccine (MCV1), World Health Organization (WHO) recommends a 2nd dose (MCV2) in the national vaccination schedules in order to reduce the accumulation of susceptible children by immunising those who failed to seroconvert from a first dose or those who missed it altogether[17]. At least 95% coverage for both MCV1 and MCV2 is recommended for elimination thus regular Supplementary Immunisation Activities (SIAs) are often required to complement the suboptimal coverage achieved by Routine Immunisation(RIs). Several target dates for elimination of transmission of endemic measles have been set and adjusted over time since 1997[18]. The Global Vaccine Action Plan 2011–2020 (GVAP) initially aimed to eliminate measles in at least five of the six WHO regions by 2020. However, with a substantial rise in measles cases in 2019, reaching levels not seen in two decades [19] and subsequent outbreaks due to disruptions in vaccination coverage during the pandemic in 2020 [20, 21],this goal became unattainable. Consequently the previous elimination target has now been revised in the immunisation agenda 2030[22]

Measle virus-specific immunoglobulin G (IgG) antibodies are established markers of immunity against infection. Immunity after a wild-type measles virus infection is generally believed to be lifelong. Vaccine-induced immunity is typically shorter, and more variable compared to natural infection, but it has been demonstrated to endure for several years, even though antibody levels may decrease over time. Both natural and vaccine-induced antibodies are indistinguishable, making it impossible to differentiate between vaccine-induced and naturally acquired antibodies. The protective thresholds for measles antibodies against infection can vary depending on the assay used, ranging from 0.12 IU/ml to 2 IU/ml[12]

In Kenya, MCV1 was introduced in 1980 and is administered at 9 months of age. Administrative coverage for MCV1 has ranged from a minimum reported national coverage of 60% to a maximum reported coverage of 93% since its introduction up to 2021[23]. MCV2 was introduced in 2013 and is administered at 18 months albeit uptake has been poor at 28% to 57%[23]. Since 2002, SIA's have been conducted every 3 to 4 yrs in either children younger than 5 or 15 years old and have typically achieved more than 80% coverage[24]. Nevertheless, measles outbreaks are regularly reported, and Kenya has not eliminated measles.

1.1.2 Rubella

Rubella is an acute illness caused by Rubivirus in the family Matonaviridae that is transmitted through respiratory droplets and direct person to person contact. Rubella infection is usually a mild illness, marked by symptoms such as fever and a rash that typically lasts for 1 to 3 days, with about half of infected individuals remaining asymptomatic[25]. Primary rubella virus infection during early pregnancy however can lead to severe consequences, including miscarriage, stillbirth, or the birth of a child with CRS which has serious public health implications[26].

The asymptomatic nature of the disease poses substantial challenges for surveillance, leading to substantial underreporting. The number of rubella cases and cases of CRS is often estimated from models based on serological data. According to WHO, the current annual global incidence of CRS is reported to be 100,000 cases [25] while case fatality attributable to CRS has been shown to range between 5% and 34% resulting in an estimated 5000 to 34000 annual deaths[26, 27].

The primary goal of rubella vaccination is to prevent CRS which is achieved either through immunising women of child-bearing age or implementing childhood immunisation programs to

disrupt rubella virus transmission and ultimately eliminate both rubella and CRS. However, if childhood rubella vaccination fails to attain sufficient herd protection due to suboptimal immunisation efforts, it can result in an increase in the average age at which individuals get infected. This scenario may elevate the risk of rubella infection during pregnancy, subsequently raising the risk of CRS[25].By 2021, 173 out of 194 (89%) WHO member countries had integrated rubella-containing vaccines into their EPIs [25].Due to the high effectiveness of a single dose of rubella vaccine of between 99.3% to 100% and the long-term persistence of protection [28],a second dose is not a routine requirement. However, because the control efforts for measles and rubella are linked, a second dose is offered as part of MCV2[25]. Additionally, plans for rubella elimination are closely linked to those for measles elimination, as both vaccines are co-administered. As of January 2021, rubella had been successfully eliminated in 93 of the 194 WHO member countries.

Rubella virus-specific IgG antibodies are recognized correlates of protection against infection. They are typically detectable about three weeks after infection and appear to persist through life [29]. While some variability exists in the IgG levels considered protective against infection, those exceeding 10 IU/ml are generally deemed sufficient [25].Vaccine-induced immunity is also assumed to be lifelong with several studies spanning 10 to 21 years documenting persistent seropositivity in more than 95% of the participants[25, 30].It is not possible to distinguish between vaccine induced and naturally acquired immunity against rubella.

In Kenya, there is no a well-established system for CRS[31].In accordance with WHO guidelines, Kenya introduced the rubella vaccine into the EPI program that coincided with a catch-up vaccination campaign for measles and rubella, targeting 19 million children aged 9 months to 14 years in November 2016[32]. Although the MR campaign was successful attaining a coverage of 95% of the target population, the impact on Rubella immunity and CRS is still largely unknown

1.1.3 Tetanus

Tetanus is a disease caused by Clostridium tetani bacterium which enters the body through wounds and cuts. It is characterized by muscle stiffness and spasms often starting in the jaw and neck[33]. The incubation period varies between three to twenty-one days depending on the extent of injury.

Unlike most VPDs, tetanus is not transmitted from person to person; rather, it is contracted through exposure to the bacterium in the environment[34].

Tetanus continues to be a major public health concern, with an 80-100% fatality rate among neonates in absence of treatment[35, 36].Surveillance is poor in many countries and the majority of reported cases have been neonatal tetanus in infants born among unvaccinated mothers. In 2018,WHO estimated that 25 000 neonates died from neonatal tetanus[36, 37]

Tetanus is preventable through immunization with Tetanus Toxoid (TT) targeting children and women of reproductive age. TT is frequently administered as part of a pentavalent vaccine through the EPI, combined with diphtheria, pertussis, hepatitis B, and Haemophilus influenzae type b(Hib). While the WHO recommends three primary infant doses starting at 6 weeks of age, along with three booster doses from the second year of life, many countries have yet to incorporate these booster doses into their vaccination programs[38]. Several targets for maternal and neonatal tetanus elimination(MNTE) through universal active immunisation of children and women of reproductive age have been set since 1989[39]. By the end of 2018,only 14 countries had not reached the MNTE status which is defined as having less than one neonatal case per 1000 live births in each country per year. Sustaining this elimination requires TTCV booster doses to be included in country immunization schedules[39].

Natural infection with tetanus does not provide any immunity. A small amount of tetanus toxin has been shown to be enough to cause an infection but insufficient to generate protective antibody levels [33, 40, 41]. Consequently, the presence of tetanus antibodies in the bloodstream is primarily a result immunisation. The duration of protection following primary immunisation varies due to differing vaccination schedules across countries. On average, tetanus immunity is observed to last about 5 years after 3 doses, 10 years after 4 doses, and 20 years after 5 doses. The protective threshold for tetanus immunity is assay-specific, with concentrations above or equal to $\geq$0.011 IU/ml normally considered protective against infection[34]

In 2018, Kenya was officially validated to have achieved MNTE [42]. However, the national estimates of DPT3 coverage, which range from 75% to 81% [23] fall short of the 90% national coverage target. Furthermore, Kenya has yet to introduce the booster vaccine doses as recommended by WHO for sustaining MNTE and ensuring long-term protection against tetanus. Recent studies have also highlighted tetanus immunity gaps among older children in some parts

of the country [43]. A more comprehensive serological study is essential to identify these immunity gaps and assess the necessity for implementing these booster doses.

1.1.4 Diphtheria

Diphtheria is caused by the bacterium Corynebacterium diphtheriae. It is characterized by symptoms such as a sore throat, fever, and the development of a grayish membrane in the throat that can obstruct breathing. Without treatment, it can lead to complications and last for several weeks. Diphtheria primarily spreads through respiratory droplets from an infected person or by coming into contact with contaminated surfaces[44, 45].

Once a major cause of childhood mortality in the era before vaccination, the global burden of diphtheria has fallen dramatically, from more than a million cases a year in the mid-1900s to about 8000 cases reported in 2017 [46]. Numerous high-income nations have effectively eradicated diphtheria which has negatively impacted surveillance in other countries making it hard to determine the accurate number of cases and associated deaths. Nevertheless, sporadic diphtheria outbreaks have been reported in developing countries[47-49] underscoring the need for renewed efforts to enhance our understanding of diphtheria and strengthen epidemic preparedness.

Diphtheria is prevented through vaccination with a diphtheria toxoid vaccine developed in 1923 and subsequently introduced in the EPIs in 1974 as a three-dose schedule administered from 6 weeks of age in combination with TT and pertussis vaccine [45]. In 2017,WHO revised its recommendations for diphtheria vaccination to include three booster doses given at 12–23 months of age, 4–7 years of age, and 9–15 years of age in addition to the 3-dose primary series[50]. A 90% national vaccination coverage target for the primary dose had been set by GVAP for the global elimination of DPT by 2020. This was however not attainable, and it has since been revised in the 2030 agenda[51]. In 2018, a total of 136 out of 194 WHO member countries offered at least one booster dose of DTP-containing vaccines. Gavi-eligible nations have the option to seek financial assistance from Gavi to establish vaccine delivery systems for the inclusion of DTP booster doses. However, there is currently insufficient data available to inform models regarding the impact of DTP booster doses.

IgG antibodies, induced by either infection or vaccination, serve as correlates of protection against disease. These antibodies are identical, rendering it impossible to distinguish between antibodies induced by vaccination and those acquired through natural infection[45]. The protective threshold levels are assay-specific, but generally, antibody levels equal to or greater than 0.01 IU/ml are considered to provide basic protection against disease, while levels above 0.1 IU/ml are associated with full protection[52]. Both naturally acquired and vaccine-induced immunity have been observed to diminish over time without boosting. Similar to tetanus, the duration of immunity following primary vaccination varies among countries, dependent on different EPI schedules, vaccine formulations, and exposure levels. However, diphtheria immunity has been shown to decline more rapidly than tetanus. One study found that 67% of children lacked sufficient immunity 3 to 13 years after a primary series of three vaccine doses[53], and another observed a five-fold declining antibody levels within the first year following a primary series[54].

In Kenya, diphtheria vaccination follows a typical schedule used in many countries, involving a pentavalent vaccine with three primary doses administered at 6-10-14 weeks of age. As of now, booster doses have not been incorporated into the vaccination program. Serological data is essential to gain a comprehensive understanding of the population's immunity status, the duration of protection, and the potential necessity for booster doses among older age groups.

1.1.5 Pertussis

Pertussis, commonly known as whooping cough, is caused by the bacterium Bordetella pertussis. It presents with symptoms that progress in stages, including a mild cold-like phase followed by severe coughing fits. The duration of illness typically lasts about six to ten weeks[55]. Pertussis is highly contagious and spreads through respiratory droplets when an infected person coughs or sneezes. Infants are particularly vulnerable to severe complications, such as pneumonia and even death, especially within the first six months of life[56]. Re-infections are common and occur throughout a person’s lifetime[55, 57].

Despite the effective implementation of infant vaccination programs, which led to a substantial reduction in pertussis cases and child mortality compared to the pre-vaccine era, pertussis remains endemic in all countries with recurring epidemic cycles every 2 to 5 years [58, 59]. Infants face the highest case fatality rates. In 2013, pertussis-related mortality in the first year of life was

estimated at approximately 63,000 deaths annually among children under 5 years of age [60] .A recent systematic review identified pertussis incidence rates in infants surpassing 1000 cases per 100,000 population during outbreaks [61] although there is paucity of reliable surveillance data especially from developing countries.

Pertussis vaccine was introduced in the EPIs in the 1974 in combination with diphtheria and tetanus toxoid vaccines[59]. The current recommendation for pertussis vaccination includes a primary schedule of 3 doses starting at 6 weeks of age and three booster doses given at 12–23 months of age, 4–7 years of age, and 9–15 years. The recommended vaccination coverage targets are set at 90%, similar to diphtheria and tetanus, to ensure effective control[59].

IgG does not predict protective immunity reliably in pertussis and as such there are no reliable correlates of protective immunity against infection. The prevalence of IgG however can serve as an indicator of exposure to pertussis [62, 63]. Following pertussis infection, IgG antibody levels can rapidly rise to over 100 IU/ml. These levels subsequently decline rapidly, with most individuals having IgG-anti-PT levels dropping below 10 IU/ml within five years[55, 62, 64]. Both natural infection and vaccination against pertussis do not confer lifelong immunity. While research on the duration of protection after primary infection is limited, re-infections have been documented 3 to 12 years after the initial exposure[62, 65]. The duration of vaccine-induced immunity varies depending on the vaccine formulation, but antibodies have been shown to commonly decrease substantially as early as one year after the initial three-dose series of vaccination[66].

In Kenya, pertussis vaccination is administered concurrently with tetanus, diphtheria, hepatitis B, and haemophilus influenzae type b(hib) in three primary doses given at 6-10-14 weeks of age, with no booster doses. Serological data will play a vital role in determining the presence of recent pertussis circulation from the timing of infection based on the levels of antibody. This information will guide decisions on whether booster doses are needed for older age groups.

1.1.6 Hepatitis B

Hepatitis B is caused by the hepatitis B virus (HBV) of the family Hepadnaviridae. HBV is primarily transmitted through contact with infected blood, unprotected sex, or from mother to child during childbirth. The virus can survive outside the body for up to seven days, making it highly contagious through contaminated surfaces and needles. It is characterized by symptoms such as

jaundice, dark urine, fatigue, and abdominal pain, while some individuals remain asymptomatic[67]. The acute phase of the illness typically lasts for three to six months, but the virus can persist beyond this period, leading to chronic infection, which substantially increases the risk of developing liver disease and, ultimately Hepatocellular carcinoma(HCC)[68, 69]. The probability of becoming chronically infected with HBV is inversely related to age. Approximately 80%–90% of people infected perinatally, about 30% of children infected before the age of six years and 5% HBV-infected adults will develop chronic HBV infection[70, 71].

HBV infection is a global public health problem impacting an estimated 257 million individuals worldwide and leading to approximately 887,000 deaths annually[72, 73]. A substantial burden of HBV is in the LMICs with the majority of the countries classified as having high or highly intermediate prevalence, characterized by serologic prevalence of hepatitis B surface antigen (HBsAg) above 8% and 5%, respectively, within the general population[74].

Although a cure for HBV infection remains elusive, effective prevention can be achieved through infant vaccination. Recombinant hepatitis B vaccines were developed in the 1980s, and in 1991, the World Health Assembly (WHA) recommended their inclusion in EPIs[75]. In addition to the three-dose primary schedule which is administered as a pentavalent vaccine with Diphtheria, Tetanus, Pertussis (DTP) and Hib, WHO recommends one dose of hepatitis B vaccine at birth, (Hep BD), to eliminate perinatal transmission, which poses the highest risk of chronic infection. Regrettably, the uptake of this birth dose, introduced in only 13 out of 47 countries in the African region remains low at approximately 6%, in contrast to the 43% global uptake[76]. The WHO global health sector strategy on viral hepatitis aims to achieve a 90% reduction in new cases of chronic hepatitis B and a 65% reduction in mortality due to hepatitis B by 2030[77].

Immunity to HBV is assessed through a panel of serological markers, including hepatitis B surface antigen (HBsAg), antibody to hepatitis B (anti-HBs), and antibody to hepatitis B core antigen (anti-HBc).HBsAg is the marker of infection and is positive in the early phase of acute infection and persistently positive in chronic infection. Anti-HBc is the serological marker of previous HBV infection. The presence of anti-HBs represents immunity to HBV infection. It is the only HBV marker detected in people who have acquired immunity through vaccination while in individuals who've recuperated from prior HBV infection it coexists with anti-HBc IgG[78, 79]. Seroprotection against HBV infection from vaccination is defined as having an anti-HBs level >10

IU/ml, when measured 1–3 months after having received a complete immunisation schedule[80]. Although anti-HBs concentrations wane quite rapidly in the initial years post-immunisation, immune memory continues to persist for an extended duration and as such there is no evidence to support the need for a booster dose of hepatitis B vaccine after completion of the primary vaccination series[75, 81, 82]

While the Kenyan childhood vaccination schedule does not include HepB BD, infants have received a three-dose hepatitis B vaccine at 6, 10, and 14 weeks of age since 2001, with coverage consistently exceeding 80% since 2006[23].Currently, there is no data on the national prevalence of HBV infection in children and the effectiveness of the current vaccination program against HBV infections is also not known..

1.2 Serosurveillance

Infectious diseases can persist within populations when a sufficient number of individuals remain susceptible to infection, allowing for the continued transmission of the disease. The eradication of these diseases necessitates reaching a herd immunity threshold which is influenced by the pathogen's transmission rate and the birth rate[83]. To maintain elimination, it is essential to sustain this level of population immunity through immunisation. Therefore, it is of utmost importance that National Immunisation Programs (NIPs) utilize high-quality data to effectively monitor population immunity, enabling the identification and response to susceptible individuals.

While mathematical modelling techniques can be employed to indirectly estimate immunity profiles using disease prevalence and vaccination coverage data [84], these methods depend on indirect inference and come with inherent challenges. For example, the accuracy of vaccination coverage data in reflecting population immunity, typically obtained from administrative records, household surveys, or individuals' recall of their vaccination history comes with challenges including errors in recording vaccine doses, incorrect assumptions about the target population's size, selection bias, underreporting, and missing data particularly for those without or with incomplete vaccination histories. Notably, vaccination does not guarantee immunisation, as depending on the vaccine and the number of doses a minority of individuals may not develop an immune response post-vaccination[85].

Serosurveillance offers the most direct method for characterizing the immunity landscape by assessing the proportion of the population protected against a specific pathogen for various infectious diseases. Additionally, it allows for the inference of infection dynamics for individual infections. It involves testing of blood and other specimens to detect the presence of antibodies and/or antigens particularly in cases where specific IgG antibodies are recognized correlates of immunity. Antibody concentrations in the blood exceeding a threshold are deemed protective against infection or disease[6, 7].

Serosurveillance studies are regularly used in HICs to assess the impact of vaccination programmes, to identify at-risk groups, and measure the burden of usual and emerging diseases. In the Netherlands, national surveys have been conducted in 1995/1996[9], 2006/2007[86], and 2019[87] to assess and improve the NIP and understand protection across population subgroups. In Australia, the Australian National Serosurveillance Program (ANSP) was established in 1997 with the 2nd and 3rd rounds in 2002 and 2008 respectively to provide national estimates of population immunity to VPDs[8]. In the UK, an age-stratified serological survey initially focused on measles, mumps, and rubella (MMR) was set up during 1986/87, but it has since expanded to include other VPDs. Each year, target serum samples are collected in the age groups 0–24 years, while every five years, sera are collected across the entire age range[10]. Additionally, the European seroepidemiology program, established in 1996, coordinates serosurveillance efforts across 18 European countries, harmonizing data collection methods despite national variations in serum collection, disease epidemiology, and immunization schedules[88].

1.2.1 Epidemiological considerations for use of Serosurveillance

The utility of serosurveillance in controlling VPDs relies on various factors. These factors include the existence of a serological marker for past infection or vaccination. It is essential to determine whether vaccine-induced antibodies can be differentiated from naturally acquired antibodies. Furthermore, understanding the extent and duration of protection provided by these antibodies and whether they serve as correlates of protection against infection or disease is vital for the interpretation of serological data[6, 7].

Certain diseases are fully immunising, conferring lifelong protection against reinfection, which simplifies the direct interpretation of serological data. Measles and rubella, for instance, are such examples where IgG antibodies are good markers of past infection or vaccination and antibody

concentration exceeding a certain threshold are deemed protective against infection[12, 29]. However, because the antibodies can either develop as a result of past infection or vaccination distinguishing the two would require accounting for historical changes in disease incidence and vaccination coverage[83].

Certain diseases are immunising, but they are characterized by intricate interactions among different strains or serotypes. For example, the dengue virus co-circulates with four distinct serotypes . While infection with one serotype provides long-term immunity against that specific serotype, it offers only short-lived cross-protection against the other serotypes after primary infection[89]. In the case of invasive bacterial diseases like pneumococci, over 90 serotypes have been identified, each possessing unique epidemiological properties including potential to cause invasive and severe disease and to trigger specific immunological responses to infection and vaccination[90] . Determining protective antibody levels and normal ranges for pneumococcal IgG antibody can be challenging, as these levels may vary among different serotypes[91]. Influenza is caused by multiple strains that can escape from immunity induced by prior infection or vaccination. While cross-reactive antibodies and T-cells demonstrate evidence of cross-immunity between influenza strains, the effectiveness of immunity from one strain against another varies. While such data can still provide valuable insights into the spread of the disease, these complexities must be considered when interpreting serological data[92].

In contrast, some diseases like tetanus do not result in sustained, measurable antibody responses following infection, but vaccination does induce long-lasting antibodies that have been identified as a correlate of protection against infection[33, 34]. Serological data, in the case of these markers, may not serve as an indicator of previous infections. However, it can still be a valuable tool for evaluating the effective coverage of vaccination programs.

Finally, there are diseases for which correlates of immune protection from infection have not yet been identified. In tuberculosis, the immune response targets can vary with the stage of infection, making it complex to identify definitive correlates of protection[93]. Similarly, HIV is highly mutable and can swiftly alter its surface proteins, making it difficult for the immune system to produce antibodies that can effectively neutralize the virus[94]. While antibodies might not be representative of immunity against a pathogen, they can be used as an indicator of current or previous infection.

Seroprevalence studies offer a direct means of measuring the age-specific profile of susceptibility, provided that the assays used have known and adequate sensitivity and specificity[6, 95]. However, these estimates can be highly sensitive to uncertainty in the specificity and sensitivity of the test, particularly for rare diseases, which may lead to biased estimates and potentially skew public health decisions and interventions. High sensitivity ensures accurate detection of true positive cases, reducing the likelihood of underestimating disease prevalence or immunity levels. Conversely, high specificity minimizes false-positive results, thus reducing the risk of overestimation. To mitigate these challenges, improved reporting of serological testing information in serosurveys is essential to maximize the availability of robust and comparable data for evidence synthesis. Additionally, statistical inferences should be employed to correct for measurement error resulting from poorly performing tests[96]. When this is not feasible, investigators should at least report the test name, manufacturer, and sensitivity and specificity values to enhance data comparability[97]

1.2.2 Serosurveillance to guide and monitor immunisation programmes

Serosurveillance plays a crucial role in monitoring and guiding immunisation programs, both before and after their implementation. Data on antibody prevalence across various age groups can be integrated into mathematical models to estimate age-specific infection rates and pre-vaccination prevalence of infections that are often asymptomatic but exhibit measurable serological markers of protection against infection. This data also aids in calculation of theoretical immunity thresholds required for the elimination of infection. After vaccine introduction, serosurveillance aids in identifying at-risk groups by providing age-specific immunity profiles that can guide targeted interventions and revisions to existing vaccination strategies. When combined with mathematical models, serological data can predict potential disease outbreaks by revealing trends in age-specific risk of infection. This, in turn, helps identify age groups in need of additional protection through supplementary vaccination activities to disrupt transmission.

Rubella virus infections in childhood typically present as asymptomatic or with mild symptoms, complicating disease surveillance due to underreporting. Inadequate coverage in rubella vaccination programs can raise the average age of rubella infection, increasing the risk of rubella cases among pregnant women and the potential development of CRS in newborns as seen in

Greece[98] and Costa Rica[99]. Researchers often utilise serological data and mathematical models to assess the pre-vaccination CRS burden and determine the optimal vaccination coverage needed to achieve herd immunity, averting an increase in CRS cases. Modelling studies utilizing serological data demonstrated that the incidence of CRS was higher when public vaccine coverage for rubella-containing vaccine (RCV) was below 50%. Conversely, CRS cases decreased substantially when RCV coverage exceeded 80% in public vaccination schedules[100-102]. These findings have prompted intensified efforts to ensure robust RCV coverage during introduction, a recommendation also endorsed by the WHO[25].

Subclinical cases of Hepatitis B Virus(HBV) infection are common although exposure can also result in acute or chronic HBV infection which substantially raises the risk of developing liver disease and, ultimately, hepatocellular carcinoma (HCC)[68]. Due to the limited availability of data on chronic liver disease and HCC in developing countries, serological data played a crucial role in assessing the global disease burden and the impact of vaccination. Modelled estimates of HBV seroprevalence projected 1.4 million HBV-related deaths in the absence of vaccination in 2000. However, with the implementation of routine HBV vaccination with 90% coverage, approximately 90% of these deaths could be prevented[103].

Serosurveillance is useful in guiding revisions to vaccination programs. A study employed modelling and serological data to identify the transmission patterns of influenza, with a focus on the potential indirect benefits of childhood flu vaccination. The model's projections suggested that expanding the existing vaccination program to include children aged 5 to 16 would enhance program efficiency, averting 0.70 infections per dose and preventing 1.95 deaths per 1,000 doses. These anticipated indirect effects, supported by serological evidence, played a pivotal role in UKs decision to extend flu vaccination to children to protect them and to reduce disease transmission, subsequently safeguarding vulnerable, under-vaccinated segments of the population[104]. Elsewhere Hib serological data in the UK revealed unexpectedly high rates of waning Hib antibodies and resurgence of Hib, particularly after the use of a less immunogenic vaccine[105] prompting the implementation of catch-up vaccination programs at 12 months of age and a revision of the booster dose policy [106].

Serosurveillance is valuable for identifying at-risk population groups in need of additional protection and verifying whether desired immunity targets have been attained. A serological

survey coupled with mathematical modelling was employed to evaluate the extent of susceptibility to measles and to gauge the potential risk of a resurgence in the UK. Findings indicated a worrisome degree of susceptibility to measles, with a looming epidemic of over 100,000 cases if no intervention were to take place. This compelling evidence provided evidence for the launch of a vaccination campaign aimed at bridging these immunity gaps[107]. Serosurveys conducted in Ethiopia unveiled immunity gaps among older children, underscoring the necessity for a catch-up campaign to mitigate the risk of measles outbreaks [108] while a tetanus serosurvey revealed immunity gaps in school going children in countries that had not introduced a booster vaccine dose[43] underscoring the need for a booster dose. Conversely, in Australia, seroprevalence studies spanning from 1996 to 2007 revealed high population immunity, corroborating the evidence from coverage estimates and disease notification data, ultimately affirming the elimination of measles [109]. Similarly, in Cambodia, a nationwide survey indicated seroprevalence rates for measles exceeding 95%, confirming the attainment of target immunity levels [110].

Serosurveys are valuable for assessing the impact of vaccination campaigns by assessing whether appropriate age groups have been reached. For instance a serosurvey conducted in Kenya after a measles campaign confirmed the effectiveness of the campaign in reducing susceptibility to measles by a range of 65-78% in the different targeted age groups[111] while a study in Europe reported a reduction in susceptibility to rubella from 15% to 3% after a measles-rubella campaign[112] . Additionally, in Zambia, a post-campaign serosurvey demonstrated a substantial increase in rubella seroprevalence among the campaign target group, surging from 51.3% to 98.3%[113].

1.2.3 Serosurveillance to guide pandemic response

Seroepidemiology plays a vital role in managing epidemics, particularly when dealing with evolving pathogens. It served as a valuable tool for assessing the extent of the COVID-19 pandemic, comprehending its dynamics, monitoring viral evolution, and guiding public health strategies and vaccination.

Population seroprevalence assessments, reflecting both past and current infections, were employed to bridge knowledge gaps between syndromic surveillance and the number of people who had become infected and thus gained (temporary) immunity [114-116]. This was especially crucial due to limited viral diagnostic testing capacity, and to develop an understanding of the rates of mild and asymptomatic infections during the early stages of the pandemic[117].Seroprevalence estimates also facilitated the robust calculation of key epidemiological variables such as infection fatality rates [118-120]. These findings were invaluable in informing the parametrization of epidemiological models, assessing the potential effects of various interventions, and informing public health decision-making.

Serological information was employed to infer protection and the effectiveness of vaccines against SARS-CoV-2 variants. For instance, a sequence of periodic serological surveys was conducted to examine the immunity acquired through natural exposure and asses the influence of prior natural exposure on vaccine responses. It was observed that a single vaccination in individuals with prior natural immunity elicited a more robust immune response than two doses in those who had not previously been exposed to the virus. Furthermore, this single vaccination was found to offer sufficient protection against the Omicron variant and other variants, thereby making a compelling case for the implementation of single-dose vaccination strategies in populations with high COVID-19 seroprevalence[121]

1.3 Modelling approaches for control of VPDS

Mathematical modelling is becoming increasingly useful in the control and elimination of VPDs. It allows researchers and public health officials to understand disease transmission dynamics[122, 123], evaluate the impact of intervention strategies like vaccination[21, 124], predict disease trajectories[4, 125] and generate estimates of disease burden and cost-effectiveness of interventions[126, 127] which is useful for managing and mitigating the impact of outbreaks. Models are also particularly valuable for emerging infectious diseases, as they offer a framework for preparedness and response.

A range of computational models are available, and the choice of model depends on the specific question at hand. Statistical models primarily aim to explore associations between variables, not the underlying reasons for their behaviour. These models become especially valuable when the causal relationships driving disease transmission are not yet fully understood[128]. In contrast,

mechanistic models seek to grasp the influence of parameters and variables on one another, allowing for the incorporation of explicit hypotheses about the biological mechanisms governing transmission[129]. An ideal model is one that is well-suited to its purpose, being as simple as possible while still effective and adaptable based on available data. Models are constrained by a delicate balance between predictive accuracy (the capacity to replicate observed infection patterns), transparency (the clarity of the model's role and its components), and flexibility (the ability to adapt to novel scenarios)[130]

In this thesis, I have employed a combination of statistical Generalised Linear Models(GLM)models and compartmental models including two variations of static cohort models and a catalytic model. I will provide a brief overview of each in the following sections.

1.3.1 Compartmental models

One of the commonly employed compartmental models is the Susceptible-Infected-Recovered (SIR) model(Fig. 1.1)which classifies a population into compartments based on their infection status[129, 130]. The models can either be stochastic, employing probabilistic methods to account for randomness and uncertainty or deterrministic,whichyields consistent outcomes with specific parameter sets, and often employs ordinary differential equations to dictate the flow between these compartments.

$$\frac{ds}{dt} = -\beta SI \quad (1.1)$$

$$\frac{dI}{dt} = \beta SI - \gamma I \quad (1.2)$$

$$\frac{dR}{dt} = \gamma I \quad (1.3)$$

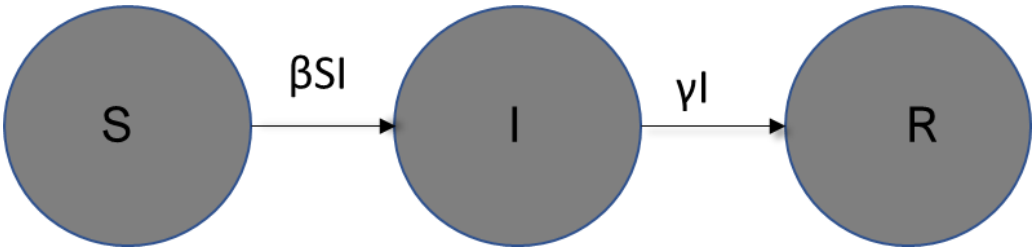

Figure 1:1 Schematic of a Susceptible (S) - Infected (I) - Recovered (R) compartmental model. (β) is the transmission rate and (1/γ) is the infectious period

The simplest model makes several simplifying assumptions such as random mixing within the population, that individuals are infected for the same duration of time, and an equal level of infectiousness among these individuals. Compartmental models are either dynamic, meaning that the rate of new infections is contingent on the current number of infections or static which assume that the force of infection (FOI) is independent of the number of infections. If we assume random mixing, FOI within the population at time t is calculated as the product of the transmission rate and effective contact rate between any two individuals in the population, denoted as β at time t, and the number of infected individuals in the population at time t, denoted as I(t).

Certain diseases may display an increased FOI in younger age groups due to the increased frequency of social interactions within these cohorts. Additionally, vaccinated individuals might experience a lower FOI and a shorter duration of infection compared to their unvaccinated counterparts. To address more complex scenarios, the model can be expanded by subdividing the population into additional compartments based on factors such as age, susceptibility levels, or vaccination status offering a versatile framework for constructing intricate pathogen-specific models.

1.3.2 Static cohort models

A special case of compartmental models is the static cohort models. These are simplified epidemiological models designed to provide a snapshot of a disease's status within a well-defined population cohort at a specific point in time. Unlike dynamic models that account for changing infection rates over time, static models assume a force of infection that is independent on the number of infections[129]. Additionally, rather than having multiple age-groups, these models are designed to follow simple, stable, and well-defined birth-cohorts overtime. The mathematics employed in static models can range from straightforward calculations of infection rates applied

to a population to determine the expected number of disease cases over a brief time span, to more intricate methods involving the solution of a system of ordinary differential equations using more complex simulation techniques like markov processes[131].

Compared to dynamic models, these models are generally less analytically complex, making them easier to work with. They require fewer epidemiological data for parameterization and are less computationally demanding to implement compared to their dynamic counterparts making them most suitable for populations where we don't expect transmission dynamics to vary in the time of the simulation [132]

1.3.3 Catalytic models

A special case of static cohort model is the catalytic model. The catalytic model, named for its structural similarity to chemical reaction equations, was introduced by Muench to estimate the rate at which susceptible individuals acquire infections from summation data[133]. The catalytic model assumes that the population is divided into two states, proportion susceptible, S(a) and proportion immune Z(a). In its simplest form, the assumption is that a disease is fully immunising and therefore the proportion immune is the same as those ever infected. The model follows individuals from birth and assumes there is a life-long constant FOI (λ) which is independent of age(a) and calendar year(fig 1:2). The equation for the simple catalytic model is shown below;

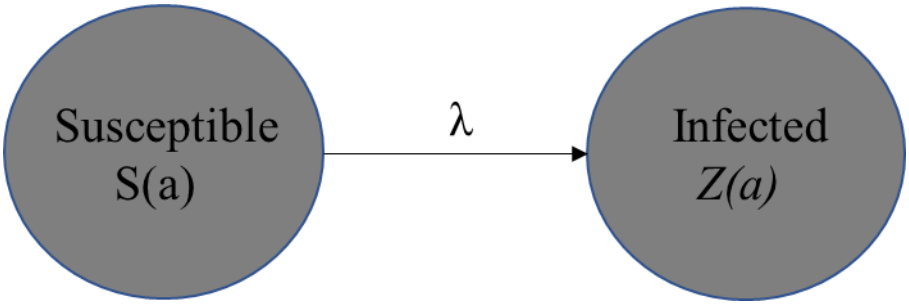

Figure 1:2 Schematic of a basic catalytic model. λ is the force of infection

$$Z(a) = 1 - e^{-\lambda a} \qquad (1.4)$$

For other diseases which are not fully immunising like Hepatitis B, the proportion immune is not the same as those ever infected. In such cases, individuals serorevert and this is characterized by the gradual loss of protective antibodies over time. The catalytic model can be expanded to

accommodate waning immunity, allowing individuals who were previously infected to become susceptible again. The rate at which antibody prevalence decreases over time can be estimated as the waning rate(ω).This variation of the catalytic model is known as a reverse catalytic model(fig 1:3) and the equation is shown below;

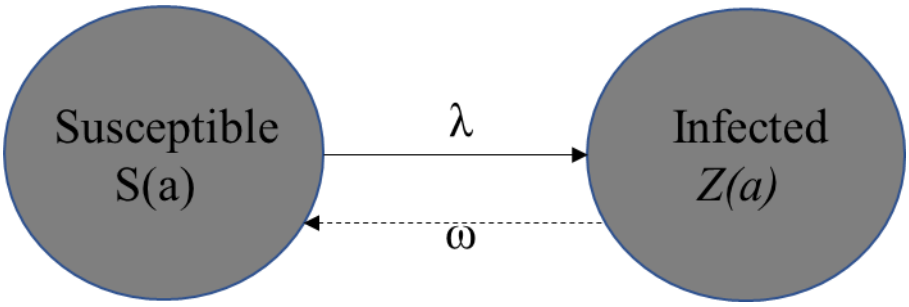

Figure 1:3 Schematic of a reverse catalytic model. λ is the force of infection, ω is the waning rate.

$$Z(a) = \frac{\lambda}{\lambda+\omega}(1 - e^{-a(\lambda+\omega)}) \quad (1.5)$$

In both the simple and reverse catalytic models, assumption is that the mortality rates for susceptible and infected individuals is the same.

Muench's pioneering work has inspired the development of various variations of the catalytic model for estimating FOI from serology data over the years. These models include both parametric and non-parametric approaches, which relax certain assumptions from the original model[134]. For example, diseases like measles may exhibit a higher FOI in younger age groups, primarily because of the heightened intensity of social contacts within these age groups. Moreover, the FOI can undergo changes over time, either as a result of substantial outbreaks or the implementation of interventions such as vaccination. In such cases, the models can be adapted to permit the FOI to vary by both age and time dependent[135, 136].

1.3.4 Bayesian fitting methods

In this thesis I use Bayesian Methods to fit models to data. Bayesian fitting methods estimate model parameters and assess uncertainties by merging prior information with observed data, often utilizing Markov Chain Monte Carlo (MCMC) to generate samples from the posterior parameter

distributions [137]. These methods are grounded in Bayes theorem which postulates that the parameter posterior distribution, $p(\theta|data)$ is proportional to the likelihood of the data given the model parameterisation $p(data|\theta)$, multiplied by the prior information on parameters $p(\theta)$[138]

$$p(\theta|data) \propto p(data|\theta)\, p(\theta) \qquad (1.5)$$

MCMC is an algorithm used to efficiently sample the posterior distribution to generate estimates of the parameter distribution. If the MCMC is converged and autocorrelation is limited, the samples form a good estimate of the full posterior distribution. One of the most frequently used MCMC sampling algorithm is the Metropolis-Hastings (MH) algorithm [139]. This typically involves proposing parameter values and subsequently accepting or rejecting these proposed parameters based on the MH ratio. These parameters are then updated using an iterative process until they converge. An example is shown below;

1. Define the initial parameter value θ_t and the proposal function
2. Generate the proposed parameter θ'using θ_t and the proposal function
3. Calculate the priors of the proposed parameter $p(\theta')$
4. Calculate the likelihood of the data given the proposed parameter $p(data|\theta')$
5. Calculate the acceptance ratio as;$\alpha = \frac{p(\theta')p(data|\theta')}{p(\theta_t)p(data|\theta_t)}$
6. Accept or reject:

   -Generate a random number, μ, from a uniform distribution in the range [0, 1].

   -If $\mu \leq \alpha$, accept the proposed parameters by setting $\theta_{t+1} = \theta'$

   -if $\mu > \alpha$,reject the proposed parameters by setting $\theta_{t+1} = \theta_t$
7. Repeat the process until the parameter estimates converge

It is essential to confirm comprehensive sampling of the parameter space. While various diagnostics methods including the effective sample size (ESS) and the Gelman-Rubin diagnostic, can be used to show a lack of convergence, it is difficult to prove convergence[140]. This is because one can never be sure that 100% of the parameter space has been explored. In cases involving complex posterior distributions, convergence might imply that the chain is trapped in

high-density regions. Hence, it is imperative to run multiple chains with different starting points for theta to ensure full exploration of the parameter space.

The first steps of the algorithm may be biased by the initial value and are therefore usually discarded for the further analysis. Monitoring the acceptance ratio which is influenced by the proposal function is crucial. In general, when proposals are close, the acceptance rate tends to be higher, whereas wider proposals lead to a lower acceptance rate. Extremely high or low acceptance rates are generally unfavourable because it means that the algorithm is staying at the same point which results in suboptimal probing of the parameter space.

Complex posterior distributions may cause chains to become trapped in high-density regions. To enhance computational efficiency and reliability, MCMC optimization strategies can be implemented, such as adaptive MCMC methods[141]. This typically involve using multivariate proposal functions that are adjusted to the correlation structure of the parameter space and setting an acceptance rate of 23.4% which has been shown to be optimal for effective exploration of the parameter space[142]

1.4 Summary motivation

As with many other LMICs, the Kenyan national immunisation program faces several challenges. These include inadequate resources for immunisation, issues with the cold chain resulting in the loss of vaccine potency, and occasional vaccine stockouts[143]. Moreover, the program has grown in size and complexity over time, necessitating increased monitoring and more efficient resource allocation[144]. Consequently, Kenya grapples with a substantial burden of VPDs and recurring outbreaks.

A substantial data gap persists, particularly regarding the population's immunity resulting from existing vaccination programs targeting Tetanus, Measles, Rubella, Diphtheria, Pertussis, and Hepatitis B. Currently, there is no data on the prevalence of HBV infection in children[145], the combined impact of routine immunisation and Supplementary Immunization Activities (SIAs) on measles age-specific immunity remains unknown, as well as the age-groups with immunity gaps. Furthermore, the impact of Measles-Rubella vaccine introduction on Rubella immunity is uncertain[24, 32]. Additionally, considering the WHO recommendations for booster vaccine doses for tetanus to enhance the life course approach to vaccination, tetanus serology would be beneficial

for identifying immunity gaps and the need for these booster doses[38]. Serosurveys are also necessary to generate information on the population immunity profile for diphtheria and pertussis due to the scarcity of data for these diseases. Finally, as Kenya transitions out of external support for its vaccination programme, it will become increasing responsible for its own vaccine policy[146]. This project will contribute context specific data to inform current and future vaccine policy by doing the following: defining population immunity to the principal vaccine preventable diseases; and by tackling topical immunisation challenges by a series of supported evidence-to-policy analyses, incorporating local evidence, and generating local models.

Mathematical models have been successfully utilised in assessing susceptibility to VPDs, quantifying the impact of intervention strategies, and projecting disease trajectories[124, 125]. These models often integrate historical transmission parameters from the literature, vaccination coverage data reported by National Immunisation Programs and socio-demographic variables to characterise disease epidemiology and pinpoint high-risk age groups. Meanwhile, serological surveys serve as valuable tools for directly estimating population protection levels against VPDs and they have been successfully utilized to design optimum vaccination strategies particularly in HICs countries[8, 86]. A combination of mathematical models and serological surveys constitutes a synergistic approach in the control and ultimate elimination of VPDs.

1.5 Aims and Objectives

1.5.1 Aim

The overall aim of this thesis is to use a series of case studies to highlight the added value of serological data, particularly if used in combination with modelling, for the understanding and ultimately ability to control of VPDs in Kenya.

1.5.2 Objectives

1. Measles

   a) Describe the seroprevalence of antibodies against Measles and Rubella over a 12-year period (2009 – 2021) in Kilifi, Kenya and the impact of Measles-Rubella (MR) campaign of 2016

   b) Assess the relative contribution of infection, routine vaccination and supplementary immunisation activities to measles seroconversion in Kenyan children

c) Evaluate the utility of SIA to mitigate the risk of a measles outbreak in the pandemic and post-pandemic period

2. Diphtheria, Pertussis, Tetanus

   a) Describe the seroprevalence of antibodies against Diphtheria, Pertussis and Tetanus(DPT) over a 12-year period in children in Kilifi, Kenya (2009-2021)

   b) Estimating tetanus immunisation coverage from vaccination records and cross-sectional serological surveys in Kilifi.

3. Hepatitis B Virus

   a) Describe HBV seroprevalence in Kenyan children and estimate the effectiveness of the current vaccination program against HBV infection.

1.6 Structure of this thesis

This thesis adopts a research paper format, with each analysis chapter following the structure of a scientific paper, either already published or slated for submission to a journal. In this introductory chapter, I establish the necessary foundation by exploring serosurveillance, various VPDs, and the diverse mathematical models employed in this research. The subsequent sections include six distinct results chapters, as detailed below, culminating in a comprehensive discussion that includes the overall findings and implications.

Chapter Two: Seroprevalence of antibodies against Measles and Rubella over a 12-year period (2009 – 2021) in Kilifi, Kenya and the impact of Measles Rubella (MR) campaign of 2016

This paper is currently in preparation for submission and focuses on addressing objective one, part a. The study employs a series of statistical tests to describe age-specific population immunity to measles and rubella in Kenyan children. Additionally, it evaluates the influence of rubella vaccine introduction on the levels of rubella immunity.

Chapter Three: The relative contribution of infection, routine vaccination and supplementary immunisation activities to measles seroconversion in Kenyan children: A modelling study

This paper is presently ready for submission and focuses on addressing objective one, part b. The study builds on the findings from objective one, part a by fitting a static birth cohort model to measles immunity profiles to track the proportion of children who are either measles-naïve or have seroconverted due to natural infection or vaccination through MCV1, MCV2, or SIAs.

Additionally, the paper explores various scenarios involving changes in the timing and coverage of the current RI program that will reduce dependence on SIAs and lower measles susceptibility.

Chapter Four: The importance of supplementary immunisation activities to prevent measles outbreaks during the COVID-19 pandemic in Kenya.

This paper was published in BMC Medicine in 2021, by Mburu et al.[20] and addresses objective one, part c. The study builds on the findings from objective one, part a by utilizing a static cohort model incorporating measles serological data, local contact patterns, and vaccination coverage data to investigate the risk of measles outbreaks amid the pandemic caused by disruptions in routine immunisation and SIAs

Chapter Five: Seroprevalence of antibodies against Diphtheria, Tetanus and Pertussis over 12 years in children in Kilifi, Kenya (:2009-2021)

This paper is currently in preparation for submission and focuses on addressing objective two, part a. The study employs a series of statistical tests to describe age-specific population immunity to diphtheria, tetanus and pertussis in Kenyan children and the possible need for booster doses of these vaccines.

Chapter Six: Estimating tetanus immunisation coverage from vaccination records and cross-sectional serological surveys in Kilifi

This paper is currently in preparation for submission and focuses on addressing objective two, part b. The study leverages the tetanus immunity profiles obtained in objective two, part a, combined with vaccination coverage estimates obtained from vaccine records to evaluate the effectiveness of tetanus serological data in identifying gaps in vaccination, especially in scenarios where vaccination records were unavailable.

Chapter Seven: HBV seroprevalence in Kenyan children and the effectiveness of the current vaccination program against HBV infection

This chapter is dedicated to addressing objective three. The study utilizes data from various HBV markers to determine HBV seroprevalence. Subsequently, the research integrates this information with vaccination records obtained from a vaccine registry to update vaccination details for participants with missing vaccine records and estimate the effectiveness of the existing vaccination program against HBV infection.

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2. Chapter 2: Seroprevalence of antibodies against Measles and Rubella over a 12-year period (2009 – 2021) in Kilifi, Kenya and the impact of Measles-Rubella (MR) campaign of the 2016

2.1 Research paper cover sheet

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Student ID Number	2003951	**Title**	Mrs.
First Name(s)	Caroline		
Surname/Family Name	Mburu		
Thesis Title	The utility of mathematical modelling of serological data in assessing the impact of vaccination programmes in Kenya		
Primary Supervisor	Stefan Flasche		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

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SECTION E

Student Signature	[redacted]
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2.2 Bridging section

Currently, in preparation for submission, this paper focuses on addressing objective one, part a. The study aims to track annual trends (2009-2021) in age-specific population immunity for measles and rubella, specifically targeting children <15 years and adults. The primary objective is to identify susceptible populations which will provide valuable insights to guide immunisation activities. The research also evaluates the impact of SIAs by examining changes in population immunity before and after campaigns for both measles and rubella.

Before this study, crucial information on measles serial age-specific population immunity profiles in Kenyan children, essential for assessing progress towards elimination, was lacking. Similarly, the impact of the rubella vaccine introduction on rubella immunity in children and adults was unknown.

The estimates generated through this research are anticipated to be of immense value in monitoring the Kenyan immunisation program and, by extension LMICs in general. The age-specific immunity profiles for measles and rubella in children and adults will significantly complement existing case surveillance data, contributing to the resolution of critical knowledge gaps in the field.

I generated the estimates using a series of statistical tests and wrote all the code. I also wrote the draft manuscript and generated the figures. Throughout the process, I received input/suggestions/edits from my supervisors.

The supplementary material is included as Appendix B in this thesis, so any references to the supplementary material can be found there.

2.3 Abstract

Background: Measles and Rubella have been targeted for elimination by the World Health Organisation**.** Age-specific population measles and rubella immunity data which are important for assessing progress towards elimination are scarce. We conducted seroprevalence surveys to identify disease-specific population immunity profiles in both children and adults in Kilifi.

Methods: Data were from cross-sectional surveys conducted in the Kilifi Health Demographic Surveillance System (KHDSS) from 2009 to 2021. IgG antibodies were measured using a fluorescent bead-based multiplex immunoassay and antibodies greater than 0.12 IU/ml for measles and 10.0 IU/ml for rubella were deemed protective. Bayesian multilevel regression with post stratification was used to obtain seroprevalence estimates adjusted for the underlying population, sensitivity and specificity of the assay. Associations between changes in seropositivity with age, gender, location and ethnic group were assessed using a mixed effects logistic regression.

Results: Measles seroprevalence increased from 73% (CI: 69-76%) in 2009 to 94% (CI: 89-97%) in 2021. Seropositivity increased with age (OR=1.44yrs,95% CI=1.28-1.69) while GMC levels decreased with age. Over the years, MCV1 ineligible children showed low seroprevalence (8-52% across surveys), while 34-90% of MCV1 eligible kids were seropositive. Adult measles seroprevalence ranged between 96-99%. Following the MR campaign, there was a non-significant decrease of 10% in measles susceptibility among age-eligible children. Protective rubella antibodies in age-eligible children rose from 45% (CI: 35-52%) pre-campaign to 79% (CI: 75-84%) post-campaign. Both rubella seroprevalence and GMC levels significantly increased across ages ($P<0.05$), with adult seroprevalence high at 88-99%.

Conclusion: These findings provide crucial insights into the measles-rubella control program; although we find evidence of improved measles protection in recent years, there is still suboptimal immunity in MCV1 and 2 eligible children, indicating insufficient vaccine uptake. Immunity gaps in older children suggest heavy reliance on SIAs, while gaps in MCV1 ineligible children imply prolonged susceptibility, possibly due to the rapid decay of maternal antibodies. The introduction of rubella vaccination shows a positive immunity impact in children which will need to be maintained to prevent immunity gaps in the older groups.

2.4 Background

Seroepidemiology can be an effective tool to monitor population immunity to vaccine-preventable diseases (VPDs). Estimates of population immunity particularly age-specific immunity profiles are used to identify high-risk groups that may require targeted intervention, identify declining antibody levels in vaccine recipients, identify the impact of different vaccination schedules and estimate effective vaccination coverage in some settings [1-4]. Serosurveys are underexploited particularly in low- and middle-income countries (LMICs) for assessing the impact of vaccination programmes and for informing vaccine policy. Consequently, there is a significant data gap given the high burden of VPDs in LMICs [5].

The Kenya Expanded Programme on Immunisation (KEPI), which was formally introduced in 1980, initially consisted of a schedule that included one dose of a Measles-Containing Vaccine (MCV1) given at 9 months. [6] The first supplementary immunisation activity (SIA) was conducted in 2002 and targeted children aged 9 months to 14 years. Since then, SIAs have been conducted periodically every 3-4 years among under 5-year-olds or 15-year-olds for accelerated control of measles in the country. However, the country has continued to experience periodic measles outbreaks over the years including a large outbreak in 2006 due to a delay in SIA [7], one due to an influx of unvaccinated refugees from neighbouring countries between 2010-2011[8] and another due to postponement of the 2020 SIA[9]. Outbreaks in 2014, 2018 and 2019 were attributed to the accumulation of susceptible children caused by sub-optimal MCV1 coverage (consistently below the elimination target of 95%) and the low uptake of MCV2 of between 28% to 57% since its introduction [10]. Rubella surveillance in the country is poor, consequently, the burden of infection is not well documented and only a few outbreaks have been reported in the country including a 2014 outbreak in a rural area in Kenya [11]. The study showed that Rubella is endemic in the country and that outbreaks are often underestimated.

Although Kenya has made significant progress towards measles and rubella elimination, it is yet to meet the milestones for measles control set by the World Health Assembly (WHA)[12]. A second dose of MCV(MCV2) was introduced in 2013 to decrease Kenya's reliance on SIAs for measles control and is recommended routinely for children aged 18 months. [13] In 2016 in response to increasing rubella cases in Kenya, a combined Measles-Rubella (MR) campaign was

conducted in <15-year-olds and achieved an estimated coverage of 95% [14]. Thereafter the MR was introduced in the routine immunisation schedule to be given at 9 months and 18 months. [15]

Measles and rubella population immunity data for Kenyan children is limited. This study was conducted to track annual trends (2009 -2021) in age-specific population immunity with the aim of identifying susceptible populations among both children <15 years and adults to help target immunisation activities. In addition, we also assessed the impact of SIAs by exploring changes in population immunity before and after the campaigns.

2.5 Methods

2.5.1 Study Setting, study population and survey design

Kilifi County on the Indian Ocean coast of Kenya is home to the Kilifi Health & Demographic Surveillance System (KHDSS) that monitors births, deaths, in-migration and out-migration in a population of about 300,000 of the County's approximately 1.4 million residents [16].

The serological data from 2009 to 2019 originated from two surveys: the Malaria Cross-Sectional Survey(2009-2013)[17], which is in part longitudinal and the Pneumococcal Conjugate Vaccine Impact Study (PCVIS)(2015-2019)[18],which is primarily cross-sectional conducted as part of an ongoing effort to actively monitor malaria and pneumonia infections in children under 15 years of age within the KHDSS. Participants in 2021 were recruited from COVID-19 serosurveillance in Kenya conducted as part of the pandemic response[19].The malaria cross-sectional surveys annually sample a random group of healthy children from various locations in the KHDSS. Since 1998, children have been recruited annually and followed until age 15. Data from 2009, 2011, and 2013 comprised an age-stratified sample of 50 children in 10 age strata (aged 0, 1, 2, 3, 4, 5, 6, 7, 8-9, and 10-14 years) randomly selected from this population[20]. Data in 2015,2017 and 2019 was from PCVIs study collected on independent age-stratified random samples of the KHDSS in similar age strata with 50 children in each stratum. For the 2021 survey, 100 individuals were randomly selected in each 5-year age band from 0-14 years, 50 individuals in each 5-year age band from 15-64 years and 50 individuals aged ≥ 65 years bringing the total to 850 participants.

About 2mls of venous blood were collected from all consenting participants then separated, aliquoted, and stored at -70oC until processing.

2.5.2 Bead coupling and fluorescent bead multiplex immunoassay

IgG antibodies against measles and rubella were determined using a fluorescent bead-based multiplex immunoassay. Bead coupling and fluorescent bead multiplex immunoassay were carried out as previously described with minor modifications[21]. Beads were vortexed and incubated in the dark at constant rotation at room temperature for 2 hours and washed 3 times in phosphate buffered saline (PBS) tween (0.05%) and resuspended in PBS, 1%BSA and 0.05% (wt/vl) sodium azide (storage buffer). Beads were then stored at 4 degrees. Samples, reference standard (RUBI 1-94) and controls were diluted in PBS,0.1% tween and 3% BSA (assay buffer).2.5ul of serum/plasma samples were diluted 1/200 and 1/4000. Standard was 3-fold serially diluted in 11 wells starting at 1:50 and in-house controls 1/200. 25ul of beads at a concentration of 1000beads/well and 25 ul of diluted samples, standard, controls and blanks were added to plate and incubated at room temperature for 45minutes at speed of 600rpm. Plates were washed 3 times using PBS and 0.1% tween (wash buffer) using a magnetic separator. 25ul of secondary antibody (R-PE) at a dilution of 1/400 was added and plates incubated in the dark for 30min. Plates were washed 3 times and 100ul of PBS added ready for reading. Plates were read using Luminex Magpix platform using XPONENT software version 4.2 (Luminex Corp). Background was subtracted from Median Fluorescent Intensities (MFI). Antibody concentration (IU/ml) was interpolated from a 5-PL reference standard curve using Milliplex analyst software version 3.5.

Participants were considered protected when IgG concentrations were above or equal to 0.12 IU/ml for measles and 10.0 IU/ml for rubella.

2.5.3 Ethical considerations

Ethical approval was obtained from the Scientific and Ethics Review Unit (SERU) of the Kenya Medical Research Institute (Protocol SERU 3847). The serological samples were collected under SERU-approved protocols with a provision for storage of residual samples and use in future research (SERU 1433 4085 2887,3149,3426). Written informed consent was obtained from parents/legal guardians of all participants before sample collection. In addition, written assent was obtained from all participants aged 13-14 years old.

2.5.4 Statistical analysis

We first tabulated the seropositive results in each survey year based on the age strata at the time of the sample collection, sex, ethnic group and location in KHDSS.

To visualize the trends in population immunity in children overtime, we collapsed the initial age strata into 6 age groups for children under 15 yrs as follows; <9m (ineligible for vaccination),9m-<1yr (eligible for MCV1). This categorization was based on a prior study conducted in KHDSS, which revealed a delay of 2 months in the administration of MCV1[22].The subsequent age groups; were1-<2yr (eligible for MCV2),2-4yrs,5-9yrs and 10-14 yrs. For immunity in adults, we maintained the 5-year age bands used in the data collection for participants above 15 years in 2021.To account for potential bias, we implemented multilevel regression and post-stratification (MLRP) by fitting a Bayesian logistic regression model that incorporated age as a variable to adjust the estimates for the underlying population using mid-year population estimates in KHDSS, sensitivity and specificity of the assay [23]. The prior distributions for the sensitivity and specificity estimates were derived from the original study, which reported values of 96.5% for sensitivity and 95% for specificity for measles, and 99% for sensitivity and 95% for specificity for rubella during the development of the multiplex assay[21]. The models were fitted using rjags software package. Age-specific seroprevalence estimates were visualized using bar graphs and differences between groups in each year determined using chi-square test and fishers exact test where appropriate . We employed a mixed effects logistic regression to examine the association between seropositivity and several demographic factors including year, sex, age, location, and ethnic group. The selection of these variables was driven by specific hypotheses regarding their potential influence on seroprevalence. For instance, we hypothesized that year might influence seroprevalence due to changes in vaccination coverage over time. Additionally, considering the biological differences between sexes that could affect immune response and susceptibility to infections, we included sex as a variable. Moreover, we anticipated that geographic variations in disease prevalence, access to healthcare, and environmental factors could impact seropositivity, thus necessitating the inclusion of location. Lastly, socio-cultural factors and differential healthcare access among ethnic groups were thought to potentially influence seropositivity rates, hence the inclusion of ethnic group as a variable.

Geometric mean concentrations(GMCs) and the 95% confidence levels were also calculated, and values tabulated after adjusting for underlying population. There were no negative IU/mL values

observed for either measles or rubella; however, some outliers were identified after visualizing the raw antibody titers using a boxplot(IgG=0.0001 IU/ml). These outliers were excluded from further analysis to enhance the accuracy of the estimates. To display the variability in age-specific GMCs, raw antibody titres were log transformed and displayed via boxplot for each survey year. One-way ANOVA was used to test differences in GMCs between groups in each year.

To assess the impact of the MR campaign carried out in May 2016, children aged between 9months to 14years at the time of the campaign, i.e. those born between mid-May 2002 and mid-August 2015 were identified. We stratified this group into pre-campaign population i.e. children whose data was collected in the 2015 survey and post-campaign population i.e. children whose data was collected in the 2017 survey. Seroprevalence estimates and GMCs were calculated in a similar way and statistical differences pre and post campaign assessed using chi-square test and two independent variable t-test. All statistical analyses were conducted using the R-statistical software.

Geometric mean concentrations(GMCs) and the 95% confidence levels were also calculated, and values were tabulated after adjusting for the underlying population. To display the variability in age-specific GMCs, raw antibody titres were log-transformed and displayed via boxplot for each survey year. One-way ANOVA was used to test differences in GMCs between groups in each year.

To assess the impact of the MR campaign carried out in May 2016, children aged between 9 months to 14years at the time of the campaign, i.e. those born between mid-May 2002 and mid-August 2015 were identified. We stratified this group into pre-campaign population i.e. children whose data was collected in the 2015 survey and post-campaign population i.e. children whose data was collected in the 2017 survey. Seroprevalence estimates and GMCs were calculated in a similar way and statistical differences pre and post-campaign assessed using a chi-square test and two independent variable t-tests. All statistical analyses were conducted using the R-statistical software.

2.6 Results

2.6.1 Characteristics of the study population

In total, there were 2,686 participants with females a slight minority. The median age was 6 years (IQR: 3-8 years). The least number of study participants was 290 recorded in year 2021 while the

highest number of participants was 520 recorded in 2019. The majority of participants were from North and South locations in Kilifi and the Chonyi and Giriama ethnic groups (Table 2:1).

2.6.2 Trends in Measles population immunity in children

The overall proportion of children with protective measles antibodies in Kilifi varied between 73% CI: (69-76%) in 2009 to 94% CI: (89-97%) in 2021 (Table 2:2 and Fig. 2:2). In all the surveys, there was significant heterogeneity in seroprevalence across the ages ($P<0.05$). The immunity trend was similar in the different survey years whereby the MCV1 ineligible children had the lowest prevalence ranging between 8% CI: (01-25%) in 2019 to 52% CI: (11-87%) in 2021. This was followed by a spike in the prevalence in the MCV1 eligible children ranging between 34% CI: (09-65%) in 2017 to 90% CI: (50-100%) in 2011. This increase across the ages continued in the MCV2 eligible children and children in 2-4- and 5-9-year age groups with all the years having a seroprevalence greater than 92% in these ages. In some of the years, seroprevalence slightly declined in the oldest age groups with the lowest value of 87% CI: (71-99%) recorded in 2009.

Similarly, GMC levels varied significantly across the ages ($P <0.05$) in all the surveys and decreased consistently with increasing age (Table s2:1 and Fig. 2:2). Significant waning of maternally-acquired antibodies was evident as seen by the low level of antibody concentrations in the MCV1 ineligible children while waning of vaccine induced immunity was reflected by the decline in antibody levels across the ages with the oldest age group in some of the survey years almost falling below the cut-off threshold (Fig. 2:2). There was a high variability in the GMCs of MCV1 eligible children across the different years. Changes in immunity following the three SIAs in our study period were evident given the increase in GMCs in the surveys conducted after the SIAs i.e. 2011,2013 and 2017 in the target age groups. There was no evidence of an increase in GMCs in the survey years before and after the introduction of MCV2.

In the mixed effects logistic regression, a year increase in age was significantly associated with higher measles seropositivity (OR=1.44,95% CI=1.28-1.69) while significantly higher measles seropositivity was found in children from Jibana ethnic group compared to Chonyi (OR=2.85,95% CI=1.62-15.98). Measles seroprevalence showed no significant variation based on gender, different locations compared to South KHDSS, or survey years compared to the baseline year 2009 (Table 2:3).

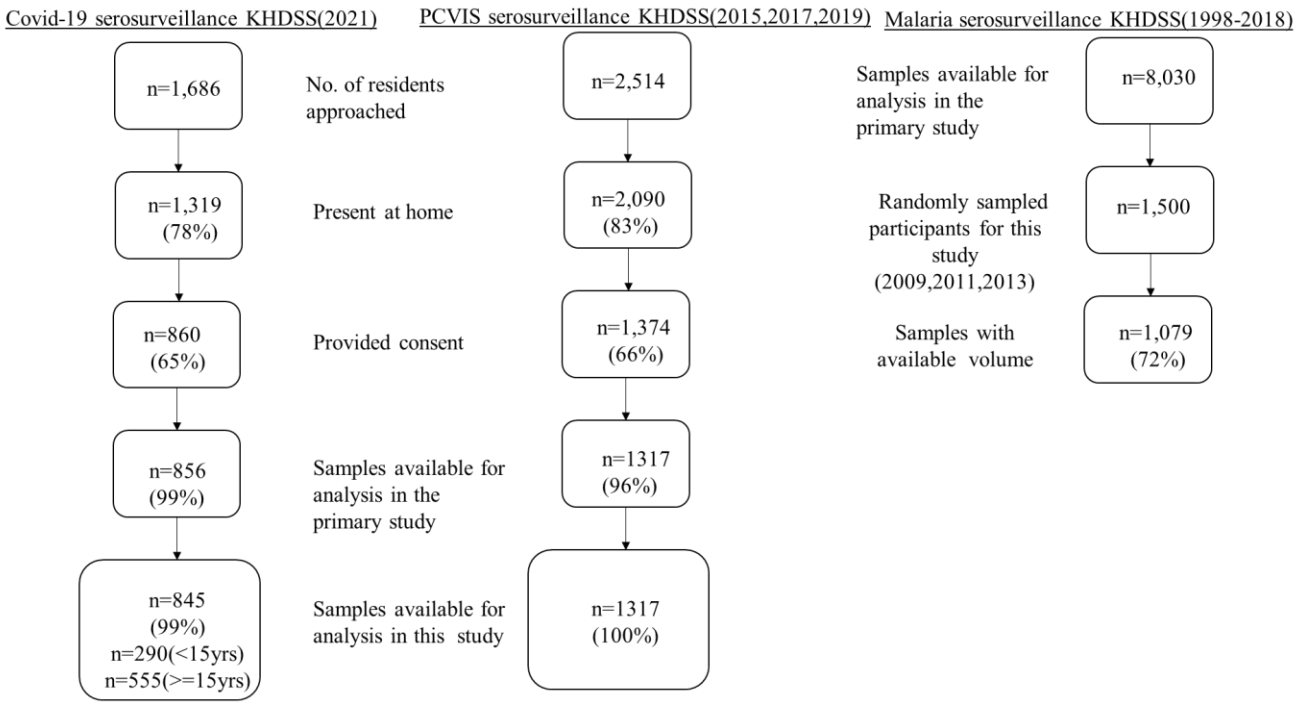

Figure 2:1 Flow diagrams illustrating the recruitment and participation processes from the three serosurveillance studies utilized in our dataset. Data from 2009, 2011, and 2013 were randomly sampled from the Malaria-cross-sectional survey population using the sampling strategy of the PCVIS study.

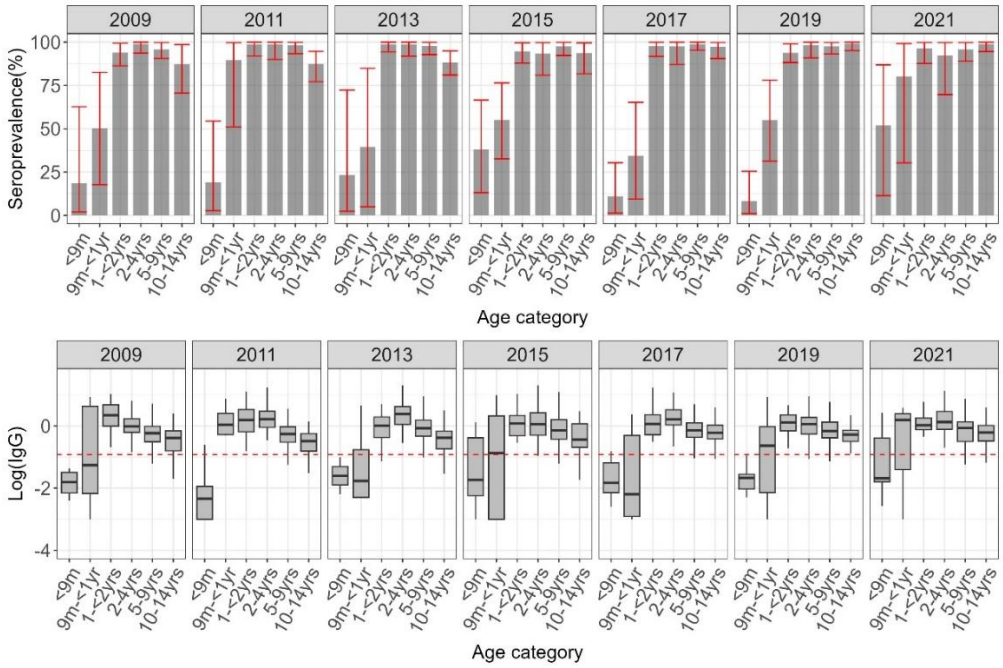

Figure 2:2 Measles seroprevalence estimates were obtained using Bayesian modelling adjusted for test performance and underlying age structure using multilevel regression and poststratification. The red error bars indicate 95% credible intervals while the red line in the below figure is the protective threshold for measles of 0.12 IU/ml

Table 2:2 Measles (M) and Rubella (R) seropositivity and total number of participants per survey year stratified by age strata during sample collection, sex, location in KHDSS and ethnic group

Survey year	**2009(5th June-19th Sep)**			**2011(25th Jun-4th Nov)**			**2013(29th Jun-10th Nov)**			**2015(1st Jul-31st Oct)**			**2017(28th Jun-8th Nov)**			**2019(29th Jun-7th Jul 2019)**				**2021(1st Jan-31st May)**		
	n	M(%)	R(%)	n	M(%)	R(%)	n	M(%)	R(%)	n	M(%)	R(%)	n	M(%)	R(%)	N	M(%)	R(%)		n	M(%)	R(%)
Age in years																						
<1	14	42	5	14	50	5	7	32	8	35	49	13	27	21	19	39	29	30	0-4	93	93	88
1	31	95	7	19	97	21	45	98	2	34	96	23	39	97	62	48	99	96	5_9	102	98	91
2	30	97	8	22	99	6	22	99	4	34	99	14	38	99	75	49	95	91	10_14	95	99	97
3	34	99	17	19	99	21	29	99	13	38	98	31	47	99	82	54	97	92				
4	38	99	21	22	99	14	27	99	8	38	97	39	36	99	87	54	99	90				
5	44	97	32	18	99	33	25	99	12	39	99	50	49	99	89	50	96	73				
6	58	99	40	23	99	48	31	99	13	29	99	49	49	99	84	54	99	82				
7	38	98	53	34	99	57	26	98	26	40	99	50	44	99	94	55	99	90				
8_9	62	95	52	67	99	71	66	99	52	45	97	58	43	99	95	61	99	94				
10_14	16	93	76	70	87	73	128	88	69	44	95	69	49	99	96	56	99	95				
Sex																						
Female	179	95	32	151	96	50	195	99	33	172	93	40	212	96	83	262	94	86		132	99	95
Male	186	94	32	157	94	44	211	93	36	204	92	41	209	95	79	258	93	84		158	95	89
Location																						
North	83	95	15	2	99	0	90	97	32	150	91	31	158	96	84	157	93	82		2	100	99
South	206	98	37	251	95	46	230	96	40	108	94	44	130	91	81	187	93	90		2	51	50
Township	5	84	19	1	99	99	4	78	24	111	91	49	119	97	77	172	96	82		0	0	0
Unspecified	71	86	41	54	93	52	82	95	21	7	99	72	14	99	86	4	51	99		286	97	92
Ethnic group																						
Chonyi	129	93	35	144	93	42	138	90	34	127	98	43	135	92	80	184	95	89		2	51	50
Giriama	105	98	19	34	96	44	112	99	36	173	87	38	195	95	81	222	94	84		2	100	99
Jibana	48	99	44	57	99	56	58	96	45	5	99	40	7	90	86	9	81	78		0	0	0
Kauma	7	99	0	12	96	59	12	99	50	48	92	35	44	99	82	72	95	78		0	0	0
Others	76	85	41	61	93	49	86	95	23	23	96	61	40	97	83	33	82	82		286	97	92
Total	365	73	40	308	80	46	406	90	33	376	91	47	421	92	82	520	92	86		290	94	90

2.6.3 Trends in Measles population immunity in adults

Measles seroprevalence in adults was high ranging between 96-100% and comparable across the different ages (P=0.41) (Table s2:2 and Fig. 2:3). Contrary to the waning of antibodies seen in the younger age groups, GMCs in adults were high and increased across the ages indicating a relatively high exposure of natural infection in adults compared to late childhood.

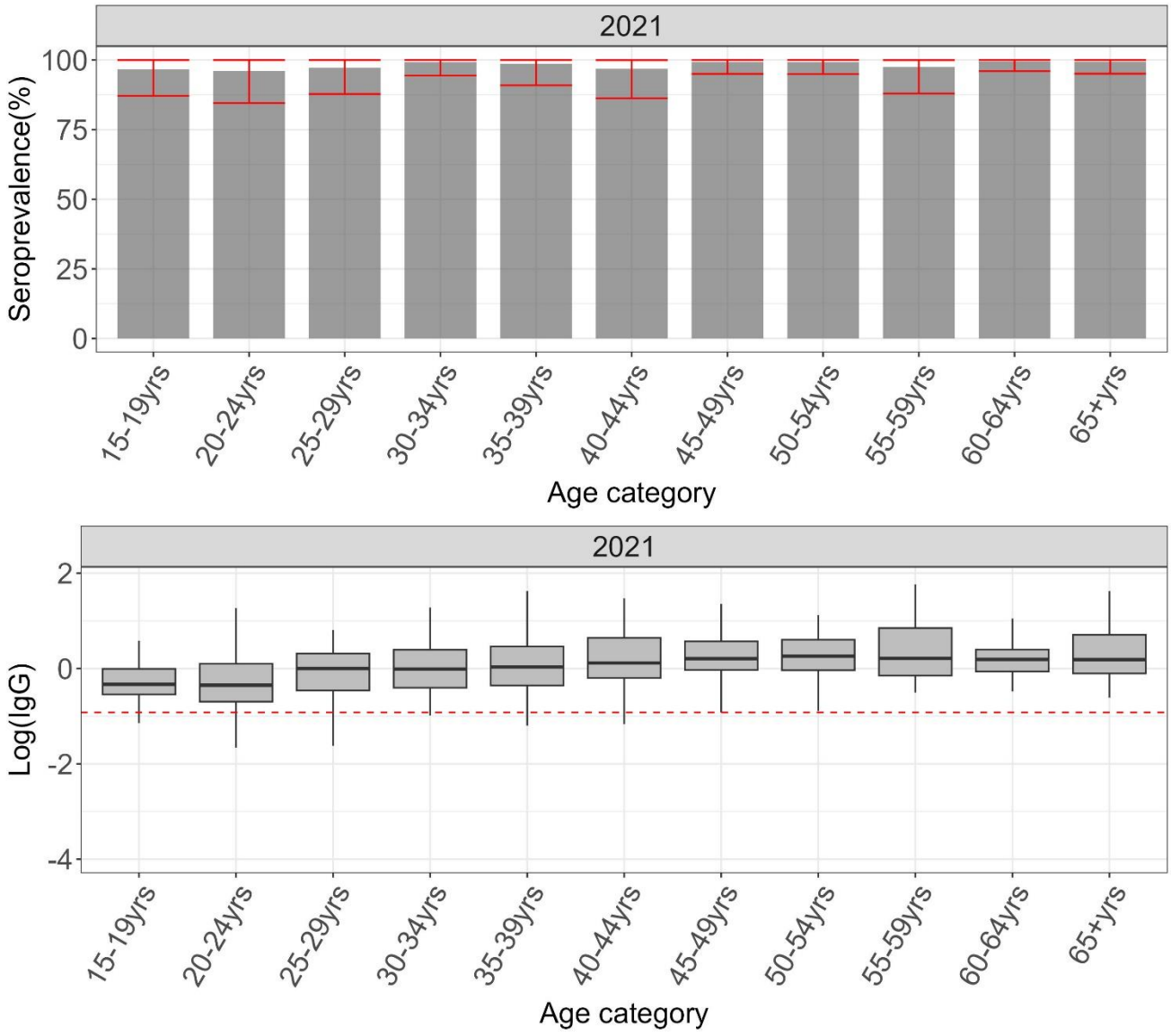

Figure 2:3 Measles seroprevalence and Geometric Mean Concentrations(GMCs) in adults in KHDSS in 2021. The red error bars indicate 95% credible intervals while the red line in the below figure is the protective threshold for Rubella of 0.12 IU/ml

2.6.4 Trends in Rubella population immunity in children

The overall proportion of children with protective rubella antibodies in Kilifi was low in the years before vaccine introduction ranging from 40% CI: (33-47%) in 2009 to 47% CI: (40-43%) in 2015. Seroprevalence significantly increased after the MR of a campaign of 2016 and subsequent introduction of rubella vaccine in the program. Overall seroprevalence ranged between 82% CI: (79-86%) in 2017 to 90% CI: (85-94%) in 2021(Table 2:2 and Fig. 2:4). Seroprevalence varied significantly across the ages in all the survey years (P<0.05).

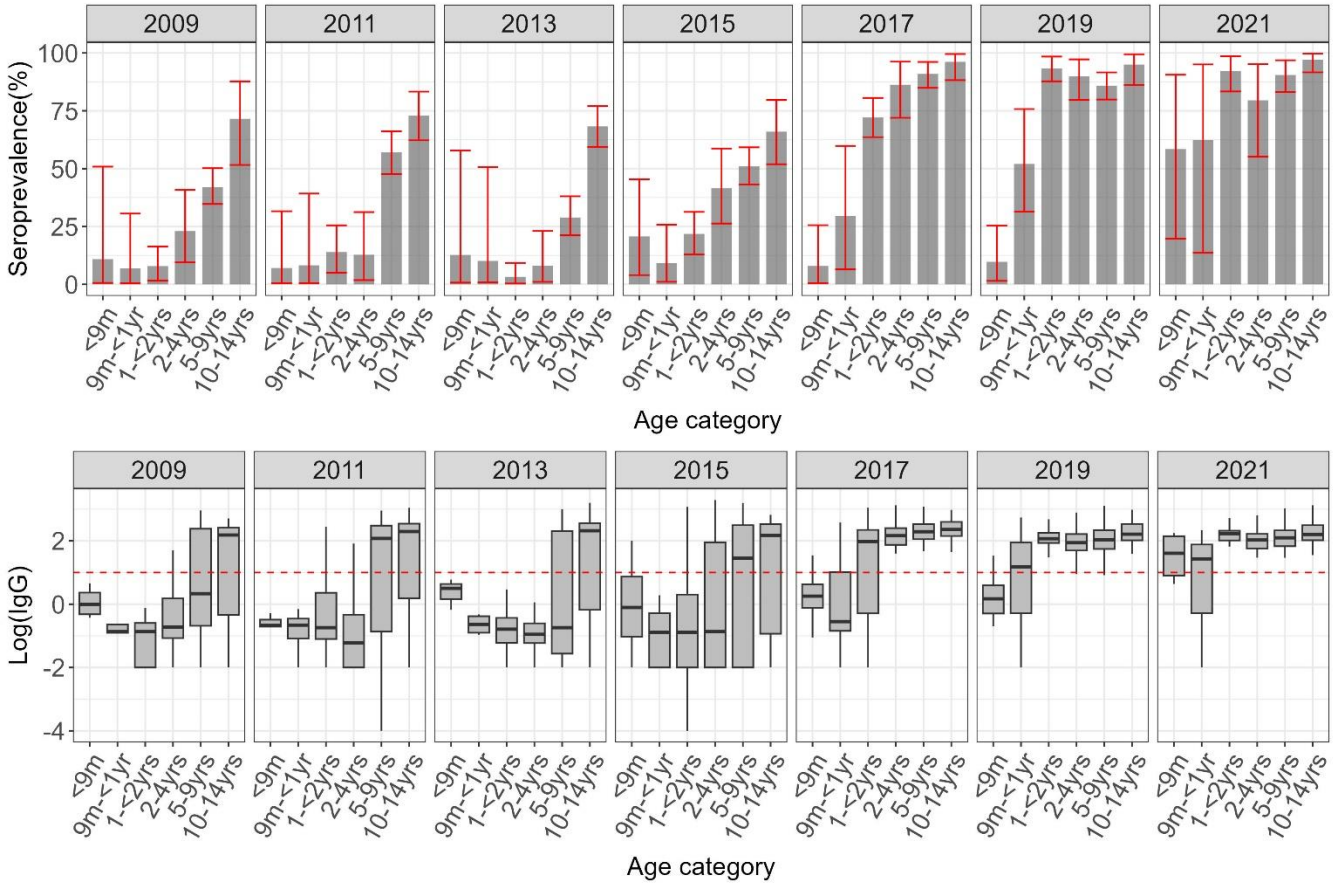

Figure 2:4 Rubella seroprevalence estimates for test performance and underlying age structure using multilevel regression and poststratification. The red error bars indicate 95% credible intervals while the red line in the below figure is the protective threshold for Rubella of 10 IU/ml.

The immunity trend was similar in the survey years before vaccine introduction. Seroprevalence in the MR1 ineligible group ranged between 08% CI: (01-32%) in 2011 to 12% CI: (0-50%) in 2009. This declined slightly in the MR1 eligible group to a range of 04% CI: (01-52%) in 2013 to

09% CI: (0-25%) in 2009 possibly due to waning of maternal immunity and lack of adequate natural exposure. From the second year of life, seroprevalence increased consistently in the older age groups.

In the years after vaccine introduction, rubella seroprevalence increased significantly with increasing age. Immunity in MR1 ineligible children had the least prevalence ranging between 9% CI: (01-21%) in 2017 to 58% CI: (18-88%) in 2021.Seroprevalence increased in the MR1 eligible children and varied between 30% CI: (08-61%) in 2017 to 61% CI: (16-96%) in 2021 and similarly in MR2 eligible children ranging between 72% CI: (62-81%) in 2017 to 92% CI: (83-99%) in 2021.This increase continued in the older age groups with all the survey years having a seroprevalence greater than 80% in the older age groups(Table 2:2).

GMC levels also increased significantly across the ages in all the survey years ($P < 0.05$), an indication of high levels of natural exposure (Table s2:1, Fig.2:4).

In the mixed effects logistic regression, a year increase in age was significantly associated with higher rubella seropositivity (OR=3.08,95% CI=2.22-4.71). Rubella seroprevalence showed no variation based on gender, different locations compared to South KHDSS, or the different ethnic groups compared to the baseline. Seroprevalence in years 2015,2017,2019 and 2021 was significantly higher compared to 2009(Table 2:3).

2.6.5 Trends in Rubella population immunity in adults

Rubella seroprevalence in adults was high ranging between 88-99% and comparable across the different ages (p=0.91) (Table s2.2 and Fig. 2:5). There was a slight decline in GMCs levels in some of the older age-groups likely due lack of childhood vaccination in these older age groups. GMCs in older females did not differ from that in the older males.

Table 2:3 Changes in population immunity overtime for Measles and Rubella. Seroprevalence estimates were adjusted for test performance and underlying population structure using Bayesian modelling.

Survey year	**2009**			**2011**			**2013**			**2015**			**2017**			**2019**			**2021**		
Measles	n	% [95% CI]		n	% (95% CI)		n	% (95% CI)		n	% (95% CI)		n	% (95% CI)		n	% (95% CI)		n	% (95% CI)	
Age in years																					
<9m	04	19	[02-63]	08	19	[03-54]	03	23	[02-72]	14	38	[13-67]	15	11	[01-30]	20	8	[01-25]	05	52	[11-87]
9m-<1yr	09	50	[18-83]	06	90	[51-100]	04	40	[05-85]	21	55	[33-76]	10	34	[09-65]	19	55	[31-78]	04	80	[30-99]
1-<2yrs	94	94	[86-99]	59	99	[92-100]	95	99	[94-100]	104	95	[88-99]	124	98	[92-100]	150	94	[88-99]	69	96	[88-100]
2-4yrs	37	99	[94-100]	22	99	[90-100]	25	99	[92-100]	38	93	[81-99]	36	98	[87-100]	54	98	[91-100]	15	92	[70-100]
5-9yrs	197	96	[91-100]	137	98	[93-100]	143	98	[93-100]	152	98	[92-100]	182	99	[95-100]	221	98	[93-100]	102	96	[89-100]
10-14yrs	24	87	[71-99]	76	88	[77-95]	136	88	[81-95]	47	94	[82-100]	54	97	[90-100]	56	99	[95-100]	95	99	[95-100]
Total	365	73	[69-76]	308	80	[77-82]	406	90	[87-94]	376	91	[87-95]	421	92	[89-94]	520	92	[89-94]	290	94	[89-97]
P_value		<0.001			<0.001			<0.001			<0.001			<0.001			<0.001			0.02	
Rubella																					
<9m	04	12	[01-50]	08	08	[0-32]	03	14	[1-61]	14	22	[04-45]	15	9	[01-29]	20	11	[01-27]	5	58	[18-88]
9m-<1yr	09	07	[01-30]	06	09	[01-40]	04	12	[01-52]	21	09	[02-25]	10	30	[08-61]	19	52	[29-76]	4	61	[16-96]
1-<2yrs	94	08	[2-16]	59	14	[5-26]	95	3	[00-09]	104	22	[13-31]	124	72	[64-81]	150	93	[88-98]	69	92	[83-99]
2-4yrs	37	23	[10-41]	22	13	[2-31]	25	8	[01-23]	38	42	[26-59]	36	86	[72-96]	54	90	[80-97]	15	80	[55-95]
5-9yrs	197	42	[34-50]	137	57	[48-65]	143	29	[21-37]	152	51	[42-59]	182	91	[85-96]	221	85	[79-91]	102	90	[83-96]
10-14yrs	24	71	[51-87]	76	73	[61-82]	136	68	[60-76]	47	66	[52-79]	54	96	[88-99]	56	95	[86-99]	95	97	[92-100]
Total	365	40	[33-47]	308	46	[41-51]	406	33	[28-38]	376	47	[40-53]	421	82	[79-86]	520	86	[82-89]	290	90	[85-94]
P_value		<0.001			<0.001			<0.001			<0.001			<0.001			<0.001			0.01	

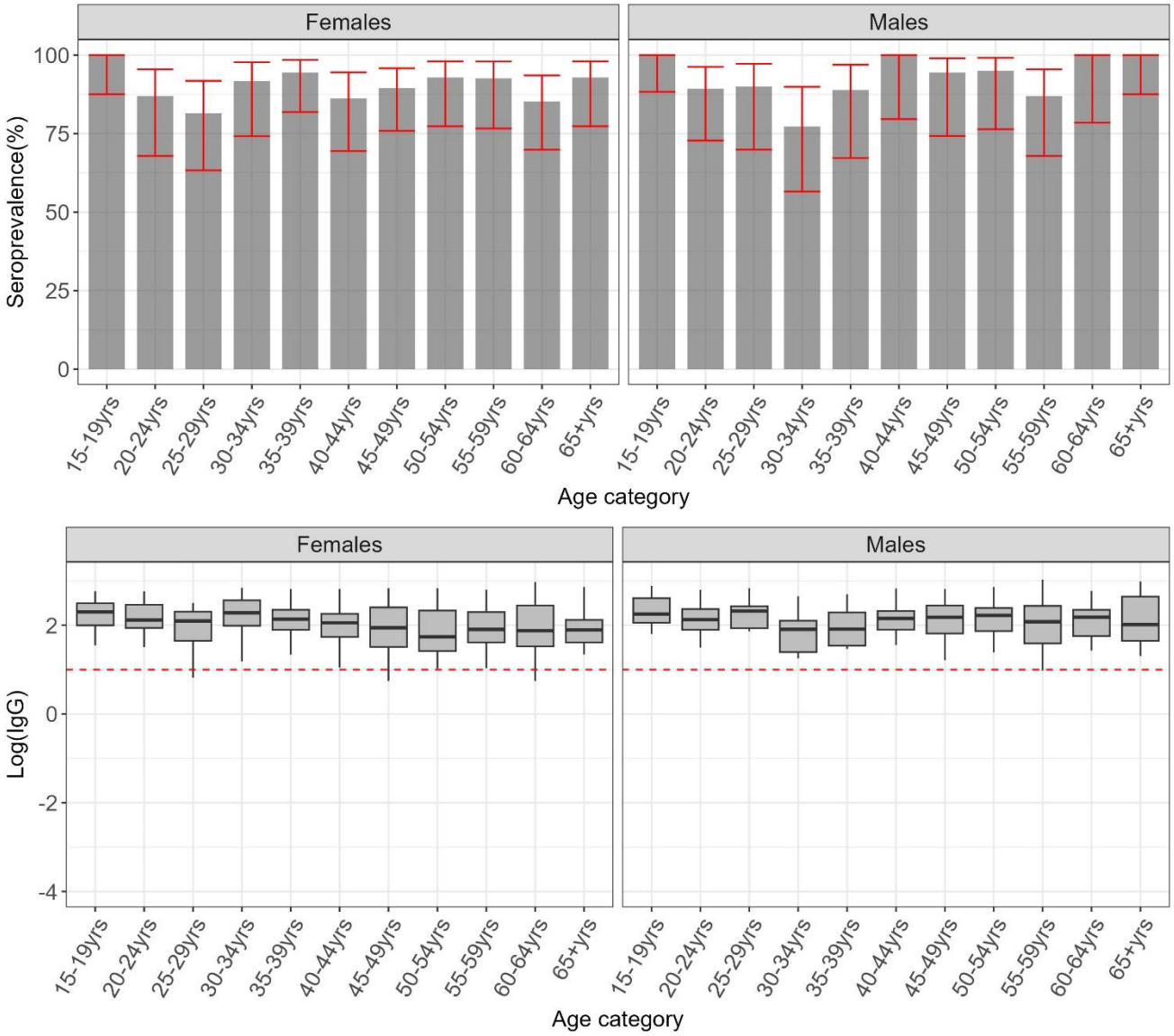

Figure 2:5 Rubella seroprevalence and Geometric Mean Concentrations(GMCs) in adults in KHDSS in 2021. The red error bars indicate 95% credible intervals while the red line in the below figure is the protective threshold for Rubella of 10 IU/ml

2.6.6 Impact of MR campaign on Measles and Rubella

The pre-campaign population had a total of 365 children compared to 370 children in the post campaign group (Table s2:3). Measles seroprevalence increased from 90% (95% CI; 85-92%) in the pre campaign period to 91% (95% CI; 87-95%) in the post campaign period translating to a reduction in measles susceptibility of 10%. Age-specific seroprevalence increased from 93% to

97% in the 1-<2year-olds, 95% to 98% in the 1-4-year olds ,97% to 98% in the 5-9year olds and 94% to 97% in the 10-14year olds. This age-specific increase in seroprevalence was not significant(P>0.05). Overall GMC levels increased significantly from 0.7 IU/ml (95% CI; 0.4-1.3 IU/ml) to 0.9 IU/ml (95% CI; 0.5-0.1.5 IU/ml) after the campaign(p<0.05). (Table s2:3 and Fig 2:6) while age-specific GMCs also increased significantly for all the age-groups other than the 5-9year age group.

Table 2:4 Disease-specific logistic regression results

	Measles	**Rubella**
Predictors	OR (95% CI)	OR (95% CI)
Gender		
Male	1	1
Female	1.49[0.77-2.97]	1.40[0.54-3.85]
Age	1.44[1.28-1.69]	3.08[2.22-4.71]
Survey year		
2009	1	1
2011	0.45[0.16-1.20]	1.13[0.79-1.61]
2013	0.26[0.09-0.69]	0.60[0.42-0.86]
2015	0.30[0.08-0.95]	1.91[1.33-2.77]
2017	0.59[0.19-1.92]	15.76[10.78-23.56]
2019	0.43[0.14-1.32]	21.42[14.74-31.47]
2021	0.15[0.01-2.63]	26.14[15.24-45.90]
Ethnic group		
Chonyi	1	1
Jibana	2.85[1.62-15.98]	3.25[0.40-28.46]
Giriama	0.85[0.31-2.18]	0.89[0.19-3.97]
Kauma	1.92[0.44-9.39]	0.39[0.04-2.83]
Other	0.67[0.12-3.68]	3.29[0.24-59.14]
Location		
South	1	1
North	1.34[0.49-3.89]	0.13[0.02-0.64]
Township	1.55[0.51-5.50]	0.32[0.05-1.79]
Unspecified	8.47[0.41-23.76]	6.25[0.07-7.70]

Overall rubella seroprevalence in the target age-group of children aged 9months to 14years was estimated to be 45% CI: (35-52%) pre-campaign and 79% CI: (75-84%) post campaign which translates to a significant reduction in susceptibility prevalence of 62% (P<0.05). GMCs increased

significantly from 4.8 (95%CI:0.5-58.8) to 108.6 (95%CI:53.9-264.5). Age-specific seroprevalence significantly increased from 22% to 45% in the 1-<2year-olds, 27% to 79% in the 1-4-year olds ,49% to 90% in the 5-9year olds and 62% to 94% in the 10-14year olds (P<0.05) (Fig. 2:6).

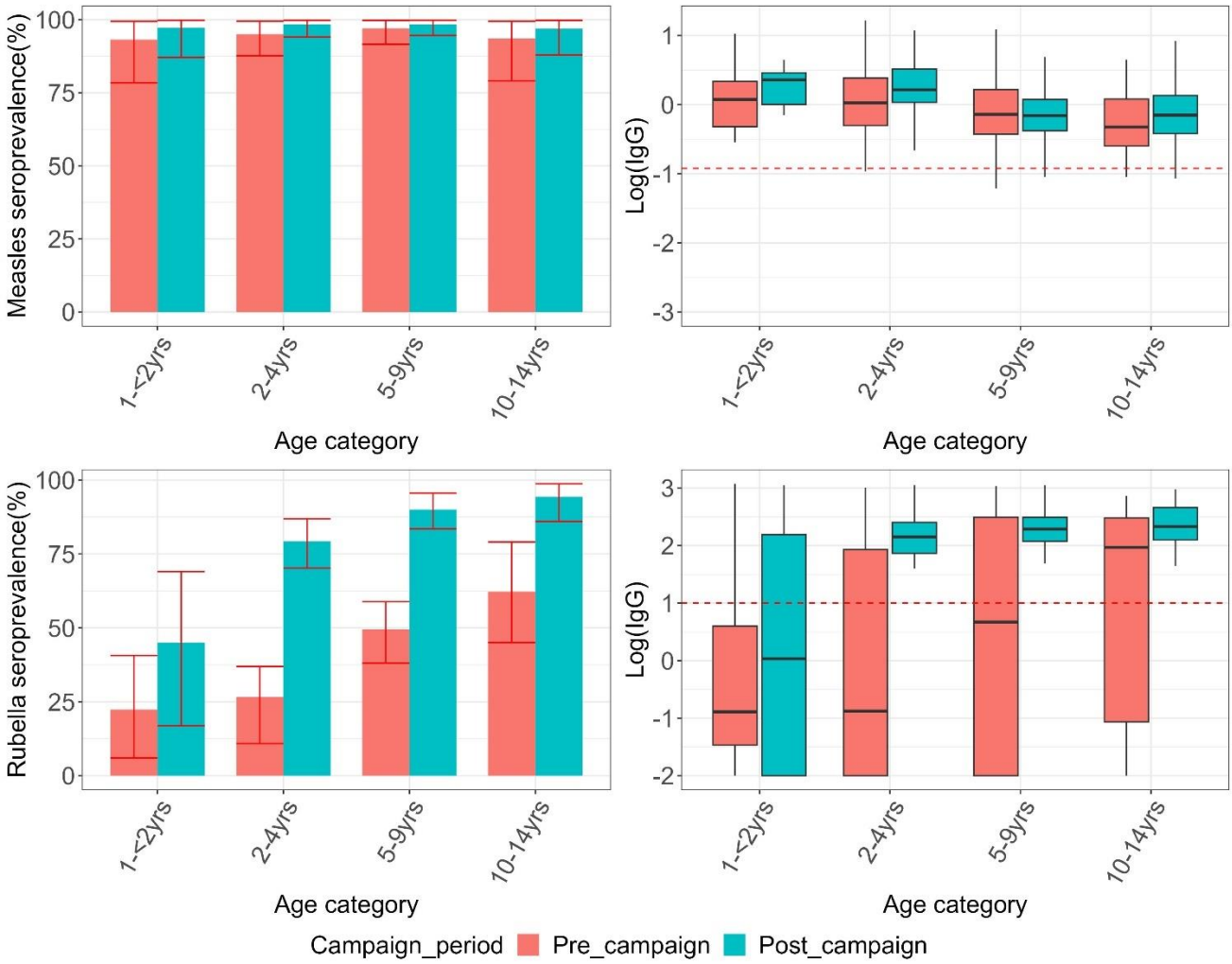

Figure 2:6 Age-specific Measles and Rubella seroprevalence and Geometric Mean Concentrations before and after MR campaign of 2016.The red bars represent the seroprevalence in pre-campaign period while the green bars represent seroprevalence in the post-campaign in the age-eligible children.

2.7 Discussion

2.7.1 Main results

Between 2009 and 2021, the estimated measles seroprevalence among children aged 0-14 years ranged between 73% to 94%. In most of the years, annual seroprevalence was lower than the herd immunity threshold for measles of 93%(82-94)[24] indicating insufficient protection levels in children against outbreaks likely due to suboptimal MCV1 and MCV2 uptake. The program is still highly relying on SIAs for immunity as shown by significant waning of IgG antibodies with age, leading to immunity gaps in older children in absence of boosting. MCV1 ineligible children had a seroprevalence lower than 50% in most of the years suggesting an extended period of susceptibility in young infants probably as a consequence of rapid decay of maternally acquired antibody. On the contrary, there was a high measles seroprevalence observed in the adult population likely due to natural infection and cumulative exposure to the virus over the years within these older age groups. For rubella, the results demonstrated the success of the vaccination program, with seroprevalence increasing from 45% to 79% in the MR campaign target group. Presently, rubella seroprevalence in children ranges between 82-90% across the survey years following vaccine introduction, aligning with the rubella herd immunity threshold of 83-86[25].This signifies adequate protection against outbreaks. In 2021, rubella seroprevalence among adults was 92%.Sustaining robust coverage will be crucial to prevent immunity gaps in women of reproductive age and CRS in infants.

2.7.2 Measles

Although Kenya has implemented a two-dose measles vaccination program and conducted regular SIAs, evidence from our study shows that herd immunity has not been achieved among children under 15 years of age, implying that efforts need to be further increased to achieve elimination goals.

The consistently low seropositivity and low antibody titres in the MCV1 ineligible group across the years ranging between 08-52% points to a rapid decay of maternally acquired antibody and increased risk of susceptibility in these age-groups. Although this phenomenon has been reported elsewhere[26, 27],it is shown to be common in areas where maternal immunity is from

immunisation rather than natural infection[28]. The recommended age for MCV1 receipt is 9 months in high transmission settings[29]. Delaying infant vaccination aims to reduce interference from maternal antibodies, allowing optimal vaccine efficacy. However, the rate of maternal antibody decline varies, posing challenges in predicting the ideal vaccination timing. Early vaccination has been suggested in high-risk settings, although this is an ongoing discussion as moderate evidence indicates potential negative impacts on seroconversion to subsequent measles vaccine doses[30].

There was a high variability in the seropositivity ranging between 40-90% and antibody titres in the MCV1 eligible group across the years likely due to the differences in MCV1 uptake. Seroprevalence across the different years in this age group was also much lower than the reported administrative coverage of MCV1 in the same period which ranged between 75-85%[31]. These discrepancies could be caused by several factors. One, receiving a vaccine does not always result in immunisation against the targeted disease. Depending on the vaccine and the number of doses a minority of individuals may not develop an immune response post-vaccination. For example, following measles vaccination, the proportions of children who develop protective antibody levels are approximately 85% at 9 months of age and 95% at 12 months of age[32]. Two, administrative coverage estimates are prone to inaccuracies including errors in recording vaccine doses and incorrect assumptions about the target population's size[33]. Three, these discrepancies could also suggest poor timeliness of MCV1 vaccination which was previously reported across 6 different birth-cohorts in KHDSS area[22] . The findings are consistent with those of several other studies that show the challenges of relying solely on vaccination coverage estimates as one might overlook these pockets of susceptibility which lead to outbreaks[1, 34].

There was no significant change in the seroprevalence in the MCV2 eligible group after the introduction of the second dose although the antibody titres increased slightly in the last two years in our study period. This could be due to the low uptake of the second dose (range of 28-52%) since its introduction[31]. This low uptake which has been reported in other areas in Africa is influenced by factors such as knowledge, perceptions, and attitudes at individual and community levels[35]. The dose timing, determined programmatically, does not align with the schedule of other RI vaccines[29]. Our findings emphasize the need for efforts to enhance awareness and improve uptake of MCV2.

Although seroprevalence was associated with an increase in age across the years, GMC levels decreased across the ages with those in the10-14yr age groups falling to the threshold level in some years as a result of waning of vaccine-induced antibodies in absence of sufficient natural exposure and boosting. This suggests a need to boost immunity in these children which is commonly done through SIAs. However, given WHO's concerns about SIA sustainability in LMICs[29, 36],efforts should also be made to improve the coverage of both MCV1 and 2. Conducting a comprehensive analysis of trade-offs in the different intervention programs will be essential to identify optimal approaches for reducing reliance on these frequent and expensive SIAs.

Overall measles seroprevalence increased from 90-91% after the MR campaign of 2016 which translates to reduction of 10% in measles susceptibility. The high seroprevalence prior to the campaign is likely due to the fact that children in the campaign target group would have benefited from the previous SIAs in 2009 and 2012[13]. Our findings suggest that the 2016 SIA was not very successful at reaching previously unvaccinated children given the small reduction in susceptibility after the campaign. This diminished impact of SIAs in LMICs due to the inability to reach zero dose children has previously been reported[37].A previous analysis looking at the impact of the 2002 measles vaccine campaign estimated an overall reduction in the susceptible population of about 65%[38]. Some of the reasons for this huge difference with our estimates could be due to the fact that the pre-campaign population in this study had relatively low immunity compared to our population. The pre and post campaign surveys in the study were conducted one month apart whereas our pre and post campaign data was collected about 2 years apart. Our findings indicate that efforts should be made to strengthen the efficiency of future SIAs so as to reach children who are missed by routine vaccinations.

2.7.3 Rubella

Our findings depict a huge impact of the MR campaign and introduction of rubella vaccine in the program. Overall seroprevalence in children increased significantly from 45% to 79% post campaign. Both the age-specific seroprevalence estimates and GMCs also increased significantly after the campaign. The findings of the impact of MR campaign were also comparable to a similar study conducted in Zambia where serosurveys conducted before and after introduction of rubella vaccine demonstrated significant increase in rubella seroprevalence from 51.3% (95% CI; 45.6-57.0%) to 98.3% (95% CI; 95.5-99.4%) in the campaign target group[39].

Age was positively associated with rubella seroprevalence even before the introduction of the vaccine. This increase of rubella seroprevalence with age which has previously been reported in other studies has been attributed to natural infection and cumulative exposure of the virus over the years[40]. The post-campaign seroprevalence in children indicates sufficient immunity levels to prevent outbreaks. However, maintaining high coverage is vital for sustained protection in adults. The vaccine introduction alters immunity dynamics, potentially reducing the circulating wild-type virus and boosting of immunity in older age groups. This is critical for rubella, as infection in women of reproductive age may lead to congenital rubella syndrome in infants which is fatal[41]. Strengthening coverage is essential to mitigate this risk and ensure comprehensive protection across all age groups.

2.7.4 Strengths

This extensive seroprevalence data utilized a random sampling strategy to ensure its representativeness within children in the KHDSS area. The dataset boasted a substantial number of participants which ensured robust statistical power. The data was also evaluated using serum specimens, which, although more invasive than oral fluid samples[42] and dried blood spots[43], have demonstrated higher sensitivity[44] . Serum samples were simultaneously tested using a highly sensitive and specific fluorescent bead-based multiplex immunoassay[21] which has been shown to be the future of sero-diagnostics for surveillance and epidemiology[45]. Additionally, the dataset was derived from a series of cross-sectional surveys conducted over different years, providing a temporal perspective on the evolving seroprevalence of measles and rubella across different age groups within the population. Overall, this dataset will significantly contribute to the understanding of immunity levels for measles and rubella in Kenya, serving as a valuable case study for LMICs

2.7.5 Limitations

The main limitation of this study lies in the generalisability of these findings to diverse settings. The study was carried out in a rural area within the African region, where most VPDs including measles and rubella are endemic. Although these results are largely representative of rural areas in measles and rubella endemic settings with similar vaccination schedules and coverage, the estimates may exhibit significant variation across rural and urban settings primarily due to

differences in vaccination coverage, levels of natural exposure, and distinct mixing patterns across various age groups.

2.7.6 Conclusions

These findings provide crucial insights into the measles-rubella control program. Suboptimal immunity in MCV1 and 2 eligible children indicates insufficient vaccine uptake while immunity gaps in older children suggest high waning rates of measles immunity in the absence of boosting. Efforts should be made to improve both the timing and the coverage of the 1st and 2nd doses and regular SIAs targeting these age groups should be implemented. The implementation of rubella vaccination has demonstrated a positive impact on immunity in children. It is crucial to sustain this immunity to prevent potential gaps in older age groups, as such gaps could lead to an elevated risk of CRS in infants.

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3. Chapter 3: Modelling the Relative Contribution of Infection, Routine Vaccination and Supplementary Immunisation Activities to Measles Seroconversion in Kenyan Children

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3.1 Research paper cover sheet

RESEARCH PAPER COVER SHEET

Please note that a cover sheet must be completed for each research paper included within a thesis.

SECTION A – Student Details

Student ID Number	2003951	**Title**	Mrs.
First Name(s)	Caroline		
Surname/Family Name	Mburu		
Thesis Title	The utility of mathematical modelling of serological data in assessing the impact of vaccination programmes in Kenya		
Primary Supervisor	Stefan Flasche		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

SECTION B – Paper already published

Where was the work published?	

When was the work published?			
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion			
Have you retained the copyright for the work?*	Choose an item.	Was the work subject to academic peer review?	Choose an item.

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SECTION C – Prepared for publication, but not yet published

Where is the work intended to be published?	Plos Computational biology
Please list the paper's authors in the intended authorship order:	Mburu CN, Ojal J, Chebet R, Ombati R, Akech D, Karia B, Tuju J, Sigilai A, Smits, G, van Gageldonk PGM, van der Klis FRM, EW Kagucia1, Scott JAG, Adetifa IMO, Flasche S
Stage of publication	**Prepared to be submitted**

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	Design, data acquisition, data analysis, write up, and submission

SECTION E

Student Signature	[redacted]
Date	

Supervisor Signature	
Date	

3.2 Bridging section

This paper is currently prepared for submission and addresses objective one, part c. Building upon the findings of objective one, part a, the study employs a static birth cohort model fitted to measles serological data, case-notification data, and information on the timing, schedule, and coverage of Routine Immunisations (RIs) and SIAs. The aim is to explore the interactions between natural infection and locally tailored interventions for measles control and elimination.

A crucial aspect of measles control is understanding the interaction between different intervention programs to assess the added value of each program and optimize resource allocation. Although, there are few studies that have explored interactions between different delivery strategies of MCV doses, these studies have failed to explicitly assess changes in RI programs that would reduce reliance on SIAs, a critical consideration given WHO's concerns about SIA sustainability in LMICs. In addition, measles epidemiology varies globally necessitating customized interventions.

Beyond significantly contributing to understanding the interplay between different MCV delivery strategies and natural infection in our specific context, this study uses a versatile modelling framework for measles control inquiries. This framework has the potential for extension to different settings, offering a valuable tool for future research in the field.

I developed the model, wrote all the code and performed the fitting analysis. I also wrote the paper and generated the figures. Throughout the process, I received input/suggestions/edits from my supervisors.

The supplementary material is included as Appendix C in this thesis, so any references to the supplementary material can be found there.

3.3 Abstract

Background: Measles outbreaks continue to cause a large burden of disease in Africa including Kenya. We used information from regular serological surveys in the Kilifi Health and Demographic Surveillance System (KHDSS) in combination with mathematical modelling to estimate the relative contribution of the vaccination programme to current measles immunity.

Methods: We developed a static birth cohort model to track the proportion of children who are either measles naïve or seroconverted due to natural infection or vaccination through MCV1, MCV2 or supplementary immunisation activities (SIAs). We fitted the model to biennial paediatric serological survey and case notification data and used vaccination coverage estimates from the KHDSS to estimate the relative contributions of vaccination and infection to measles immunity in Kilifi, a rural area in Kenya between 2009 and 2021.

Results: We estimated that between 2009 and 2021, 60% (95%CI 55-64%) of measles seroconversion in Kilifi was attributable to MCV1, with MCV2 contributing 1.0% (CI 0.9-1.1%) since its introduction in 2013. Natural infection and SIAs accounted for 24% (95%CI 17-31%) and 16% (95%CI 14-19%), respectively. A hypothetical 10% increase in MCV1 coverage increased the seroconversion attributed to MCV1 to 67% (95%CI 63-71%), with concurrent reductions in seroconversion from natural infection and SIAs to 13% (95%CI 9-18%) and 10% (95%CI 9-12%), respectively. Importantly, this same 10% increase in MCV1, if administered promptly at 9 months, could potentially reduce seroconversion from natural infection and reliance on SIAs by half to approximately 11% (CI 07-15%) and 8% (CI 7-10%), respectively.

Conclusion: Optimising routine coverage timing and uptake is crucial for reducing SIA dependence and measles susceptibility. A 10% MCV1 coverage increase could have halved susceptibility and lessened SIA demand, highlighting the potential of minor improvements in coverage to alleviate measles and reduce costly SIAs.

3.4 Background

Measles remains a major cause of morbidity especially in low and middle-income countries (LMICs)[1] and one of the major causes of mortality in children younger than 5 years[2]. Measles outbreaks continue to be reported in Africa[3], including Kenya[4, 5], and in many high-income countries too[6, 7]. A decline in immunisation coverage and delays in supplementary immunisation activities (SIAs) during the COVID-19 pandemic have further increased the risk of outbreaks[8, 9]. Owing to the very high transmissibility of measles, local elimination requires high population immunity of about 90-95% in a randomly mixing population [10] which is, often beyond the coverage that can be achieved by Routine Immunisation (RI). At least 95% coverage for both the 1st dose of measles-containing vaccine (MCV1) and the 2nd dose (MCV2) is recommended by WHO for elimination[11]. Thus, regular SIAs are often required to complement the suboptimal coverage achieved by RI. In addition, the WHO recommends continued monitoring via serological surveys to identify immunity gaps [11].

In Kenya, MCV1 was introduced in 1980 and is administered at 9 months of age. Administrative coverage for MCV1 has ranged from a minimum reported national coverage of 60% to a maximum reported coverage of 93% since its introduction up to 2021[12]. MCV2 was introduced in 2013 and is administered at 18 months albeit uptake has been poor at 28% to 57%[12]. Since 2002, SIA's have been conducted every 3 to 4 years in either children younger than 5 or 15 years old and have typically achieved more than 80% coverage [4]. Nevertheless, measles outbreaks are regularly reported, and Kenya has not eliminated measles.

In Kilifi, where health and demographic surveillance has been running since 2000[13], regular serological surveys have been conducted since 2009 alongside monitoring for vaccine uptake and measles surveillance. We use this unique data in combination with mathematical modelling, to estimate the relative contribution of the MCV1, MCV2, SIAs and natural infection to age-specific measles immunity profiles. We then use that model to predict how increases in MCV1 and MCV2 coverage may reduce the need for SIAs and the burden of measles infection.

3.5 Methods

3.5.1 Serological data

The serological data utilised for model development spanning from 2009 to 2019 originated from two primary cross-sectional surveys: the Malaria Cross-Sectional Survey [14, 15] and the Pneumococcal Conjugate Vaccine Impact Study (PCVIS)[16]. These surveys were conducted within the Kilifi Health Demographic Surveillance System (KHDSS)[13] as part of an ongoing effort to actively monitor malaria and pneumonia infections in children under 15 years of age. The data in 2021 was from participants who were recruited from COVID-19 serosurveillance in Kenya conducted as part of the pandemic response [17]. IgG antibodies against measles were determined using a fluorescent bead-based multiplex immunoassay[18]. The comprehensive details on data collection and sample testing procedures have been described elsewhere (thesis chapter 2).

3.5.2 Vaccination coverage

We used MCV1 and MCV2 vaccination coverage estimates from a birth-cohort analysis conducted in KHDSS between 2010 to 2017[19]. We extrapolated MCV1 coverage estimates for the years that were not included in the birth-cohort analysis from the administrative national MCV1 coverage in Kenya [20] after adjusting for differences based on the comparison for the years we had both datasets (see note s3:1). We relied on the schedule and coverage estimates of historical national SIAs over the course of our study period. These included an SIA in 2002 that offered a single dose of MCV to children younger than 14 years and achieved an estimated 94% coverage followed by SIAs in 2006, 2009 and 2012 in children under 5 years with 90%, 82% and 92% coverage respectively[4]. Additionally, a 2016 measles SIA in children aged 9 months to 14 years achieved a coverage of 95%[21] (Fig. 3:1b).

3.5.3 Measles case data

Both national and sub-national measles case data rely on hospital admissions. Clinicians report suspected measles cases to disease surveillance coordinators in each county, and laboratory-confirmed cases are entered into a central database and reported to WHO[4]. KHDSS measles case notification data was only available from 2014 onwards. We used national measles case data

reported to WHO[22] (Fig. 3:1c) as a proxy for cases in KHDSS after identifying matching trends for the years in which both datasets were present (Fig. s3:1).

3.5.4 Model structure

We used a static birth cohort model to track the proportion of children who are either susceptible to measles seroconversion (S) or seroconverted due to natural infection (NI) or due to vaccination with MCV1 (MCV1), MCV2 (MCV2) or SIA (SIA) (Fig. 3:1a). The population is structured by age and calendar year. The age structure is composed of monthly age groups for the first 2 years of age with annual age groups thereafter up to 14 years. We fitted the model to serological data and measles case notification data to estimate the relative contributions of these seroconversion routes to measles immunity in Kilifi.

We assumed that all infants are born with maternal immunity that lasts for 6 months [23] after which they become susceptible and can get infected with a probability governed by the force of infection (FOI). The FOI was assumed to be age-independent but proportional to the year-to-year change in the reported number of measles cases and thus defined as $FOI = \overline{FOI} * C/\overline{C}$, where FOI is the annual and $\overline{FOI}$ the average FOI during the study period, and C and $\overline{C}$ the annual and average reported number of measles cases respectively. We assumed that case ascertainment sensitivity remained similar during the study period.

MCV1 vaccination in the model was assumed to be administered at 11 months based on estimates of the timeliness of vaccinations in the KHDSS[19] and elsewhere[24, 25]. MCV2 in the model was given at 18 months as there was insufficient evidence of delay in timeliness. SIA were implemented at the time of their conduct in Kilifi. We modelled vaccine efficacy as all or nothing whereby a proportion of the vaccinated population seroconverted, and the remainder was considered a vaccine failure and remained susceptible. We did not assess the build-up of immunity in an individual from subsequent doses but rather assumed that individuals who recover from measles infection or seroconvert after vaccination are immune[11].

3.5.5 Model calibration

The model was initiated in 1996, corresponding to the birth year of the oldest age group in the initial serological survey (2009) and run until 2021. Utilizing monthly timesteps, the model adopted a fixed age of vaccine administration at 11months for MCV1 and 18 months for MCV2. Additionally, it incorporated the schedules and timing of SIAs over the study period to derive modelled seroconversion rates for the age cohorts in 2009 and subsequent surveys. We fitted the model to observed age-and year-specific seroprevalence data by estimating four parameters: the probability of seroconversion following (i) a dose of MCV to children younger than 12months (MCV1 or SIA), (ii) a dose administered to children between 12 to 18 months old (MCV2 or SIA) and (iii) a dose administered to children older than 18months (SIA) as well as (iv) the average force of infection.

We assumed a beta distribution for the priors of seroconversion of the vaccine administered to children younger than 12months with mean of 84% and with a mean of 93% for older children [26]. We used a non-informative prior for $\overline{FOI}$. Posterior distributions for the estimated model parameters were inferred using a Markov chain Monte Carlo (MCMC) approach with adaptive Metropolis-Hastings algorithm to maximise the binomial likelihood of the observed measles serological profile. The Gelman-Rubin statistic was used to evaluate MCMC convergence using a threshold of <1.1 while effective sample size (ESS) calculation was conducted to check for autocorrelation in the MCMC chains. The model was coded in R and the code is available in https://github.com/CarolineNM/Measles-seroconversion.git. Model and likelihood equations are provided in the supplementary material.

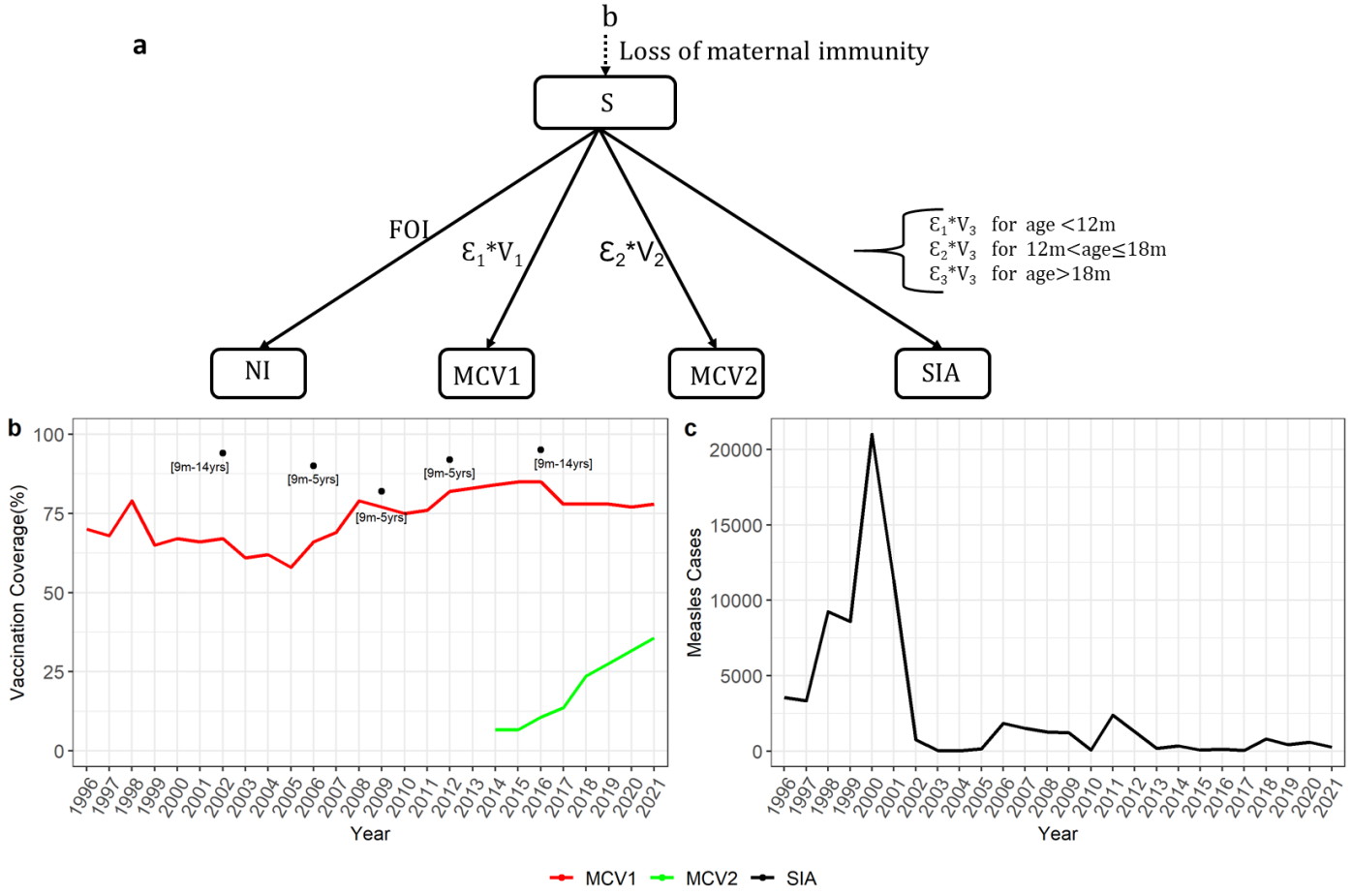

Figure 3:1 a) shows a static cohort model of measles immunity. Individuals can be divided into 5 mutually exclusive states: S-susceptible, NI=Naturally Infected, MCV1=Seroconverted from MCV1 dose, MCV2=Seroconverted from MCV2 dose, SIA=Seroconverted from SIA dose. ε_1=1-vaccine failure of one dose given at less than 1 year, ε_2=1-vaccine failure of one dose given between 12-18months and ε_3=1-vaccine failure of one dose given at 18months or older. V_1, V_2 and V_3 are the birth-cohort coverages of MCV1, MCV2 and SIA, b is the birth rate and FOI is the force of infection. b) shows the MCV1, MCV2 coverage and timing and target age groups of past SIAs in Kenya. c) shows the measles case notification data.

3.5.6 Model projections (counterfactual scenarios)

We used the fitted model to assess the impact of two of the main challenges in the current vaccination programme: suboptimal MCV1 and MCV2 coverage and suboptimal timeliness of MCV1.

Drawing from the posterior parameter estimates we re-ran the model during the post-MCV2 introduction period with the same FOI and the same timing and coverage of SIAs but

1) assumed that MCV2 coverage had been higher, at half way between the actual coverage and the MCV1 coverage (scenario: “higher MCV2”),
2) assumed that MCV2 coverage had been as high as the observed MCV1 coverage (scenario: “very high MCV2"),
3) assumed that MCV1 coverage had been 10% higher than observed and matched by MCV2 coverage. (scenario: “higher MCV1/2”),
4) the same as scenario 3) but with timely delivery of MCV1 as scheduled at 9 months (scenario: “timely & higher MCV1/2"),
5) assumed timely delivery of MCVI at 9months and MCV1 and MCV2 coverage of 95% (scenario:” ideal MCV1/2”)

3.5.7 Sensitivity analysis

We conducted a sensitivity analysis to assess the impact of the age cut off of priors of vaccine failure rates on the relative contribution of MCV1 and MCV2 to seroconversion by varying the age limit associated with each dose by 2 months and by 4 months. We then assessed the sensitivity of our findings to timeliness in receipt of MCV1 by varying the age of administration of an MCV1 dose to 9 and 10months. Finally, we evaluated the sensitivity of the seroconversion rates following vaccination to determine if the data significantly updates the priors. This was achieved by assuming non-informative priors, with a mean of 50%, for vaccines administered to both younger and older children.

3.5.8 Ethical considerations

Ethical approval was obtained from the Scientific and Ethics Review Unit (SERU) of the Kenya Medical Research Institute (Protocol SERU 3847). The serological samples were collected under a SERU-approved protocols with a provision for storage of residual samples and use in future research (SERU 1433 4085 2887,3149,3426) Written informed consent was obtained from parents/legal guardians of all participants prior to sample collection. In addition, written assent was obtained from all participants aged 13-14 years’ old.

Table 3:1 Overview of the model parameters and sources for the primary analyses

Symbol	Definition	Value	References
ε_1	1-vaccine failure rate of one dose given at <12months	Estimated, prior Beta distributed: 84% (72 - 95%)	[26]
ε_2	1-vaccine failure rate of one dose given between 12-18months	Estimated, prior Beta distributed: 93% (85 - 97%)	[26]
ε_3	1-vaccine failure rate of one dose given at >18months	Estimated, prior Beta distributed: 93% (85 - 97%)	Assumed
$\boldsymbol{FOI}$	Force of infection	$\overline{FOI} * C / \overline{C}$	
$\overline{\boldsymbol{FOI}}$	Average force of infection	Estimated, uninformative prior	
$\boldsymbol{C}$	Annual reported cases of measles	WHO reported cases for Kenya in1996-2021	[12]
$\overline{\boldsymbol{C}}$	Average annual number of reported measles cases	$C / 26$	
V_1	Annual birth-cohort coverage of MCV1	See figure s1 & Note s3:1	[19]
V_2	Annual birth-cohort coverage of MCV2	See figure s1 & Note s3:1	[19]
V_3	Coverage of SIA	94%, 2002, 9months-14yrs	[4, 21, 27]
		90%, 2006, 9months-<5yrs	
		82%, 2009, 9months-<5yrs	
		90%, 2012, 9months-<5yrs	
		95%, 2016, 9months-14years	
	Average age of routine vaccination	MCV1: 9 months+8weeks	[19, 24, 25]:
		MCV2: 18 months	
	Duration of maternal immunity	6months	[23]

3.6 Results

3.6.1 Description of the data and parameter estimates

Overall, 2414 (90%) of 2686 samples tested in this study had measles IgG antibody concentrations indicative of previous exposure either through infection or vaccination. The proportion of children with protective measles antibodies in Kilifi increased from 73% (CI 69-76%) in 2009 to 94% (CI 89-97%) in 2021 (Fig. s3:2). In all the surveys, seroprevalence increased early in life, in the months following the scheduled administration of MCV1 with little discernible trend thereafter. MCV1 coverage ranged from 75% to 85% between 2009 and 2021. Notably, it increased between 2012 and 2016 but subsequently declined, remaining below 80% for the remainder of the study period. The number of reported measles cases was higher in the early years and averaged 2000 cases

annually between 1996 and 2021 (Fig. 3:1c). The observed peaks in 2011, 2014, 2018 and 2020 coincided with declared national measles outbreaks in Kenya (Fig. s3:2).

The model was able to fit serological data well (Fig. 3:2a and Fig. s3:3). The estimated probability of seroconversion following vaccination was similar in the first two years of life at 93% (CI 89-97) for a dose administered to children less than one year and 93% (CI 86-97) for a dose administered to children between the ages of 12 to 18 months. The estimated probability of vaccine induced seroconversion in older children was 76% (CI 71-84) (Table 3:2). The average monthly

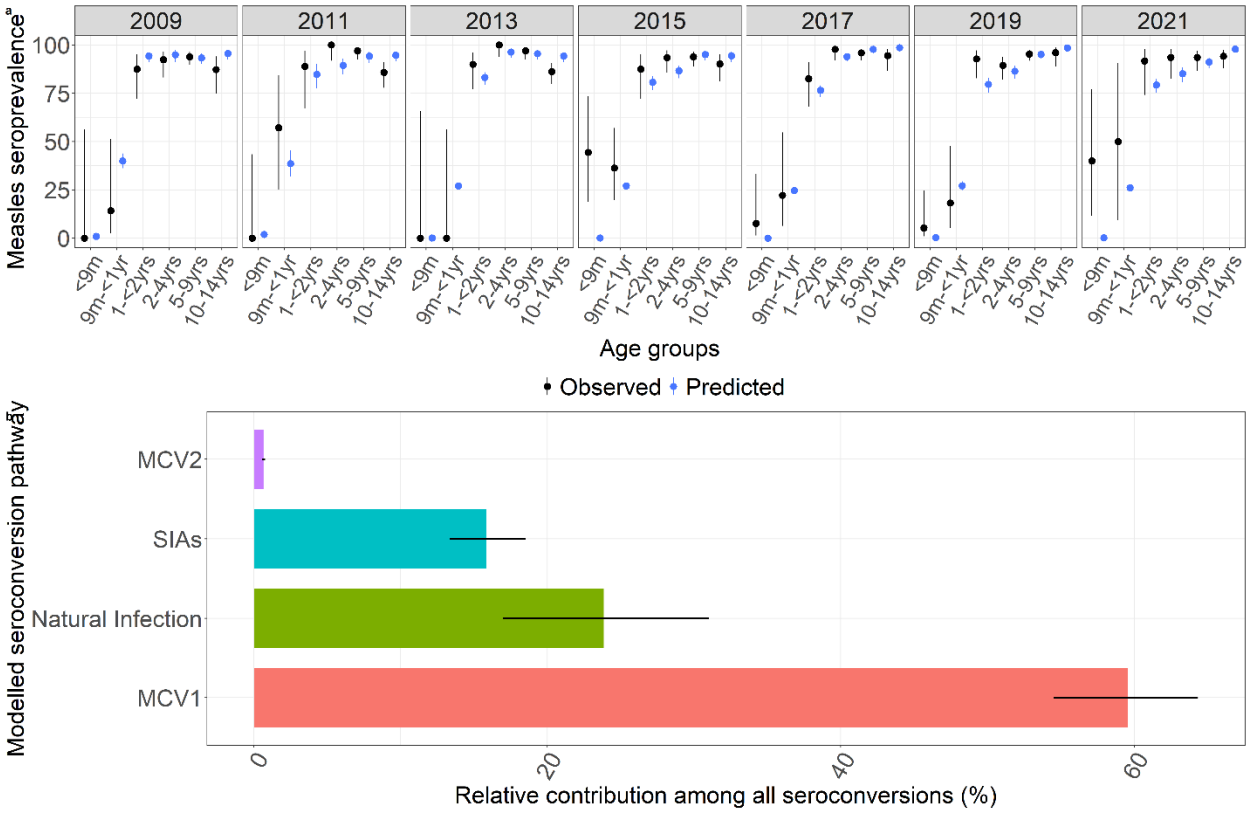

Figure 3:2. a) shows the age-specific serological data in children grouped into <9m (ineligible for vaccination), 9m-<1year (eligible for MCV1),1-<2 years (eligible for MCV2), 2-4 years (eligible for SIA in under 5), 5-9 and 10-14 years (eligible for SIA in under 14). Black is the observed data with 95% confidence intervals. The blue is the estimated seroprevalence sampled from the fitted model with 95% credible interval of the predictive posterior distribution. Model shows a good fit with majority of the predicted seroprevalence falling within the 95% CI of the observed seroprevalence. b) is the modelled output showing percentage of children that seroconverted either through MCV1, MCV2, SIA or natural infection between 2009 and 2021. Error bars indicate the credible interval of the predictive posterior distribution

FOI was estimated to be 6.0% (3.5% - 9.1%) between 1996 and 2021 consistent with an average of 16% of children in Kilifi being exposed to measles annually in the period between 2009 and 2021(Table 3:2 and Note s3:3).

Table 3:2 Parameter estimates from the static cohort model. Gelman-Rubin statistic is <1.1 implying successful convergence of the chains.

Parameters	**Definition**	**Low**	**Median**	**Upper**	**Rhat**	**Effective Sample size**
$\mathfrak{E}_1$	1-vaccine failure of one dose given at <12months	0.885	0.931	0.966	1	2164
$\mathfrak{E}_2$	1-vaccine failure of one dose given between 12-18months	0.860	0.930	0.970	1	1884
$\mathfrak{E}_3$	1-vaccine failure of one dose given at >18months	0.710	0.756	0.849	1	2002
$\overline{FOI}$	Average monthly force of infection	0.035	0.060	0.091	1	2035

3.6.2 Relative contributions of vaccination to measles seroconversion

The model estimated that administration of MCV1 in Kilifi accounted for 60% (CI 55-64%) of the seroconversion between 2009 and 2021 while natural infection contributed 24% (CI 17-31%) (Fig. 3:2b). The contribution of natural infection declined towards the end of the study period accounting for 12% (CI 8-16%) of seroconversion in 2021 compared to 42% (CI 32-49%) in 2009 (Fig. s3.4). SIAs were estimated to have contributed 16% (CI 14-19%) of all seroconversions between 2009 and 2021 with similarly decreasing contribution towards the end of the study period. MCV2 only contributed 1.0% (CI 0.9-1.1%) in the years since its introduction in part due to its low uptake to date.

3.6.3 The potential impact of increased routine MCV coverage

When MCV2 coverage was increased, the model predicted a decline in residual susceptibility and the contribution of SIAs to seroconversion (Fig. 3:3). The relative contribution of MCV2 in the period since MCV2 introduction increased from 1.0% (CI 0.9-1.1%) currently to 3.2% (CI 2.8-3.7%) and 5.3% (CI 4.6-6.1%) when assuming higher MCV2 and very high MCV2 coverage. This increase corresponded to a decline of the proportion susceptible from 8.9% (CI 8.2-9.8%) currently to 7.6% (CI 7.0-8.3%) and 6.2% (CI 5.7-6.8%) in the two scenarios respectively.

Increased MCV1 and MCV2 coverage was predicted to substantially reduce the contribution of SIAs and natural infection to seroconversion (Fig. 3:3). In addition, increased and timely MCV1 and MCV2 was estimated to reduce seroconversion from natural infection and SIAs even further. A 10% increase in the MCV1 coverage increased the proportion of seroconversion attributed to MCV1 from 61% (CI 57-65%) to 67% (CI 63-71%) and further to 73% (CI 69-77%) if MCV1 is administered at the recommended 9 months of age. With 95% coverage for each of MCV1 and MCV2, 83% (CI 78-87%) of seroconversions were attributable to MCV1 and this reduced the contribution of natural infection to seroconversion by 43% and that of SIAs by 79%.

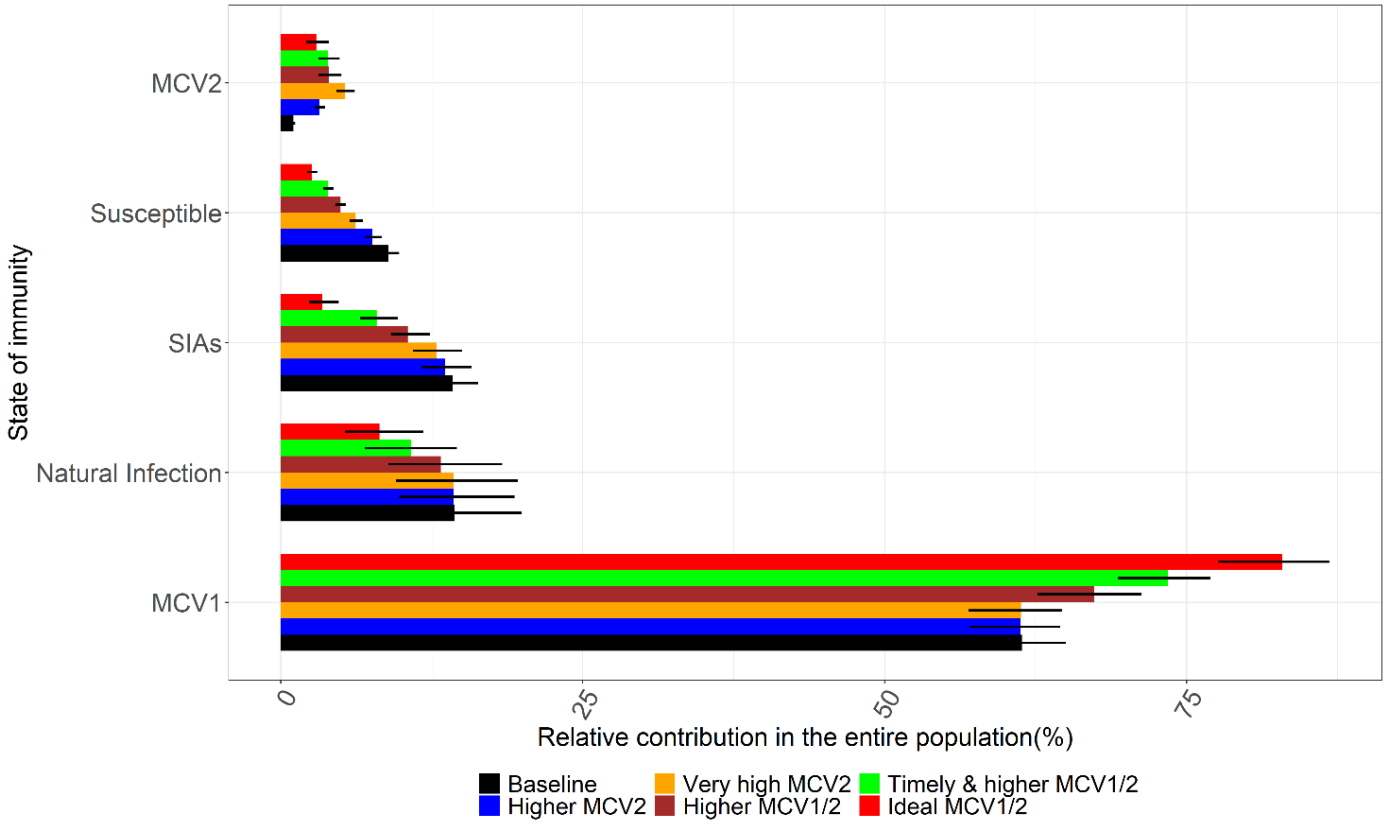

Figure 3:3 Estimated seroconversion profiles from the projection scenarios on increased MCV1 and MCV2 coverage. In the higher and very high MCV2 scenarios, there is a substantial increase in the relative contribution of MCV2 and a slight decline in the proportion susceptible. Increasing both MCV1 and MCV2 coverages as well as MCV1 timeliness has a considerable impact on the proportion seroconverted from all the pathways

3.6.4 Sensitivity analysis

The relative contribution of MCV1 and MCV2 towards seroconversion was not sensitive to alternative assumptions of age limits for the priors of the vaccine failure rates in the model.

Increasing the age limits of vaccine failure of a dose from <12months, 12-18months and >18months to vaccine failure of a dose from <16months, 16-22months and >22months resulted in highly similar estimates of the relative contribution of the different programs (Fig. s3:8).

Timeliness of MCV1 however had a considerable impact on the modelled seroconversion estimates. Seroconversion attributable to MCV1 increased from 60% (95%CI 55-64%) to 64% (95%CI 59-68%) if we assumed that MCV1 was administered at 10months and further to 66% (95%CI 60-71%) if assumed to be administered at 9 months in the baseline model (Fig. s3:9).

The estimated probability of seroconversion following vaccination was not sensitive to alternative assumptions of priors of the vaccine failure rates in the model. Similar to the main model, the estimated probability of vaccine induced seroconversion in the first two years of life was 93% (CI 89-97) for a dose administered to children less than one year and 93% (CI 87-97) for a dose administered to children between the ages of 12 to 18 months. The estimated probability of vaccine induced seroconversion in older children was 76% (CI 69-84).

3.7 Discussion

Availability of historical serological data from a series of cross-sectional surveys over a period of 12 years in a Kenyan population enabled us to estimate the relative contribution of natural infection and the different immunisation programs to measles seroconversion. We found that MCV1 accounted for more than 50% of the seroconversions in the study period. Natural infection and SIAs led to 24% and 16% of all seroconversions respectively, illustrating the reliance of the current RI programme on SIA and the size of the immunity gap allowing measles circulation. We find that increasing the coverage of MCV2 and particularly that of MCV1 and most importantly improving the timely administration of MCV1 has the potential to substantially reduce the need for SIAs and reduce the burden of measles in Kilifi.

We based our analysis on a static cohort model of measles immunity fitted to observed serological data and incorporating measles case-notification data and records on timing, schedule, and coverage of RIs and SIAs in the country. Coverage and timing of the different vaccine doses allowed us to estimate the increasing rate of seroconversion from vaccination in the different age

groups while increasing rate of seroconversion as a result of natural infection was estimated by a probability governed by FOI which was assumed to be proportional to the year to year change in the reported number of measles cases. We did not consider the additional vaccine protection as a result of a second dose in already immunised individuals but rather, and in line with the rationale of WHO to recommend a second dose, assumed that the main benefit of MCV2 would be to provide protection to those unprotected from MCV1 either due to vaccine failure or because of missed vaccination[11].

Our main findings and projection scenarios align with the WHO recommendations around measles vaccination strategies[11]. MCV1 remains the most crucial of the immunisation opportunities in the current program for measles control, accounting for by far the greatest contribution of the seroconversions if given early in life and at high coverage. These findings are in line with similar studies where contribution of MCV1 to seroconversion was estimated to be as high as 90% in some countries[27]. We estimate that if MCV1 coverage had been 10% higher during the study period residual susceptibility to infection would have been about 50% lower and at the same time would have reduced the need for reliance on SIA, highlighting the potential of seemingly small improvements in coverage to reduce the burden from measles and reduce the costs associated with SIAs. A possible way to increasing MCV1 uptake could be the roll out of the new RTS,S malaria vaccine which is scheduled to be given to infants aged between 5 to 17 months in a four dose schedule [28]. This follows from previous findings that have shown strengthening of service delivery in already existing routine vaccine programs as a result of new vaccine introduction [29].

The recommended age of MCV1 receipt is 9 months in a high transmission setting. In the model, delivery of MCV1 at 9 months resulted in 7% more seroconversions compared to MCV1 delivered at 11months with a similar coverage suggesting that efforts on ensuring optimal timing needs to be emphasized as even one-month delay may have substantial impact on the risk for natural infection [19]. We did not assess the effect of administering MCV1 to infants younger than 9 months. Although this early vaccination has been proposed in high risk settings, there is a moderate evidence showing that it might negatively impact seroconversion to subsequent measles vaccine doses [30].

In 2019, WHO initial recommendation was for introduction of MCV2 when countries achieved 80% MCV1 coverage but this was revised in 2017 to a recommendation to introduce MCV2

regardless of MCV1 coverage. The goal was to target unvaccinated children missed by earlier doses, particularly in LMICs where SIAs are not often implemented on schedule [11]. We show that, so far, this 2nd dose has led to 1% to the total seroconversions since it was first introduced, in part due to the low vaccination coverage However, there has been a gradual rise in the annual relative contribution to seroconversion, increasing from 0.2% in 2015 to 3% in 2021 primarily due to the gradual but consistent improvement in coverage over the years. Similarly low MCV2 coverage has been reported across Africa and has been attributed to several factors including the knowledge, perception and attitudes towards the vaccination at both the individual and community level [31]. The timing of the dose which was arrived at based on programmatic considerations[11] is also a factor as it does not correspond with the schedule of the other routine immunisation vaccines at the moment. Similar to MCV2, the roll-out of RTS,S malaria vaccine may provide an opportunity to increase awareness and coverage of MCV2 [28]. However, we show that increasing MCV2 coverage, while important to minimise the immunity gap from MCV1 failures, is likely to have much less impact than any increase in MCV1 coverage.

Frequent SIAs are recommended to close immunity gaps especially in communities who are hard to reach with RI programmes. However, SIAs are expensive and their impact is often diminished by delays or disruptions especially in LMICs as well as the inability to reach zero dose children[32]. In total five SIAs all with high coverage were conducted in the 12-year study period and were estimated to have contributed about 14% of seroconversions. This impact of SIAs while still crucial to reduce the immunity gaps left by insufficient RI coverage would likely be smaller if SIAs are delivered to children who are already vaccinated. This has been shown to be one of the main challenges with measles SIAs [32] and among the reasons for recommendations by WHO to phase out SIAs once countries achieve a 95% coverage of both doses. Our findings concur with the recommendation as increasing RI coverage to 95% in the model would reduce the contribution of SIAs to total seroconversions to only 3%.

A key strength of our study was the availability of good quality historical serological data spanning over a decade which provided excellent means of directly estimating levels of population protection. The KHDSS population registry was crucial in informing assessment of vaccination coverage [19] while mathematical modelling allowed us to combine these different pieces of evidence to make meaningful conclusions on the current immunity profile in children in Kilifi.

Our study had a few limitations. First, we used a static cohort model in our projections which is bound to reduce the impact of vaccination as we do not account for the indirect herd effects of vaccination; these will particularly underestimate the ability to control measles circulation at high vaccine coverage. Our results can be interpreted as the minimum decrease in seroconversions due to natural infection. We used nationwide measles case data in our projections as we did not have estimates from KHDSS for all years of study. However, there were similar trends of measles cases in KHDSS and nationwide for the years in which both datasets were present and there was also no evidence suggesting that measles case finding changed during the study period. Our analysis also relied on rural data, restricting the generalisability to diverse contexts. While the results represent rural measles-endemic areas well, variations may occur in urban settings primarily due to differences in vaccination coverage and measles susceptibility profiles in urban and rural settings. The MCV1 and MCV2 coverage estimates used in the analysis were informed by a birth-cohort analysis and administrative nationally reported coverage estimates. Given the limitations associated with the methods of collecting this data, it is likely that the estimates could either be an overestimate or underestimate. If both MCV1 and MCV2 were overestimated, this would likely mean that we underestimated the impact of SIA in our model. Conversely, if both MCV1 and MCV2 coverages were underestimated, this would imply that we overestimated the relative contribution of SIAs to the total seroconversions. Finally, we did not incorporate build-up of immunity from subsequent doses in individuals. Instead, our assumption was that immunity from a single dose was lifelong and that the administration of MCV1, MCV2, and SIAs is uncorrelated. In reality, health-seeking behaviour could lead to correlations, as children receiving MCV1 are likely to receive MCV2 and participate in SIAs, an observation reinforced by the fact that SIAs have not significantly decreased the number of children with zero vaccine doses[32]. Although this assumption simplified our cohort model and is not expected to marginally change the outcome, it is likely that we slightly overestimated the impact of MCV1 and underestimated the impact of MCV2.

In conclusion, a combination of serological, vaccine coverage and measles surveillance data and mathematical modelling allowed us to assess the impact of the current measles prevention programme in Kilifi by deciphering the relative contributions of MCV1, MCV2, SIAs and natural infection to measles seroconversions. We showed that a slight increase in routine vaccination coverage and timeliness, especially of MCV1, can result in a substantial decline on the reliance on

SIAs as well as the prevention of natural infection. Particularly the roll out of RTS, S may provide an opportunity to increase routine Measles vaccination coverage to improve measles control and reduce costs associated with frequent SIAs.

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4. Chapter 4: The importance of supplementary immunisation activities to prevent measles outbreaks during the COVID-19 pandemic in Kenya

4.1 Research paper cover sheet

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RESEARCH PAPER COVER SHEET

Please note that a cover sheet must be completed for each research paper included within a thesis.

SECTION A – Student Details

Student ID Number	2003951	**Title**	Mrs.
First Name(s)	Caroline		
Surname/Family Name	Mburu		
Thesis Title	The utility of mathematical modelling of serological data in assessing the impact of vaccination programmes in Kenya		
Primary Supervisor	Stefan Flasche		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

SECTION B – Paper already published

Where was the work published?	BMC Medicine
When was the work published?	03/02/2021
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion	

Have you retained the copyright for the work?*	Yes	Was the work subject to academic peer review?	Yes

*If yes, please attach evidence of retention. If no, or if the work is being included in its published format, please attach evidence of permission from the copyright holder (publisher or other author) to include this work.

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Where is the work intended to be published?	
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For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	Design, data acquisition, data analysis, write up, and submission

SECTION E

Student Signature	
Date	
Supervisor Signature	
Date	

4.2 Bridging section

Published in BMC Medicine in 2021 this paper addresses objective one, part b. Expanding upon the findings of objective one, part a, the study employs a static cohort model that integrates measles serological data, local contact patterns, and vaccination coverage data. The objective is to investigate the risk of measles outbreaks during the COVID-19 pandemic, caused by disruptions in routine immunisation and SIAs.

Given the highly contagious nature of measles, large outbreaks are typical following disruptions to healthcare systems. Following the COVID-19 pandemic, the WHO recommended benefit–risk assessments to sustain RI services, considering local disease transmission dynamics and health system characteristics. Despite the proven benefits of maintaining RI services and assessments of disruptions to other VPDs elsewhere, a local assessment of the actual risk of measles outbreaks based on existing measles seroprevalence and the measles control program was lacking.

This work not only provided insights into the expected impact of COVID-19 on measles outbreaks but also offered valuable considerations for the utility of post-pandemic SIAs, which was subsequently implemented in June 2021.

I developed the model, wrote all the code and performed the fitting analysis. I also wrote all the draft papers and generated the figures. Throughout the process I received input/suggestions/edits from my supervisors.

The supplementary material is included as Appendix D in this thesis, so any references to the supplementary material can be found there.

4.3 Abstract

Background: The COVID-19 pandemic has disrupted routine measles immunisation and supplementary immunisation activities (SIAs) in most countries including Kenya. We assessed the risk of measles outbreaks during the pandemic in Kenya as a case study for the African Region.

Methods: Combining measles serological data, local contact patterns, and vaccination coverage into a cohort model, we predicted the age-adjusted population immunity in Kenya and estimated the probability of outbreaks when contact-reducing COVID-19 interventions are lifted. We considered various scenarios for reduced measles vaccination coverage from April 2020.

Results: In February 2020, when a scheduled SIA was postponed, population immunity was close to the herd immunity threshold and the probability of a large outbreak was 34% (8-54). As the COVID-19 contact restrictions are nearly fully eased, from December 2020, the probability of a large measles outbreak will increase to 38% (19-54), 46% (30-59), 54% (43-64) assuming a 15%, 50% and 100% reduction in measles vaccination coverage. By December 2021, this risk increases further to 43% (25-56), 54% (43-63) and 67% (59-72) for the same coverage scenarios respectively. However, the increased risk of a measles outbreak following the lifting of all restrictions can be overcome by conducting a SIA with ≥ 95% coverage in under-fives.

Conclusion: While contact restrictions sufficient for SAR-CoV-2 control temporarily reduce measles transmissibility and the risk of an outbreak from a measles immunity gap, this risk rises rapidly once these restrictions are lifted. Implementing delayed SIAs will be critical for prevention of measles outbreaks given the roll-back of contact restrictions in Kenya.

4.4 Background

The SARS-CoV-2 pandemic has damaged the economy, and disrupted social interaction and important health services in Kenya and elsewhere.[1, 2] The cumulative incidence of COVID-19 cases continues to rise in many parts of Africa suggesting the current mitigation measures will be maintained or reintroduced for periods at least until the pandemic peaks.[3]

Despite the World Health Organization (WHO) advisory to sustain routine immunisation (RI), vaccine coverage temporarily declined in many countries including Kenya that reports a 33% disruption of RI.[4-7] Following guidance from the WHO, all countries suspended scheduled measles SIAs.[6-8] Measles control in Kenya is achieved by giving children a first dose of Measles Containing Vaccine (MCV1) at 9 months, and a second dose (MCV2) from 18 months. SIAs, first introduced in 2002 are conducted periodically among children <5 years or <15 years for accelerated control of measles.[9] Based on accumulation of susceptible children, the timing of such campaigns has typically been chosen to close immunity gaps in time to prevent potentially large measles outbreaks. A Measles SIA originally planned for 2019 was rescheduled for February 2020 due to a shortfall in funding and postponed again following the COVID-19 pandemic.

Following identification of the first COVID-19 case on March 13, 2020, Kenya imposed various mitigation measures: ban on large gatherings, suspension of international flights, closure of bars, cessation of movement from hotspot counties, restriction of restaurant operating hours and a nationwide curfew from 7pm to 5 am. While it is plausible that these physical distancing and lock down measures may reduce the risk of measles outbreaks, they are temporary and may be associated with rebound risk periods. The availability of recent measles serological data provided the opportunity to use Kenya as a case study to estimate the impact of reduced measles vaccination coverage and suspended SIAs due to COVID-19 on the risk of measles outbreaks.

4.5 Methods

This study used a cohort mathematical model that combined measles serological data, local contact patterns, and vaccination coverage estimates.

4.5.1 Serological data

We estimated measles immunity profile in children using serum samples collected during serological surveys among residents of Kilifi Health and Demographic Surveillance System

(KHDSS) Kilifi, Kenya [10] for the Pneumococcal Conjugate Vaccine Impact Study (PCVIS) [11] These serosurveys, conducted every two years since 2009, target 50 KHDSS randomly selected children in ten age strata (0, 1, 2, 3, 4, 5, 6, 7, 8-9 and 10-14 years) and blood samples <2ml were collected from participants. The sample size for the PCVIS serosurveys was calculated to obtain narrow confidence intervals around the estimate of prevalence of immune response both overall and by age-category for each serosurvey year. For instance, for a proportion of 0.80, the 95% confidence intervals (CIs) would be 0.77- 0.84 overall and 0.69-0.91 in each age stratum.

In the 2019 serosurvey, there were 497 participants and the blood samples were collected in July (165), August (162), September (130) and October (40). We tested for measles immunoglobulin G (IgG) antibodies using a fluorescent-bead-based multiplex immunoassay. Antibody concentrations ≥0.12 IU/ml were considered protective against measles. [12]

We assumed these results reflected measles immunity in Kilifi in August 2019 and assumed 96% of persons >15 years had protective measles antibodies concentrations, similar to findings in adults in Nairobi in 2007-2009[13] (Table 4.1). We also assumed protection from maternal immunity was similar to the proportions of the infants <9 months old who had protective antibodies.

4.5.2 Vaccination coverage

MCV1 national coverage in Kenya has been between 75% and 80% since its introduction in 1985. [14] MCV2 was introduced in Kenya in 2013 and coverage rose up to 45% in 2018. [9] The last measles SIA in children aged 9 months to 14 years took place in 2016 and achieved 95% coverage. [15]

We assumed national MCV1 and MCV2 coverage were 79% and 45% respectively in 2018, and that these stayed at the same level from August 2019 until the end of March 2020 when COVID-19 contact restrictions were introduced in Kenya. From April 2020, we explored the following routine vaccination coverage scenarios alongside a suspended SIA

A. routine vaccination coverage remained the same

B. routine vaccination coverage reduced by 15% for both MCV1 and MCV2

C. routine vaccination coverage reduced by 50% for both MCV1 and MCV2

D. routine vaccination was suspended

4.5.3 Contract matrix

We used an age-mixing matrix which consisted of the number of contacts between six different age groups. The matrix was generated from diary studies conducted in Kilifi, Kenya [16] using a bootstrap of 4000 samples by randomly sampling n individuals with replacement from the n participants of the contact survey.

4.5.4 Projecting immunity

We adapted a static cohort model of measles immunity [17] to estimate age-stratified population immunity profile in Kilifi by combining recent measles serological data with new vaccine-derived immunity during the prediction period using the local vaccination schedule, MCV1 and MCV2 uptake, and vaccine efficacy. We assumed waning immunity or additional acquired immunity from natural exposure, and demographic changes in the short time frame were negligible. Hence, the key mechanisms of the projection model were that individuals are born at a constant rate, gained immunity through vaccination at the recommended age and at the observed coverage, and grow older.

In extrapolating immunity for young infants under 9 months old, maternal immunity was assumed to be the same as the observed data. For ages 9 months to 17 months, immunity was estimated in accordance with the assumed MCV1 vaccination uptake and a vaccine effectiveness of 93%. For those ≥18 months, we estimated the immunity based on the assumed uptake of MCV2 and the same vaccine effectiveness. We aggregated projected immunity to age groups given by contact data and weighted each age group according to population estimates before averaging them to estimate overall immunity. We did not explicitly model MCV2 delivery but rather assumed that the MCV1 effectiveness is an average of MCV1 and MCV2 efficacy weighted by proportion of children who receive MCV1 only or both doses. The underlying assumption here was that the same children who received MCV2 had also received MCV1. We predicted age-stratified and population level immunity until December 2021.

To derive a contact-adjusted estimate for the proportion of the population who are immune to measles, the predicted age-stratified immunity profile was weighted by age-stratified social contact patterns observed in Kilifi. This was implemented using the epimixr package with the contact

matrix, population size and plain immunity levels as the function input. Contact adjusted immunity (r') was given by ;

$$r' = 1 - R/R_0$$

where R/R_0 is the ratio of the effective reproduction number R to the basic reproduction number R_0.In an age-structured population, the basic reproduction number is related to the contact matrix via

$$R_0 = \mathrm{p}(\boldsymbol{K})$$

where p denotes the spectral radius and $\boldsymbol{K}$ is a matrix with elements

$$K_{ij}=\phi_{ij}(N_i/N_j)$$

Where ϕ_{ij} is the number of contacts that an individual in age group *i* makes with that in age group *j*, N_i is the size of age group *i* and N_j is the size of age group *j*. The effective reproduction number R is obtained in the same way, except that the matrix **K** is multiplied with a vector of susceptibility to yield an effective matrix $\mathbf{K'}$

$$K'_{ij} = K_{ij}(1 - r_i)$$

where r_i is the proportion immune in age group *i.* This method has been previously shown to yield robust projections for measles immunity to transmission in the population.[17]

The herd immunity threshold (HIT) for measles during the COVID-19 pandemic was calculated assuming an R_0 of 12 to 18 with a median of 14[18] and that COVID-19 prescribed contact restrictions caused a 50% reduction in measles transmissibility similar to the observed reduction in physical contacts in Kenya.[19] We also explored a 25% and 75% reduction in measles transmissibility in a sensitivity analysis. The HIT is calculated as $\boldsymbol{\mathcal{H}}_0 = (R_0\text{-}1)/R_0$.

4.5.5 Quantitative impact of outbreak risk

We obtained a crude estimate of the outbreak risk using the predicted immunity and HIT. The probability of a large outbreak, p, sparked by a single infected individual was given by p = 1-(1/R)

^{I0} where I0 is the initial number infected, and R is the effective reproductive number. R<1 implies that probability, p, is negative which is defined to be 0 for no outbreak.

4.5.6 The effectiveness of a post-lockdown SIA in reducing outbreak risk

We assessed the impact of SIAs in two age categories; 9 months to 5 years and 9 months to 15 years, consistent with typical age ranges in previous SIAs conducted within the country by predicting the post-SIA immunity profile and the corresponding risk for a large measles outbreak.

We simulated SIAs in either November 2020, December 2020 or December 2021, assumed a coverage of 95% similar to the most recent national SIA in 2016,[15] and applied vaccine efficacy of MCV1. The SIA was simulated by reducing the age specific pool of susceptible by the effective coverage of the SIA.

In simulating the SIA, we used the age-specific predicted immunity to calculate the age specific pool of susceptible at the different time-points. We reduced this age specific pool of susceptible in the age-groups of interest by the effective coverage of the SIA. We aggregated the results and adjusted the overall crude immunity using the social contact matrix. Finally, we calculated the outbreak probability assuming a SIA is conducted before restrictions are lifted (using the reduced HIT based on 50% reduction in contacts) and assuming an SIA is conducted after restrictions are lifted (using the normal HIT based on 0% reduction in contacts)

4.5.7 Uncertainty analyses

We assessed the sensitivity of our findings to uncertainty inherent in several of our assumptions via probabilistic re-sampling. We included uncertainty for population immunity profile in the older age groups (>15yr old), combined MCV1 and MCV2 vaccine effectiveness, and MCV1 and MCV2 coverage (Table 4:1). As part of each parameter bootstrap, we also bootstrapped participants of the serological survey and hence the age stratified population immunity at the start of the simulation. We present median estimates including uncertainty quantified as per the 95% quantiles of the 4000 bootstrap samples.

4.5.8 Sensitivity analyses

We conducted a sensitivity analysis to assess the impact of a delay in receipt of MCV1 on outbreak probability. We delayed the age of receipt of MCV1 in our model by three months as reported for delayed vaccination in Kilifi [20] and also by six months. We also predicted unadjusted population immunity in Kilifi and estimated the corresponding probability of a large outbreak

Table 4:1 Model parameters. An overview of the key model parameter assumptions and their sources. Parameter ranges are those used in the sensitivity analyses

Parameter	**Value** (95% quantiles)	**Source**
Vaccine schedule	MCV1: 9 months MCV2: 18 months	[21]
Vaccine effectiveness (Beta distributed)	MCV1: 85% (80 - 90%) +MCV2: 98% (95 - 100%) Combined effectiveness 93% (88-96%)	[22, 23]
Age-Immunity profile in <15y old (Bootstrapped from data)	Observed in 2019	[11]
Proportion immune among >15y old (Beta distributed)	96% (90 - 99%)	[13]
Vaccine coverage Aug 2019 to March 2020 (assumed to be same as in 2018) (Beta distributed)	MCV1: 79% (75-85%) MCV2: 45% (40-50%)	[9, 14, 20]
Vaccine coverage from April 2020	MCV1 & MCV2 0%, 15%, 50% or 100% reduced	assumption
R_0 measles (Log-normally distributed)	14 (12 - 18)	[18]
Reduction in contacts during COVID-19	50% (25% and 75%)	[24]
Age demographics	from KHDSS in 2019	[10]
Social mixing matrix	from 2011/12	[16]

4.6 Results

4.6.1 Measles seroprevalence in Kilifi in late 2019

The proportion of MCV1-eligible children with protective measles antibody concentrations was high in 2019 (Fig. 4:1 and Table s4:1). 71 of 74 (96%) children >=9 years had protective levels. Similarly, 228 of 237 (96%) 4-8-year-olds were immune. Among under-fours eligible for MCV1, 145 of 166 (87%) were immune while one of 20 (0.05%) children under 9 months old, who were ineligible for MCV1, had protective antibodies.

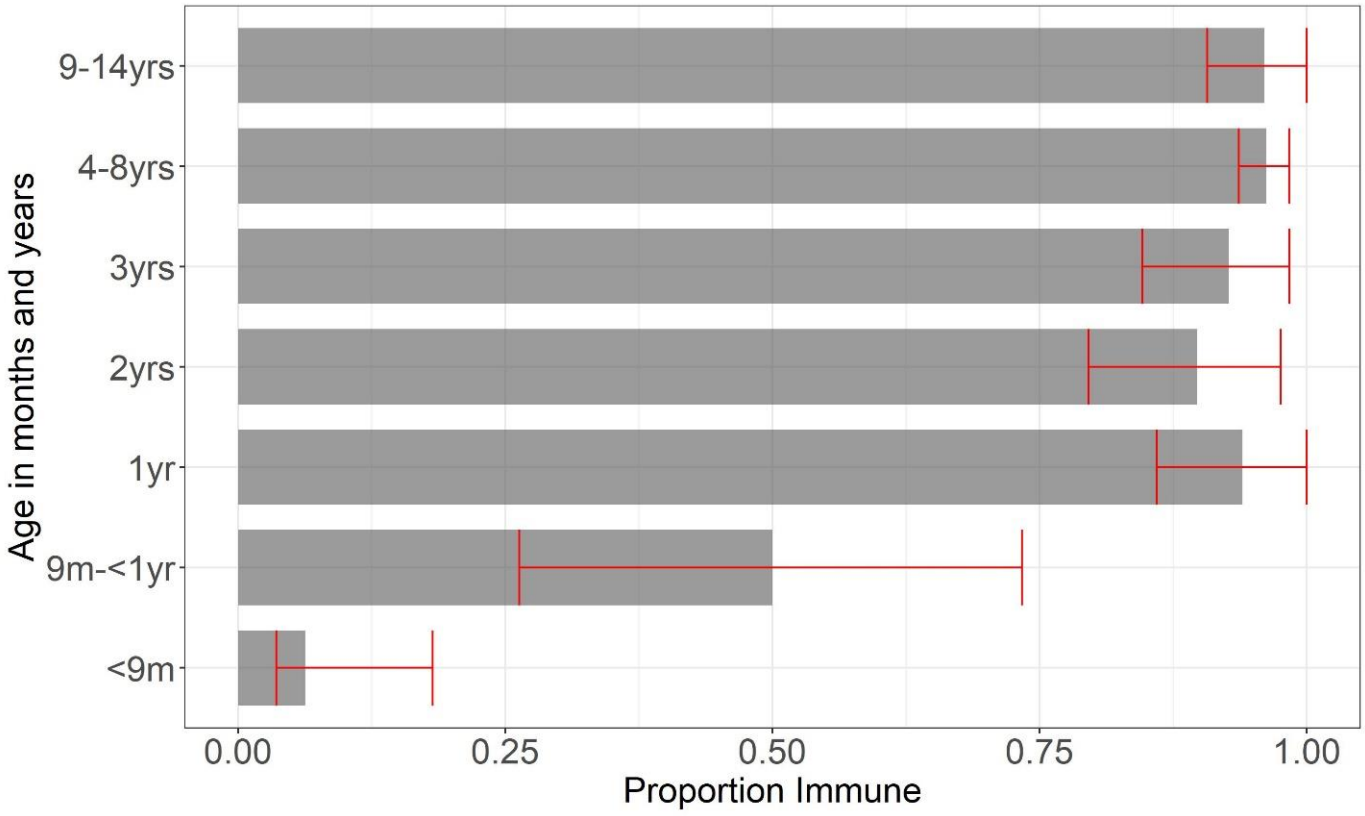

Figure 4:1 Age-stratified population immunity profile. Estimated age-stratified proportion of the Kilifi County population who were immune to measles infection in August 2019 from data. Antibody concentrations ≥0·12 IU/ml were defined as protective. Confidence bounds displayed (in red) are the 95% quantiles of a nonparametric bootstrap that is used to propagate uncertainty into the modelling framework. MCV1 is recommended to be administered at 9 months as per the Kenyan immunisation schedule and MCV2 from 18 months

4.6.2 Age adjusted immunity

We estimate that in late 2019, population immunity adjusted for age-differences in social contacts was 90% (85-92). Predicted immune proportions was unchanged in February 2020, at the time of originally planned SIA.

Following the start of COVID-19 pandemic and restriction measures that caused a decrease in vaccination coverage, we estimate that population immunity decreased quickly, depending on extent of reduction in vaccination coverage. If coverage reduced by 15% from April 2020, the contact-adjusted population immunity would decline to 88% (85-91) by December 2020 and 87% (84-90) by December 2021. A 50% reduction in vaccination coverage would lead to a more rapid decline in this immunity to 87% (83-89) in December 2020 and 85% (81-87) in December 2021(Fig. 4:2)

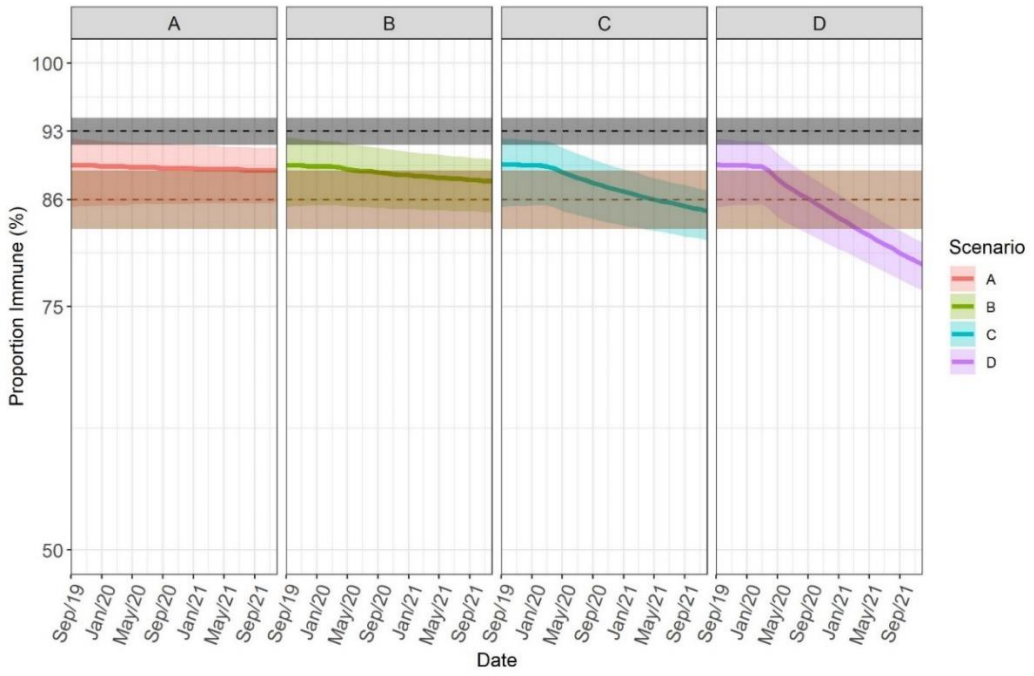

Figure 4:2 Monthly projected age adjusted immunity profiles for all the age groups from September 2019 to December 2021. The changes in coverage took effect in April 2020. The black dotted line shows the herd immunity threshold for measles before the COVID-19 physical distancing measures, 0·93 (0·92 to 0·94) and the brown dotted line shows the herd immunity threshold during COVID-19 physical distancing measures, 0·86[0·83-0·89], assuming the lockdown measures are still in effect. The bold lines and shaded region in each scenario i.e. A. No reduction, B. 15% reduction, C. 50% reduction and D. 100% reduction indicate the median estimates and the uncertainty of the predicted immunity quantified as the 95% quantiles of the bootstrap analysis. There was a quick decline of predicted immunity over the study period that was based on assumed reduction in routine coverage

4.6.3 Age adjusted immunity vs herd immunity threshold

A basic reproduction number of 14 (12-18) implies a HIT of 93% (82-94) and if, as a result of physical distancing, measles transmission is reduced by 25%, 50% and 75% this HIT drops to 90% (89-93), 86% (83-89) and 71% (67-78) (Fig.s4:1). Before contact restrictions came into effect in April 2020, age-adjusted immunity was below the HIT: in 99% of simulations this immunity was below the HIT. Reduction in HIT temporarily mitigated the immediate risk for measles outbreak as in April 2020, 94% of simulations were above the 50% reduced transmission HIT, 20% were above the 25% reduced HIT and 100% of simulations were above the 75% reduced HIT.

Depending on vaccination coverage maintained during COVID-19 pandemic, population immunity may decline quickly in young children (<2 years). By April 2020, age-adjusted immunity fell below the normal transmission HIT in all simulations under all the scenarios. (Fig. s4:3).

Similarly, the risk of a large measles outbreak from introduction of a single infectious individual increased quickly if routine vaccination coverage declined (Fig. 4:3). If in December 2020, measles transmissibility is similar to pre-COVID-19 levels and routine measles coverage since April 2020 reduced by 15%, 50% or 100%, we estimate probability for a large measles outbreak as 38% (19-54), 46% (30-59), 54% (43-64) respectively in the age-adjusted analysis. By December 2021, this risk would increase to 43% (25-56), 54% (43-63) and 66% (59-72) respectively. The probability of a large measles outbreak was much lower if measles transmissibility reduced by 25%, 50% and 75% (Fig. s4.4). In December 2020, if routine measles coverage since April 2020 reduced by 50%, we estimate probability of a large measles outbreak as 28% (7-45), 0% (0-18) and 0%(0-0) assuming a 25%, 50% and 75% reduction in transmission.

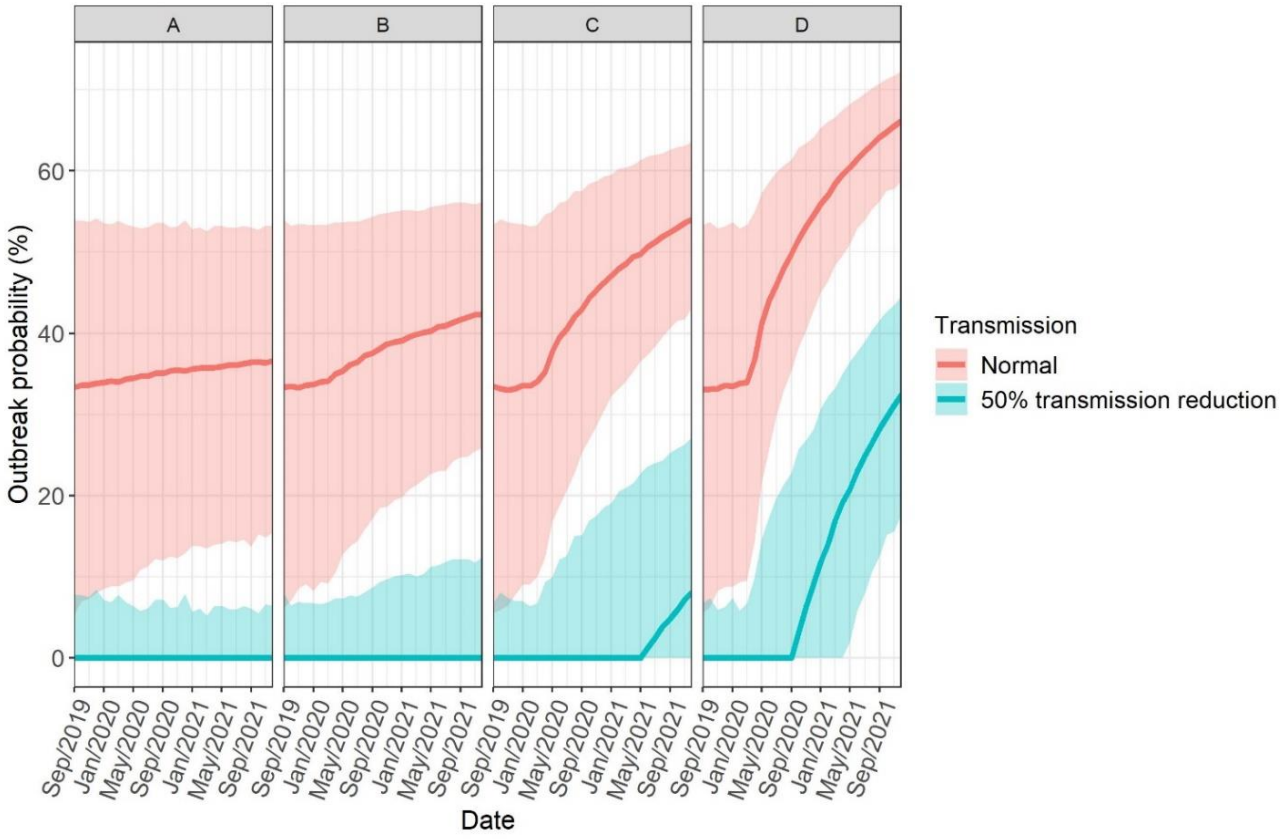

Figure 4:3 Probability of a large measles outbreak sparked by a single infected individual. Outbreak probability was calculated using the predicted immunity and herd immunity threshold before (red) and during (green) COVID-19 movement restriction measures. Zero probability indicates no possibility of an outbreak. The bold lines and shaded region in each scenario i.e. A. No reduction, B. 15% reduction, C. 50% reduction and D. 100% reduction indicate the median estimates of outbreak risk and the uncertainty quantified as the 95% quantiles of the bootstrap analysis. The risk of a large measles outbreak from the introduction of a single infectious individual increased quickly based on the level of impairment of routine vaccination coverage

4.6.4 Effectiveness of a SIA

A SIA in 9-month to 5-year-old children or 9-months to 15-year-olds both during and immediately after lifting transmission-reducing COVID-19 restrictions can substantially reduce outbreak risk (Fig. 4:4).

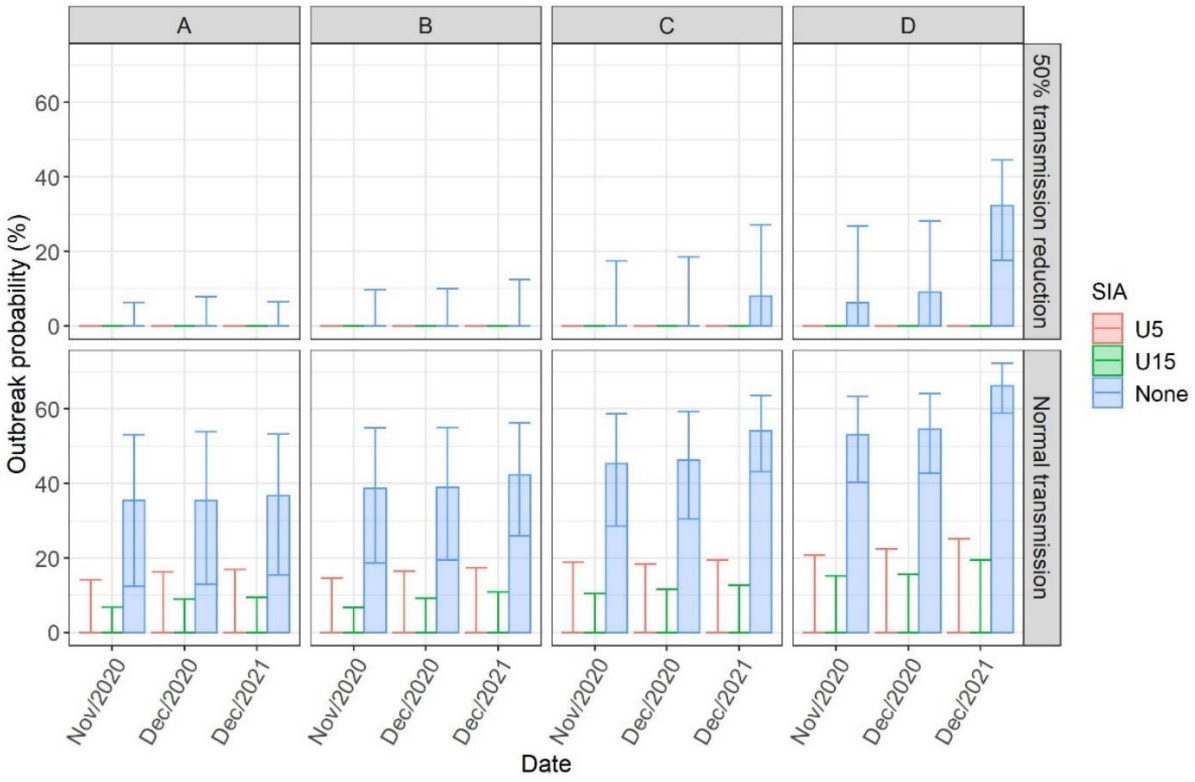

Figure 4:4 Probability of a single infectious person seeding a large outbreak before (none) and after implementing a SIA in children 9 months to 5 years old (U5) and in 9 months to 15 years old (U15) at different time points post-lockdown (Normal transmission) and during lockdown (50% transmission reduction). Outbreak probability was calculated by comparing the proportion immune with the herd immunity threshold. The shaded area is the median estimate of the outbreak risk and the error bars indicate the uncertainty in outbreak risk quantified as the 95% quantiles of the bootstrap analysis. In all the scenarios, i.e. A. No reduction, B. 15% reduction, C. 50% reduction and D. 100% reduction, the risk of a large measles outbreak would be largely mitigated through delivery of a SIA among children <5 years old or <15 years old.

If measles vaccine coverage declines by 15%, 50% or 100% from April 2020, a post lockdown SIA delivered to children 9 months to 5 years old in December 2020 with 95% coverage would reduce the risk of an outbreak to 0% (0-17), 0% (0-20) and 0% (0-22) respectively in age-adjusted analysis. A similar SIA would reduce the risk of an outbreak to 0% in all the scenarios assuming a 50% reduction in contacts in December 2020 (Fig. 4:4).

Even if RI coverage is low through to December 2021, the risk for a large measles outbreak would be mitigated through an SIA for under-fives if delivered as soon as possible (Fig. s4:5)

4.6.5 Impact of delayed vaccination on outbreak probability

A 3-month and 6-month delay in the receipt of MCV1 in age-eligible children caused a marginal increase in the risk of a large measles outbreak (Fig. s4:6). This increase in outbreak risk associated with delay in receipt of MCV1 was also evident for different assumptions of transmission reduction during lockdown (Fig. s4:6)

4.6.6 Crude Population immunity

The predicted crude population immunity was slightly higher compared to age-adjusted immunity but followed the same declining trend over time (Fig. s4:7). Before contact restrictions came into place, 73% of simulations were below the HIT and by October 2020 and July 2020, this immunity fell below the HIT in more than 95% of simulations under scenario C and D respectively (Fig. s4:8).

4.7 Discussion

Our analysis suggests a decline in population immunity during COVID-19 pandemic will result in an increased risk of a measles outbreak depending on the extent to which routine vaccination coverage is reduced. We estimated the probability of a large measles outbreak from the introduction of a single infectious individual to be 38% (19-54), 46% (30-59), 54% (43-64) in December 2020 assuming a 15%, 50% or 100% reduction in routine measles vaccination coverage respectively since April 2020. This risk, which will increase to 43% (25-56), 54% (43-63) and 67% (59-72) by December 2021 will be greatly reduced if a SIA among children <5 years old is conducted before or immediately after all COVID-19 related restrictions on physical contact are lifted.

We based our analysis on an immunity model that combined serological data and age-specific mixing patterns in Kenya. Combining the two is a better strategy for predicting outbreaks as opposed to using immunity profiles alone as it allows adjustment of overall immunity by taking into account contribution of each age-group to transmission.[17]

As there is considerable uncertainty in actual reduction of routine vaccination uptake, we predicted population immunity for scenarios of routine vaccination coverage since April 2020 i.e., 15%, 50% and 100% reductions, and the corresponding outbreak risk. Our assumption of 15% reduction in vaccine coverage rates is based on reduction in vaccine clinic visits in Kilifi County (DHIS2 Routine Report) while the 50% reduction lies in the range of reported disruption in vaccination services from WHO immunisation pulse poll.[6] We assumed a 50% reduction in measles transmissibility given that COVID-19 mitigation measures implemented on 25th March 2020 were reported to have reduced social contacts and disease transmission by the same margin.[24] Although some restriction measures remain in place e.g. nationwide curfew, others like the partial lockdown have since been eased and ban on international flights was lifted on 1st August 2020. While the assumption of a 50% reduction in measles transmission was applicable at the beginning of the epidemic due to stringent measures imposed, current herd immunity threshold may be much higher than originally assumed but still lower than pre-COVID-19 threshold.

To account for the uncertainty in measles transmissibility during lockdown, we explored two other scenarios, 25% and 75% reduction in measles transmission in a sensitivity analysis. We found that a 75% reduction in measles transmission would result to zero outbreaks in all the scenarios

during the entire study period, which was much lower compared to the outbreak probability in our baseline analysis. A 25% reduction in measles transmission resulted to a much higher probability of measles outbreak compared to our baseline analysis. For instance, in December 2020, the estimated outbreak risk was 28% (7-45) compared to 0% (0-18) in our baseline analysis assuming a 50% reduction in routine vaccination coverage.

In the calculation of a quantitative impact of outbreak risk, our estimate of the probability of a large outbreak was based on the introduction of a single infectious individual in a population where there is hardly any measles circulation. Based on our results, the outbreak probability would be much higher and severe if multiple cases were introduced.

SIAs in Kenya are generally conducted every 2-4 years and provide a second opportunity for vaccination in children regardless of their vaccination history and are ideally timed to close immunity gaps arising from accumulation of susceptible and vaccine failures.[25] They have been shown to be effective in increasing immunisation equity by reaching children from poor households.[26] In February 2020, at the time of the planned national SIA, we estimated that 90% (85-92) of the population were immune after adjusting for age-differences in social contact. This immunity which was equivalent to a 34% (8-54) probability of a large outbreak suggests the SIA would have been timely in closing immunity gaps. The risk of an outbreak which was accelerated by immunity gaps arising in children who missed their routinely delivered MCV1 and MCV2 continued to increase in subsequent months following the start of COVID-19 and by December 2020, the estimated risk had increased to 38% (19-54), 46% (30-59), 54% (43-64) assuming a 15%, 50% and 100% reduction in measles vaccination coverage respectively. Based on limited information on additional reductions in vaccination coverage as the pandemic progressed in Kenya's devolved counties and marked reduction in vaccination services in Kenya in May 2020 compared to January and February 2020 reported in the second WHO immunisation poll, it is highly probable most areas will experience an outbreak risk of 46% (30-59) corresponding to a 50% reduction in routine coverage.

Assuming all COVID-19 restrictions remain in place, the risk of outbreaks would only be experienced in the suspended RI scenario in 2021. The severity and timing of these outbreaks would be largely reduced if a measles vaccine campaign is delivered but it will also depend on time delay of catch-up campaigns and speed at which a campaign can be organised. In December 2020 for instance, a SIA would reduce outbreak risk to zero in all scenarios with an upper bound

risk of 15% while in December 2021, outbreak risk would reduce to zero with an upper bound risk of 25% after delivery of SIA.

The current disruption to vaccination services will cause further delays to vaccination, which is a challenge even in normal circumstances. We had previously reported consistently poor timeliness of MCV1 vaccination across 6 different birth-cohorts (2011-2016) in Kenya.[20] Here, a delay in age of MCV1 by 3 months resulted in a marginal increase in outbreak risk. For instance, assuming a 50% reduction in routine vaccination, a delay in vaccination would see the risk increase from 46% (30-59) to 53% (40-64) by the end of the year. This reiterates the importance of timeliness in administration of vaccines in children as even a slight delay may cause considerable immunity gaps.

Our results emphasize the importance of maintaining high RI coverage during this pandemic because the benefits of sustaining RI services far outweighs the risks of any excess COVID-19 deaths that may arise from vaccination clinic visits.[27] Due to the highly infectious nature of measles, massive outbreaks following disruptions to health care systems and reduced MCV1 coverage are typical. Following the West Africa Ebola outbreak in 2014-2015, Liberia, Sierra Leone and Guinea reported more than a 25% reductions in MCV1 coverage.[28, 29] Reported cases also occurred in a lower age group compared to pre-Ebola period suggesting accumulation of susceptible children who missed their vaccine doses was a key contributor. Immunity gaps continued to be felt in these countries two years later even after successful implementation of SIAs. Recently, measles outbreaks have been reported in five counties in Kenya[30] even with COVID-19 restrictions which suggests an adverse synergistic interaction between pre-existing gaps of susceptibility due to lower vaccination coverage in some counties (compared to national estimates) and a precipitous drop in RI coverage during this period. These outbreaks and our results are well aligned with recent Kenya measles risk assessment report by the Measles and Rubella Initiative, and recent WHO guidance on catch-up vaccination to close the immunity gaps caused by the COVID-19 pandemic.

As expected, majority of vaccine eligible children had protective antibody concentrations against measles while only one of 20 (0.05%) infants under 9 months old had protective levels. This suggests that there is an extended period of susceptibility in young infants probably a consequence of rapid decay of maternally acquired antibody. This will require further investigation in Kenya.

However, this phenomenon has been previously reported in areas where maternal immunity is increasingly from immunisation rather than natural infection.[31]

Our analysis was based on data from a rural area in African region. Although these results are largely representative of rural areas in measles endemic settings, they may vary in an urban setting especially as measles susceptibility profiles have been shown to vary across urban and rural settings mainly due to heterogeneity in vaccination coverage and the different mixing patterns between and within age-groups.

A key strength of our study is availability of recent serological data which provides an excellent means of directly estimating levels of population protection against infection and can also be used to guide post-COVID-19 SIAs. In addition, availability of an age-mixing matrix from the same area allowed us to estimate overall immunity by taking into account the level of contact between different age-groups.

Our study has a few limitations. Population immunity was only available for children <15 years but we varied observed immunity estimates in adults from a previous study in our model which resulted in a slight shift in overall immunity. Our results showing SIAs conducted in under-fives will mitigate the risk of measles outbreak risk are based on the assumption that majority (96%) of the older age groups have measles immunity. Susceptibility gaps in this older age-groups will require SIAs for a wider age range (e.g., 9 months to 15 years) to close population immunity gaps and reduce the outbreak risk. The serological data estimates and the mixing matrix used in our study may not be fully representative of the country although we utilised national estimates of vaccination coverage, which was the main driver of predicted immunity. We did not explicitly model MCV2 delivery but assumed the overall effectiveness was an average of MCV1 and MCV2 efficacy weighted by proportion of children who either receive MCV1 only or both doses. Finally, there is some uncertainty around the actual reduction in transmission due to variability in compliance with physical distancing measures in place. However, we accounted for uncertainty by varying both the reduction in transmission and the R0.

In conclusion, measles SIA originally scheduled for February 2020 in Kenya would have been well-timed as population immunity was below herd immunity threshold. Interruptions to RI since the start of COVID-19 pandemic restrictions in Kenya have now widened the measles immunity gap, but associated risk of large measles outbreaks were partially mitigated by COVID-19 contact restrictions in place. As these measures have almost been fully lifted, we estimate that measles

outbreak risks will dramatically increase, and an immediate SIA will be required to close measles immunity gaps.

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5. Chapter 5: Seroprevalence of antibodies against Diphtheria, Tetanus and Pertussis over a 12-year period in children in Kilifi, Kenya (2009-2021)

5.1 Research paper cover sheet

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RESEARCH PAPER COVER SHEET

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SECTION A – Student Details

Student ID Number	2003951	**Title**	Mrs.
First Name(s)	Caroline		
Surname/Family Name	Mburu		
Thesis Title	The utility of mathematical modelling of serological data in assessing the impact of vaccination programmes in Kenya		
Primary Supervisor	Stefan Flasche		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

SECTION B – Paper already published

Where was the work published?	
When was the work published?	
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion	

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SECTION C – Prepared for publication, but not yet published

Where is the work intended to be published?	To be determined
Please list the paper's authors in the intended authorship order:	Mburu CN, Ojal J, Chebet R, Ombati R , Akech D, Karia B, Tuju J, Sigilai A, Smits G, van Gageldonk PGM, van der Klis FRM, Scott JAG, Flasche S, Adetifa IMO
Stage of publication	**Prepared to be submitted**

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	Design, data acquisition, data analysis, write up, and submission

SECTION E

Student Signature	[redacted]
Date	
Supervisor Signature	
Date	

5.2 Bridging section

Presently in preparation for submission, this paper is centered on addressing objective two, part a. The study endeavors to monitor annual trends (2009-2021) in age-specific population immunity for diphtheria, tetanus, and pertussis, with a specific focus on children under 15 years and adults. The primary goal is to identify susceptible populations, providing crucial insights to inform immunisation activities. Additionally, the research evaluates the necessity of a booster pentavalent vaccine dose recommended by the World Health Organization (WHO).

Prior to this study, essential information on DPT serial age-specific population immunity profiles in Kenyan children was unavailable. Limited disease surveillance, a lack of seroprevalence data, and uncertainties in reported vaccination coverage estimates raise concerns about potential immunity gaps and the risk of future outbreaks for these diseases.

The estimates derived from this research are expected to be of significant value in monitoring the Kenyan immunisation program and, by extension, LMICs in general. The age-specific immunity profiles for DPT in both children and adults will substantially enhance existing case surveillance data, contributing to the resolution of critical knowledge gaps in the field.

I generated the estimates using a series of statistical test and wrote all the code. I also wrote the paper and generated the figures. Throughout the process I received input/suggestions/edits from my supervisors.

The supplementary material of the paper is included as Appendix E in this thesis, so any references to the supplementary material can be found there.

5.3 Abstract

Background: Current methods for evaluating performance of immunisation programmes including disease surveillance and administrative measures of vaccination coverage do not provide age-specific immunity profiles. We conducted serial seroprevalence studies to estimate age-specific population immunity trends for diphtheria, pertussis and tetanus in both children and adults in Kilifi.

Methods: Study population consisted of randomly selected participants from cross-sectional surveys conducted in Kilifi Health Demographic Surveillance System (KHDSS). IgG antibodies were measured using a fluorescent bead-based multiplex immunoassay, with protective thresholds set at 0.011 IU/ml for tetanus and diphtheria. These were further grouped into minimal, full, and long-term seroprotection levels. Pertussis antibodies were grouped based on the likely time since infection based on the lack of a defined threshold level of protection. Bayesian multilevel regression with poststratification was used to obtain seroprevalence estimates adjusted for underlying population, sensitivity and specificity of the assay. Associations between changes in seropositivity with age, gender, location and ethnic group were assessed using a mixed effects logistic regression.

Results: Only 5% of children, had diphtheria antibody titres associated with long-term seroprotection. Across surveys the proportion of children with full seroprotection against diphtheria ranged from 11% (95% CI: 05-22%) to 34% (95% CI: 26-43%) and with minimal seroprotection from 40% (95% CI: 34-46%) to 52% (95% CI: 45-58%). Tetanus seroprevalence in children was higher. The proportion of children with long-term seroprotection ranged from 10% (95% CI: 06-15%) to 39% (95% CI: 33-45%) but a substantial proportion had minimal seroprotection, ranging from 23% (95% CI: 17-28%) to 33% (95% CI: 27-38%) in survey years. Only 1% of adults had long-term seroprotection titres against diphtheria, while 36% of adults had long-term seroprotection against tetanus.

Older age was significantly associated with lower tetanus (OR=0.81, 95% CI: 0.74-0.88) and diphtheria (OR=0.86, 95% CI: 0.82-0.89) seroprevalence in children. About 5% of children and 1% of adults showed evidence of recent pertussis infection, indicated by IgG levels above 62.5 IU/ml.

Conclusion: The rapid decline in antibody titres with age, particularly for diphtheria indicates inadequate protection against disease outbreaks. This coupled with a substantial proportion of older children having minimal tetanus seroprotection, and even minimal evidence of pertussis circulating in the population, emphasizes the potential need for prolonged protection through booster pentavalent doses which are recommended at 12-23 months, 4-7 years and 9-15 years of age.

5.4 Background

Vaccination remains one of the most important and cost-effective public health interventions for the prevention and control of Vaccine Preventable Diseases (VPDs)[1]. Current methods for evaluating performance of national immunisation programmes which include disease surveillance studies and administrative measures of vaccination coverage do not show age-specific disease immunity profiles. Serological surveillance is an essential tool for monitoring VPDs as it offers an effective measure of population immunity by directly estimating the prevalence of antibodies. Seroprevalence studies in children are particularly important due to their vulnerability to infections and their potential to spread diseases given the multiple contacts in learning institutions[2].

Routine childhood immunisation schedule in Kenya was formalised in 1980 with the introduction of Kenya Expanded Programme of Immunisation (KEPI)[3] . The Diphtheria-Pertussis-Tetanus (DPT) schedule initially consisted of three doses of a trivalent vaccine at 6,10 and 14 weeks. Presently, the schedule includes three doses of pentavalent vaccine after the introduction of *Haemophilus influenzae* type B (hib) and Hepatitis B vaccines in the programme in November 2001[4]. Although WHO recommends 3 booster doses for DPT to be administered at 12-23 months, 4-7 years and 9-15 years of age to ensure long term protection, Kenya is yet to implement any of these in its immunisation programme[5, 6]

Kenya's national immunisation program has made significant progress towards VPDs elimination. For instance, maternal and neonatal tetanus were verified to be eliminated in 2019[7] while the World Health Organization (WHO) data show a decline in the number of reported cases of pertussis over the years although surveillance data is unavailable for some of the years leading to uncertainty about the quality of the data [8]. Despite these progress, Kenya national estimates of pentavalent coverage which ranged between 75-81% from 2009 to 2018 [9] are below the 90% national coverage target set by the Global Vaccine Action Plan (GVAP) for DPT elimination [10]. In addition, heterogeneities in vaccination coverage may imply some regions have an even lower coverage which could result to disease outbreaks. Poor disease surveillance, lack of seroprevalence data and uncertainties in the reported vaccination coverage estimates raises concerns about potential immunity gaps and the risk of future outbreaks for these diseases.

This study was conducted to evaluate age-specific seroprevalence for diphtheria, pertussis and tetanus in order to detect potential immunity gaps among both children and adults and assess the need of a booster vaccine dose.

5.5 Methods

5.5.1 Survey design and study population

Data was from a series of cross-sectional surveys conducted in Kilifi Health Demographic Surveillance System (KHDSS) [11] which was established in 2000 to monitor births, deaths, in-migration and out-migration in a population of approximately 280000 over an area of 891km^2.

These serosurveys including Malaria Cross-Sectional Survey[12]and the Pneumococcal Conjugate Vaccine Impact Study (PCVIs) [13] have been conducted as part of an ongoing effort to actively monitor malaria and pneumonia infections in children under 15 years of age within the KHDSS. The sample size is calculated to ensure precise estimates of seroprevalence, both overall and by age-category, for each serosurvey year. Data from 2009, 2011, and 2013 comprised an age-stratified sample of 50 children in 10 age strata (aged 0, 1, 2, 3, 4, 5, 6, 7, 8-9, and 10-14 years) randomly selected from Malaria cross-sectional survey[14].Data in 2015,2017 and 2019 was from PCVIs study collected on independent age-stratified random samples of the KHDSS in similar age-strata with 50 children in each stratum. Samples in 2021 were from a surveillance study of COVID-19 in Kenya conducted as part of the pandemic response[15] which consisted of 850 randomly selected participants including a 100 participants in 5-year age band from 0-14 years,50 participants in each 5-year age band from 15-64 years and 50 individuals aged 65 years and above.

Blood samples of about 2mls were collected from all consenting participants then separated, aliquoted, and stored at -70oC until processing.

5.5.2 Bead coupling and fluorescent bead multiplex immunoassay

IgG antibodies against diphtheria, tetanus and pertussis were determined using a fluorescent bead-based multiplex immunoassay. Bead coupling and fluorescent bead multiplex immunoassay was carried out as previously described with minor modifications[16, 17]. For tetanus and diphtheria, susceptibility is indicated by antitoxin levels below 0.011 IU/mL while protective levels range between 0.01 and 1.0 IU/mL [18, 19]. Fully protective levels are considered at or above 0.1 IU/mL,

with concentrations exceeding 1.0 IU/mL indicating long-term protection[20, 21]. Utilizing these thresholds, participants with IgG concentrations equal to or greater than 0.011 IU/mL for tetanus and diphtheria were classified as protected. These individuals were further categorized into three levels of seroprotection: minimal-seroprotection ($0.011 \leq IgG < 0.1$ IU/mL), full-seroprotection ($0.1 \leq IgG < 1$ IU/mL), and long-term seroprotection ($IgG \geq 1$ IU/mL).

No cut-off was used for pertussis but based on previous studies in Netherlands and the Gambia[22, 23],anti-pertussis toxin (PTx) IgG antibodies were categorised into 4 groups reflecting the time since infection; ≥125 IU/mL (infection in the past 6 months), 62.5 to <125 IU/mL (infection in the past 12 months), 20 to <62.5 IU/mL (infection in the past >12 months and/or vaccination response) and 0 to < 20 IU/mL (no recent infection).

5.5.3 Ethical considerations

Ethical approval was obtained from the Scientific and Ethics Review Unit (SERU) of the Kenya Medical Research Institute (Protocol SERU 3847). The serological samples were collected under a SERU-approved protocols with a provision for storage of residual samples and use in future research (SERU 1433 4085 2887,3149,3426). Written informed consent was obtained from parents/legal guardians of all participants prior to sample collection. In addition, written assent was obtained from all participants aged 13-14 years old.

5.5.4 Statistical analysis

Crude seroprevalence estimates were obtained by dividing the number of participants with positive samples by the total number tested for each survey year based on the age strata during sample collection, gender, ethnic group and location in KHDSS.

We collapsed the data into 4 age groups for children under 15yrs of age as follows; <1yr,1-4yrs,5-9yrs and 10-14yrs in order to compare the changes in seroprevalence overtime and maintained the 5-year age bands used in the sample collection for participants above 15 years of age. Multilevel regression and post-stratification (MLRP) implemented by fitting a Bayesian logistic regression model [24] that incorporated age as a variable and was used to adjust the estimates for the sensitivity and specificity of the assay and underlying population. The prior distributions for the sensitivity and specificity estimates were derived from the original study, which reported values

of 99% for sensitivity and 92% for specificity for diphtheria, 99% for sensitivity and 93% for specificity for tetanus and 99% for sensitivity and 94% for specificity for pertussis during the development of the multiplex assay[16].The models were fitted using rjags software package. Age-specific seroprevalence estimates were visualized using bar graphs and differences between groups in each year determined using chi-square test and fishers exact test where appropriate. We employed a mixed effects logistic regression to examine the association between seropositivity and several demographic factors including year, sex, age, location, and ethnic group. The selection of these variables was driven by specific hypotheses regarding their potential influence on seroprevalence. For instance, we hypothesized that year might influence seroprevalence due to changes in vaccination coverage over time. Additionally, considering the biological differences between sexes that could affect immune response and susceptibility to infections, we included sex as a variable. Moreover, we anticipated that geographic variations in disease prevalence, access to healthcare, and environmental factors could impact seropositivity, thus necessitating the inclusion of location. Lastly, socio-cultural factors and differential healthcare access among ethnic groups were thought to potentially influence seropositivity rates, hence the inclusion of ethnic group as a variable.

Geometric mean concentrations(GMCs) and the 95% confidence levels were also calculated, and values tabulated after adjusting for underlying population. To display the variability in age-specific IgG concentrations, raw antibody titres were log transformed and displayed via boxplot for each survey year. One-way ANOVA was used to test differences in GMCs between groups in each year. Statistical analyses were conducted using the R-statistical software[25].

5.6 Results

5.6.1 Characteristics of the study population

The study population comprised of 2686 participants of which 1381(51%) were males. Median age of all participants was 6 yrs. (IQR: 3-8 yrs.). Ages were comparable between men (median:5 IQR:3-8) and women (median: 6, IQR:3-8, p=0.198). The number of participants across the survey years ranged between 290 in year 2021 and 520 in 2019. Crude estimates of proportion of participants with at basic seroprotection against diphtheria were much lower compared to tetanus and declined across the ages. Majority of participants were from North and South location in Kilifi and the Chonyi and Giriama ethnic groups (Table 5:1).

5.6.2 Immunity against Diphtheria

Majority of the children had minimal seroprotection against diphtheria in all the survey years which ranged between 40% CI: (34-46%) in 2011 and 52% CI: (45-58%) in 2013 (Table s5:1 and Fig. 5:2). Proportion of children with full seroprotection ranged between 11% CI: (05-22%) in 2009 to 34% CI: (26-43%) in 2021. Only less than 5% of children had long-term seroprotection against diphtheria in all the years.

Significant heterogeneity in seroprevalence levels across ages was observed in all surveys ($P<0.05$). The immunity trend across ages remained consistent across survey years whereby susceptible children and those with minimal seroprotection increased with age, while the proportion with full seroprotection decreased with age. The proportion of children with long-term seroprotection also generally decreased with age, although this trend was not consistently observed in all years (Table s5:1 and Fig. 5:2).

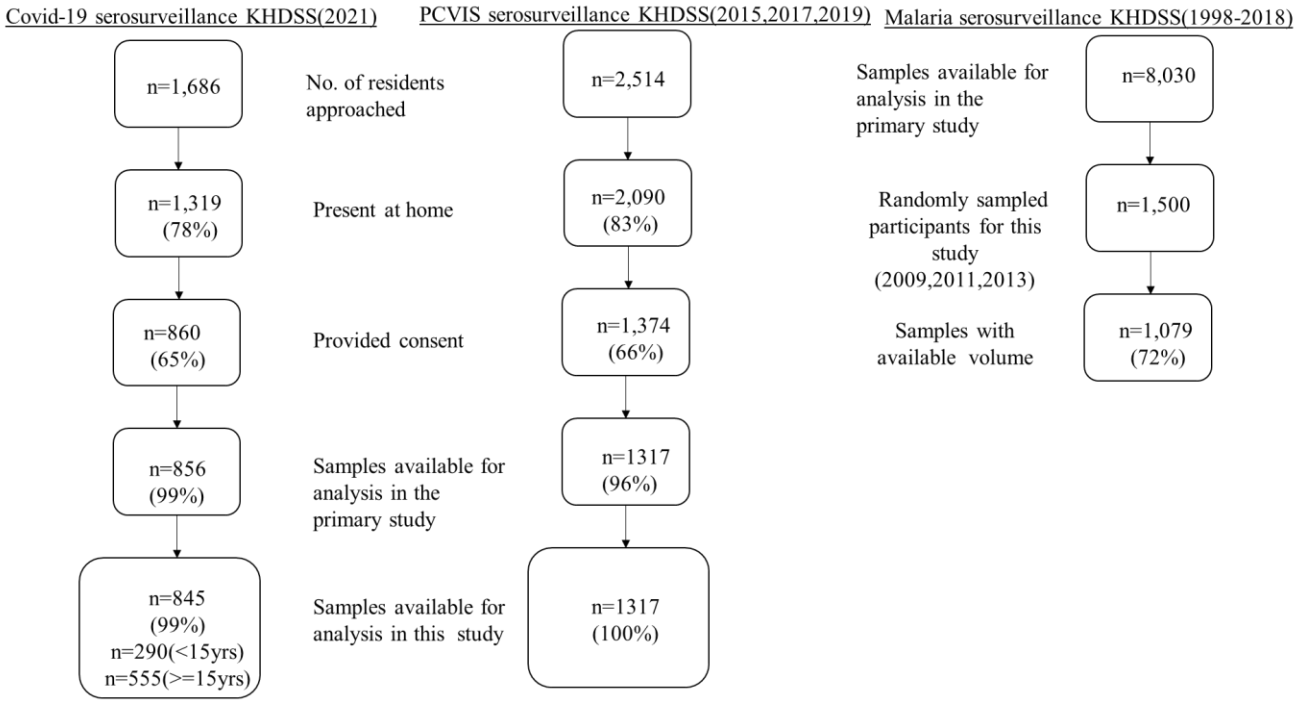

Figure 5:1 Flow diagrams illustrating the recruitment and participation processes from the three serosurveillance studies utilized in our dataset. Data from 2009, 2011, and 2013 were randomly sampled from the Malaria-cross-sectional survey population using the sampling strategy of the PCVIS study

Table 5:1 Proportion of participants with minimal seroprotection ((≥0.011IU/ml) against tetanus (TT) and diphtheria (DP) and total number of participants per survey year (n) stratified by age strata during sample collection, sex, location in KHDSS and ethnic group

Survey year	**2009(5th June-19th Sep)**			**2011(25th Jun-4th Nov)**			**2013(29th Jun-10th Nov)**			**2015(1st Jul-31st Oct)**			**2017(28th Jun-8th Nov)**			**2019(29th Jun-7th Jul 2019)**			**2021(1st Jan-31st May)**			
	n	DP(%)	TT(%)	n	DP(%)	TT(%)	n	DP(%)	TT(%)	n	DP(%)	TT(%)	n	DP(%)	TT(%)	n	DP(%)	TT(%)		n	DP(%)	TT(%)
Age in years																			Age in years			
<1	14	92	98	14	92	98	7	90	98	35	95	98	27	92	98	39	96	98	0-4	93	91	98
1	31	89	98	19	91	98	45	89	98	34	93	98	39	95	98	48	96	98	5_9	102	93	98
2	30	79	96	22	91	98	22	94	98	34	89	98	38	90	99	49	92	98	10_14	95	81	95
3	34	67	98	19	79	98	29	93	98	38	85	98	47	91	99	54	91	97				
4	38	62	97	22	81	98	27	95	98	38	85	98	36	80	98	54	89	97				
5	44	67	98	18	73	98	25	92	98	39	76	97	49	68	98	50	81	97				
6	58	51	96	23	66	96	31	83	97	29	78	95	49	88	99	54	61	97				
7	38	73	98	34	61	96	26	70	98	40	71	98	44	74	98	55	72	98				
8_9	62	80	97	67	77	96	66	70	94	45	67	98	43	86	92	61	77	90				
10_14	16	66	98	70	77	99	128	77	95	44	74	95	49	62	95	56	77	93				
Sex																						
Female	179	71	97	151	73	96	197	84	98	172	81	98	212	80	98	262	85	93		132	86	94
Male	186	74	97	157	85	99	209	86	95	204	85	98	209	89	98	258	85	99		158	95	98
Location																						
North	83	63	94	2	99	99	90	84	94	150	78	99	158	84	98	157	84	93		2	99	99
South	206	71	98	251	81	98	230	82	97	108	86	97	130	83	98	187	85	98		2	46	46
Township	5	79	79	1	0	99	4	46	74	111	84	97	119	83	98	172	84	98		Na	Na	Na
Unspecified	71	76	99	54	67	95	82	92	99	7	70	99	14	93	99	4	46	19		286	90	96
Ethnic group																						
Chonyi	129	75	96	144	82	97	138	83	96	127	86	98	135	82	98	184	85	98		2	49	46
Giriama	105	64	95	34	83	99	112	85	95	173	81	98	195	85	97	222	84	96		2	99	99
Jibana	48	75	99	57	83	99	58	76	95	5	80	99	7	86	85	9	90	99			Na	Na
Kauma	7	72	99	12	84	99	12	99	92	48	84	97	44	85	99	72	91	95			Na	Na
Others	76	77	99	61	67	94	86	91	99	23	83	99	40	91	99	33	76	81		286	99	96
Total	365	72	97	308	79	98	406	85	96	376	83	98	421	84	98	520	84	96		290	91	96

A comparable pattern was observed in GMC levels, showing significant variation across age groups (P <0.05) and a decline with age (Table s5:2 and Fig. 5:2). GMC levels in children under one year exceeded the full protection threshold in majority of the surveys, while those in children aged one to nine were below the full seroprotection threshold but above the minimum seroprotection threshold. In some years, GMCs in the oldest age group dropped below the minimum seroprotection threshold (Fig. 5:2).

Figure 5:2 Age-stratified proportion of the children in Kilifi County Population who were immune to diphtheria. The red dotted line in the below figure is the threshold for minimal seroprotection (0.011≤IgG<0.1 IU/ml), the green dotted line is the threshold for full seroprotection (0.1≤IgG<1 IU/ml) and the orange dotted line is the threshold for long-term protection (IgG≥1 IU/ml). From both the bar graphs and boxplots, a high proportion of the children had either minimum or full seroprotection while only a small proportion had long-term seroprotection. Immunity gaps were evidence from the increase in proportion of susceptible across the ages.

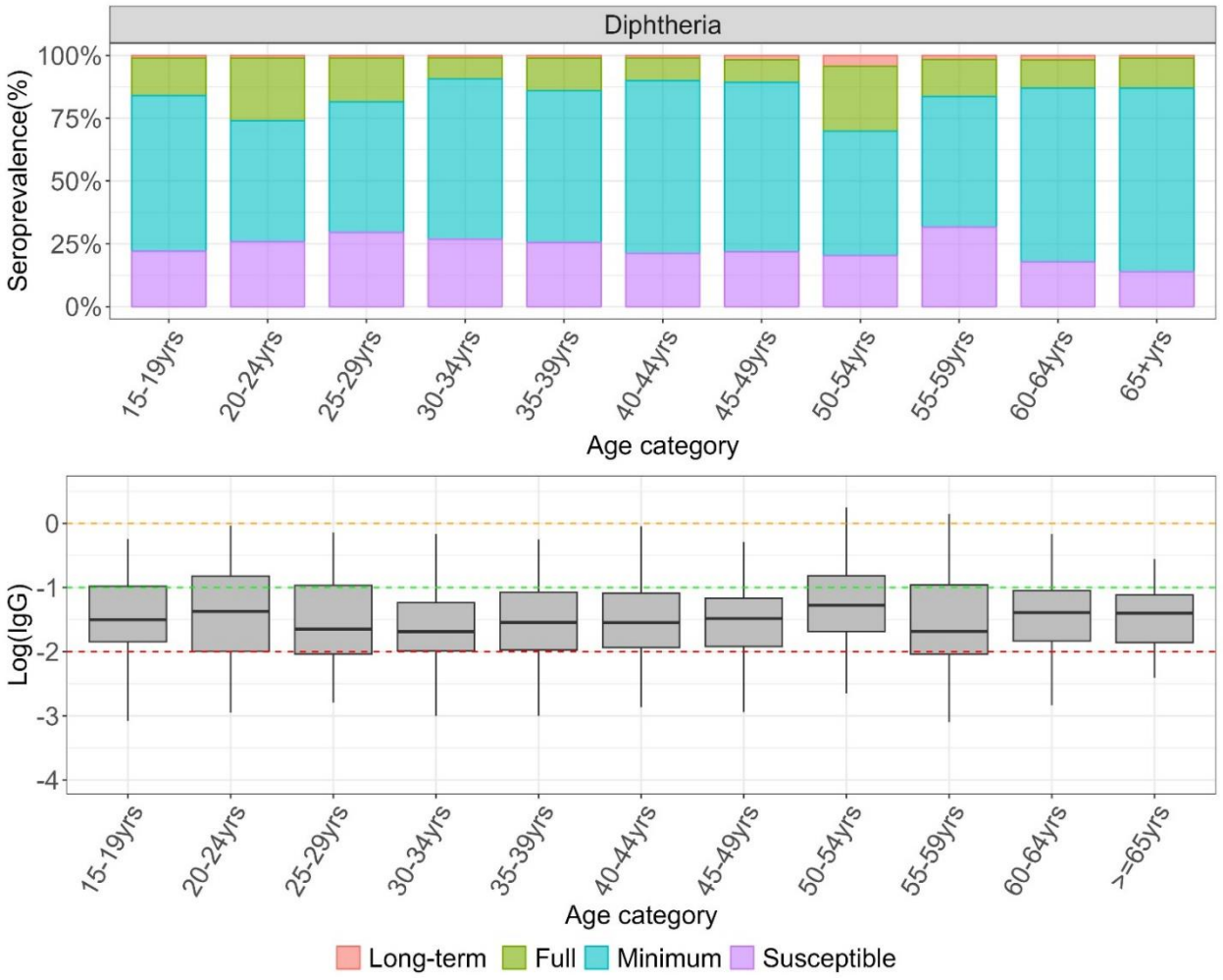

Figure 5:3 Age-stratified proportion of adults in Kilifi County Population who were immune to Diphtheria in 2021.Immunity gaps are evident across all the age groups and only less than one percent adults have long-term immunity

In the multivariate logistic regression analysis, a year increase in age was significantly associated with lower seropositivity (OR=0.86,95%CI:0.82-0.89), males had significantly lower seropositivity compared to females (OR=0.73,95%CI:0.58-0.91) and all subsequent years demonstrated significantly higher seropositivity than the baseline year 2009 (Table 5:2). Diphtheria seroprevalence showed no variation based on different locations or the different ethnic groups compared to the baseline.

Diphtheria immunity gaps were even more pronounced after inclusion of older groups (≥15 years) (Table s5:3 and Fig. 5:3). Less than 1% of adults had long-term seroprotection against diphtheria, compared to 17% with full seroprotection and 50% with minimal seroprotection.

Table 5:2 Diphtheria and Tetanus multivariate logistic regression results

	Tetanus	**Diphtheria**	**Pertussis**		
			<20 IU/ml	20-<62.5 IU/ml	≥62.5 IU/ml
Predictors	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Gender	1	1	1		
Male	0.91[0.55-1.50]	0.73[0.58-0.91]	0.78[0.65-0.91]	1.14[0.93-1.40]	1.37[1.03-1.84]
Age	0.81[0.74-0.88]	0.86[0.82-0.89]	0.97[0.94-1.01]	1.01[0.98-1.04]	1.05[1.00-1.10]
Survey year					
2009	1	1	1	1	1
2011	1.42[0.58-3.66]	1.85[1.28-2.72]	0.90[0.64-1.24]	1.58[1.07-2.35]	0.57[0.34-0.94]
2013	1.46[0.64-3.24]	3.12[2.13-4.57]	0.90[0.65-1.22]	1.42[0.98-2.06]	0.70[0.45-1.11]
2015	1.98[0.71-6.06]	2.25[1.46-3.57]	0.87[0.60-1.24]	1.55[1.01-2.36]	0.66[0.38-1.14]
2017	2.39[0.90-6.75]	2.50[1.65-3.89]	1.03[0.72-1.46]	1.50[0.99-2.25]	0.42[0.24-0.75]
2019	1.43[0.61-3.29]	2.56[1.76-3.82]	0.99[0.72-1.37]	1.38[0.94-2.03]	0.61[0.37-1.00]
2021	1.21[0.54-2.80]	5.01[3.22-8.01]	1.05[0.93-1.43]	2.03[1.40-2.96]	0.95[0.59-1.51]
Ethnic group					
Chonyi	1	1	1	1	1
Jibana	2.35[0.74-9.90]	0.91[0.61-1.43]	0.86[0.66-1.13]	0.82[0.52-1.26]	0.73[0.39-1.28]
Giriama	1.18[0.57-2.41]	0.93[0.70-1.37]	1.31[0.92-1.86]	1.15[0.84-1.56]	1.08[0.70-1.67]
Kauma	1.20[0.37-4.64]	1.33[0.79-2.45]	0.58[0.34-0.99]	1.12[0.70-1.76]	0.44[0.17-0.99]
Other	0.65[0.19-2.90]	0.86[0.41-1.73]	0.13[0.75-0.83]	1.24[0.66-2.28]	2.06[0.95-4.26]
Location					
South	1	1	1	1	1
North	0.41[0.19-0.88]	0.97[0.68-1.44]	1.15[0.85-1.53]	0.96[0.68-1.34]	0.77[0.48-1.23]
Township	0.84[0.28-2.54]	0.80[0.55-1.37]	1.06[0.74-1.52]	0.97[0.65-1.44]	0.89[0.49-1.60]
Unspecified	2.85[0.48-14.4]	1.64[0.71-3.61]	1.76[0.97-3.30]	0.90[0.45-1.83]	0.36[0.15-0.90]

5.6.3 Immunity against Tetanus

The proportion of children with protective tetanus antibodies in Kilifi was relatively high (Table s5:1 and Fig. 5:4). In all survey years, the majority of children maintained full seroprotection against tetanus, ranging from 24% CI: (09-37%) in 2011 to 46% CI: (34-56%) in 2019. The proportion with long-term seroprotection varied from 10% CI: (06-15%) in 2017 to 39% CI: (33-45%) in 2011. A substantial proportion of children exhibited minimal seroprotection, ranging from 23% CI: (17-28%) in 2021 to 33% CI: (27-38%) in 2017.

Significant heterogeneity in seroprevalence levels across ages was observed in most of the years($P<0.05$). The trend in immunity across ages was consistent, with children under one year having the highest levels of long-term seroprotection, those aged one to nine displaying the highest

levels of full seroprotection, and the oldest age group exhibiting the highest levels of minimal seroprotection. The proportion of children susceptible to tetanus was consistently less than 1% in all surveyed years (Table s5:1 and Fig. 5:4).

While GMC levels exhibited significant variation across age groups (P <0.05) and decreased with age (Table s5:2 and Fig. 5:4), the waning observed was less pronounced compared to diphtheria. Log IgG concentrations across all age groups remained above the full seroprotection thresholds, except for the oldest age group, where GMC levels fell below the full seroprotection threshold in certain years (Fig. 5:4).

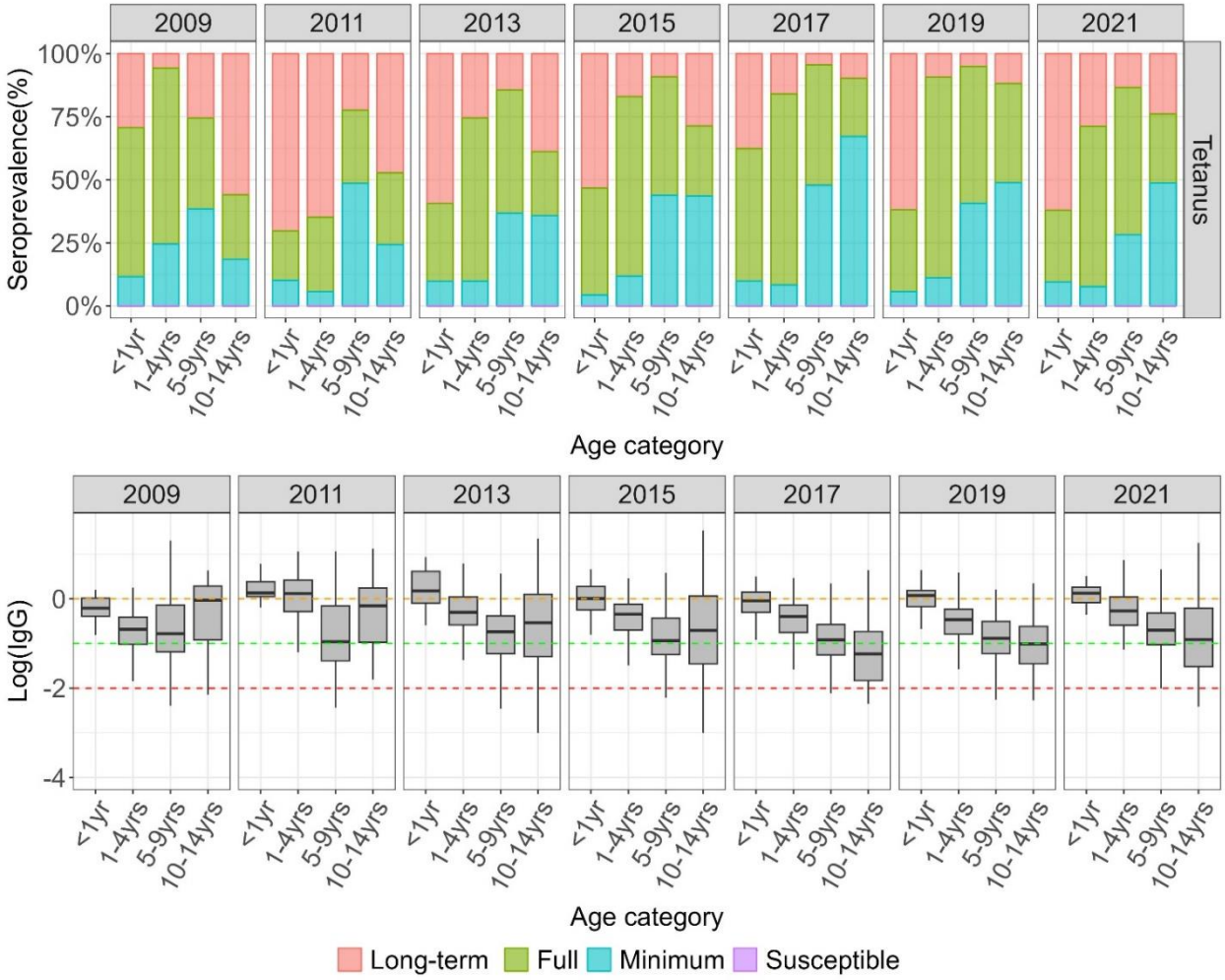

Figure 5:4 Age-stratified proportion of the children in Kilifi County Population who were immune to Tetanus. The red dotted line in the below figure is the threshold for minimal seroprotection (0.011≤IgG<0.1 IU/ml), the green dotted line is the threshold for full seroprotection (0.1≤IgG<1 IU/ml) and the orange dotted line is the threshold for long-term protection (IgG≥1 IU/ml). From the figures, there is a high level of immunity evidenced by the high proportion of children with full and long-term immunity and the low proportion of susceptible

In the logistic regression analysis, a year increase in age was significantly associated with lower tetanus seroprevalence (OR=0.81,95%CI:0.74-0.88) while significantly higher tetanus seroprevalence was found in north of KHDSS compared to south (OR=2.43,95% CI:1.15-5.21). Tetanus seroprevalence showed no variation based on gender, different years compared to 2009, or the different ethnic groups compared to the baseline (Table 5:2).

Proportion of adults with long-term seroprotection against tetanus was 36% while only less than 1% were susceptible. Adult females had slightly higher seroprotection levels compared to adult males (Fig 5:5).

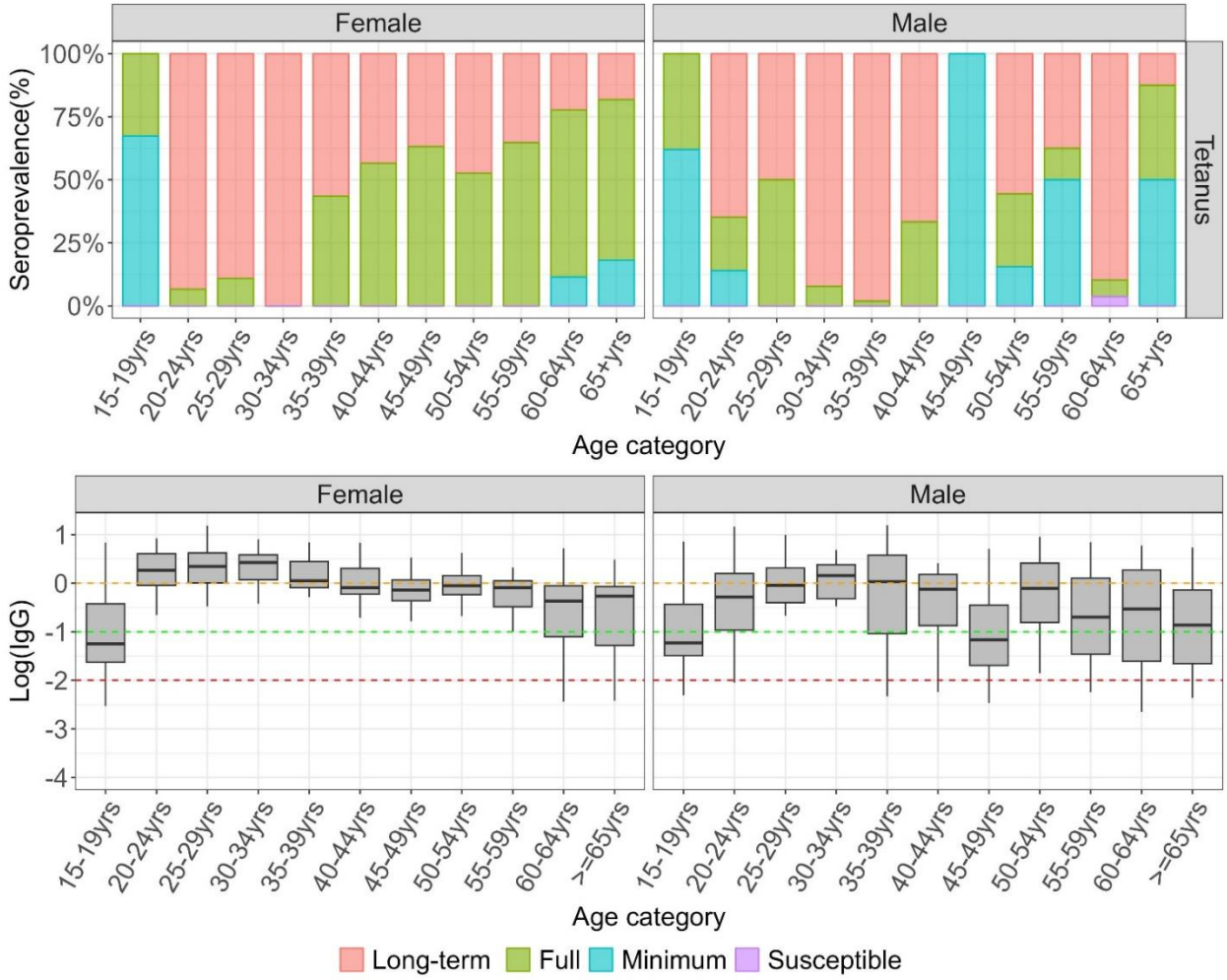

Figure 5:5 Age-stratified proportion of adults in Kilifi County Population who were immune to Tetanus in 2021 stratified into Males and Females. Majority of adults in Kilifi have long-term protection against tetanus while only less than one percent is susceptible.

5.6.4 Likely time of infection for Pertussis

Majority of children in Kilifi have not recently experienced a pertussis infection, evident from the high proportion of IgG levels between 0-20 IU/ml across all age groups in all survey years (Table s5:4 and Fig. 5:6). This proportion varied from 49% CI: (42-55%) in 2021 to 63% CI: (34-73%) in 2017. Conversely, about 5% of children across different age groups exhibited recent pertussis infection in the past 12 months (IgG≥62.5 IU/ml) in all survey years. The proportion of children with either a vaccination response and/or infection more than one year ago ranged from 11% CI: (03-21%) in 2009 to 21% CI: (16-27%) in 2021.

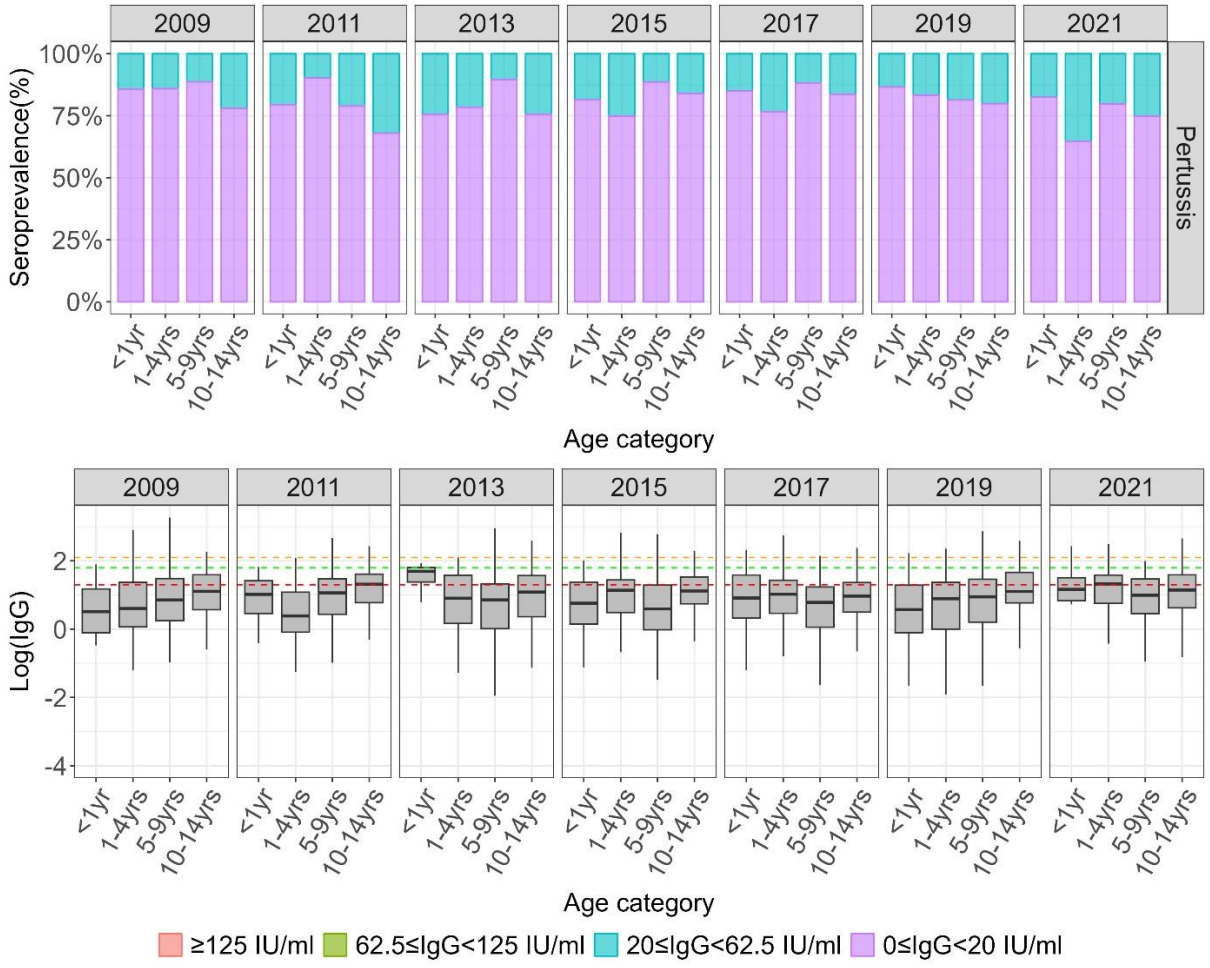

Figure 5:6 Age-stratified distribution of the Pertussis IgG intervals in children in Kilifi County Population. The orange dotted line is the threshold for IgG levels equal or above 125 IU/mL (infection in the past 6 months), the green dotted line is the threshold for IgG levels equal or above 62.5 (infection in the past 12 months) while the red line is the threshold for IgG levels equal or above 20 IU/mL (infection in the past>12 months or vaccination response). Log IgG levels below the red line are below 20 IU/mL (no recent infection). From the results a high proportion of the population has not had any recent infection.

There was a significant heterogeneity in GMCs levels across the ages in some of the years where levels decreased with increasing age up to around 4 years and then increased afterwards but this trend was not consistent across the years (Table s5:4 and Fig. 5:6). Among adults, 63% (CI: 58-67%) have not experienced a recent infection while 23% (CI: 19-27%) had an infection more than a year ago (Table s5:5 and Fig. 5:7).

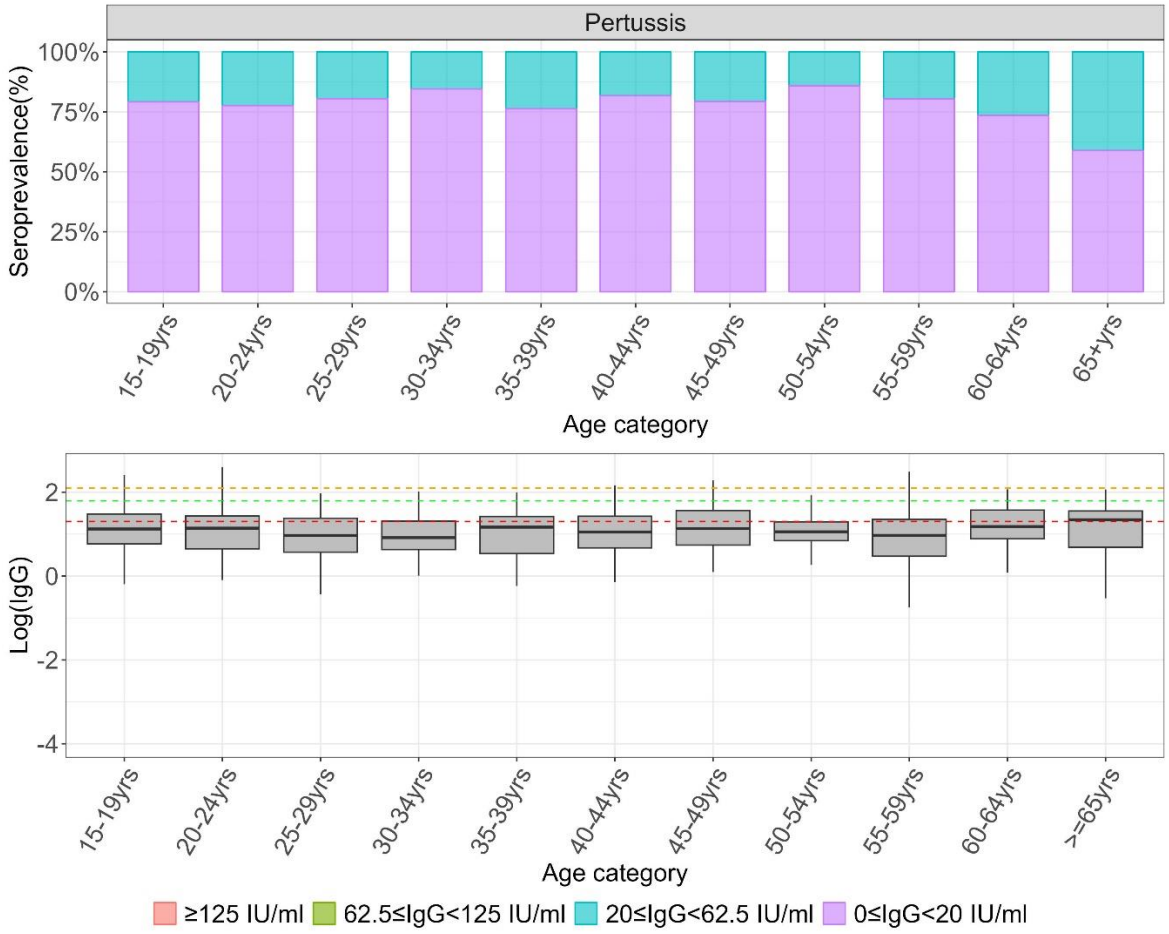

Figure 5:7 Age-stratified distribution of the Pertussis IgG intervals in adults in Kilifi County Population in 2021.Majority of adults in Kilifi have not had any recent infection

In the logistic regression analysis, increasing age was significantly associated with higher odds of having antibody concentrations ≥62.5 IU/ml (OR=1.05,95%CI: 1.00-1.10) while females had significantly higher odds of having antibody concentrations ≥62.5 IU/ml compared to males (OR=1.37,95% CI:1.03-1.84) (Table 5:3)

5.7 Discussion

5.7.1 Main results

The above study whose aim was to evaluate the current age-specific seroprevalence for diphtheria, tetanus and pertussis among children in Kilifi over a decade provides important data for Kenya's immunisation programme and LMICs in general. The study also gives a snapshot of immunity levels in adults based on one year of study. We identified inadequate diphtheria immunity levels, with the proportion of children having at least a full level of protection falling below the 84-89% herd immunity threshold[26, 27] in all the survey years, suggesting insufficient protection against outbreaks. This trend was mirrored in the adult population, where only about 20% had full seroprotection, and merely 1% maintained long-term seroprotection against diphtheria. On the contrary, immunity against tetanus remained consistently robust throughout all the years. Approximately 70-80% of children consistently demonstrated at least full protection across different years. However, there was still a substantial proportion, especially among older children, with minimal seroprotection. Our study also showed low evidence of recent pertussis infection in the last one year as only 5% of children and less than 1% of adults had antibody titres above or equal to 62.5 IU/ml.

In all antigens, post-primary vaccination antibody titres were notably lower than the reported administrative coverage of the pentavalent vaccine between 2009 and 2019, ranging from 75% to 85%[28]. This discrepancy can be attributed to various factors. Firstly, administrative coverage estimates are susceptible to inaccuracies, including errors in recording vaccine doses and assumptions about the target population size, leading to discrepancies[29]. Secondly, the lower antibody levels may result from the failure to seroconvert, as vaccination does not always guarantee immunisation against the targeted disease. For example, after the primary series, 94-100% of infants have antibody levels higher than 0.01 IU/ml for diphtheria [30] while for tetanus seroconversion rates after primary immunisation has been shown to range between 98% to 100% in children who receive two or more vaccine doses [31, 32].For pertussis, there are no established Correlates of Protection(CoPs).The immune response to pertussis vaccination exhibits notable variations, and vaccine efficacy rates after vaccine administration have been observed to range from 53% to 92% depending on the pertussis case definition used[33, 34]. Thirdly, discrepancies may stem from poor CoPs for diphtheria and tetanus. CoPs, determined based on assay

instructions, are pre-established without considering the context of the populations of interest, potentially introducing bias as IgG responses can vary due to genetic heterogeneity[35]. Lastly, heterogeneities in subnational and national coverage estimates could contribute to the observed discrepancies as suggested in other studies[36].

5.7.2 Diphtheria

The diphtheria immunity gaps among children in Kilifi is especially worrying, as it indicates a lack of population protection by herd immunity among children and suggests high risk of outbreaks. Low diphtheria seroprevalence among adults has been observed in other countries[37, 38]. However, herd immunity among children has been suggested as protective factor preventing diphtheria outbreaks in these populations. This phenomenon was evident in countries in western Europe, where despite immunity gaps in adults, the absence of diphtheria outbreaks was attributed to the high levels of immunity among children[38]. In contrast, low diphtheria seroprevalence among adults, coupled with suboptimal population immunity in children, contributed to significant outbreaks in several countries during the 1990s[39-41].

The lower immunity observed in diphtheria, in contrast to tetanus post-primary vaccination, despite their simultaneous administration in the pentavalent vaccine during infancy, has also been documented in serosurveillance studies among children in Tajikistan[39] and in the US[42]. These variations may stem from differences in the immune response to each antigen, influencing the levels of immunity achieved. Notably, tetanus toxoid vaccine has been shown to be one of the most immunogenic and highly stable antigens in normal room temperatures with very low reported rates of primary vaccine failure[18, 39].

Seroprevalences for diphtheria and tetanus in older children also differ substantially. Diphtheria exhibited more pronounced gaps in population immunity and lower levels of full seroprotection compared to tetanus, suggesting potentially higher waning rates. This aligns with prior research indicating that diphtheria immunity declines more rapidly than tetanus. One study found that 67% of children lacked sufficient diphtheria immunity 3 to 13 years after a primary series of three vaccine doses[43], and another observed a five-fold declining antibody titres within the first year following a primary series[44].In contrast, tetanus antibody titres have been shown to decline with a half-life of 11 to14 years[45] although the duration of protection following primary immunisation

varies due to differing vaccination schedules across countries. Overall, diphtheria findings indicate high susceptibility levels in both children and adults.

5.7.3 Tetanus

While tetanus immunity was much higher than that of diphtheria, a substantial proportion of older children still exhibited minimal seroprotection. Similar immunity gaps have been identified in other regions of Kenya[46],an indication that despite the success in eliminating maternal and neonatal tetanus in the country, sustaining long-term immunity remains crucial. These findings underscore the importance of administering a robust third pentavalent dose and introducing booster doses for school-going children, aligning with WHO recommendations[5]. Booster doses have been previously shown to be effective in closing these immunity gaps in older children where a survey showed tetanus seroprotection to be lower among children aged 5-14 years compared to 1-4 years of age in Kenya and Tanzania but not in Mozambique which has incorporated the recommended pentavalent booster doses in school going children in their immunisation program[46]. Tetanus immunity showed a slight elevation in adult females compared to males. These observed gender differences were anticipated and have been previously documented in countries providing a Tetanus Toxoid-containing vaccine (TTCV) for women of reproductive age [46, 47].

5.7.4 Pertussis

The majority of children exhibited low pertussis antibody titres, less than 20 IU/ml, suggesting an overall low level of pertussis transmission in the population. Our findings indicate that, on average, 5% of children and less than 1% of adults had a pertussis infection in the past year, as evidenced by antibody titres above or equal to 62.5 IU/ml. This recent infection was significantly associated with older children and females. Higher antibody concentrations observed in older children compared to young children align with an increased risk of infection with age, implying that immunity from childhood vaccination or disease may not be lifelong. However, since distinguishing between antibodies induced by vaccination or infection is challenging, observed high antibody titres in older children could also result from natural boosting[48].

Our findings of circulating pertussis in the population albeit minimal, align with observations in other African countries, such as The Gambia. A 2008 survey in The Gambia reported a 6% prevalence of recent pertussis infection within the last year, despite high vaccination coverage for the primary doses and a booster at 18 months[23].The study suggested that current pertussis vaccination strategies might not be optimal, advocating for alternative approaches like incorporating adolescent booster vaccinations. Similarly, a study in Senegal identified evidence of recent pertussis infection in the population which was said to be indicative of endemic pertussis in the population[49].

5.7.5 Strengths

This extensive seroprevalence data utilized a random sampling strategy to ensure its representativeness within children in the KHDSS area. The dataset also boasted a substantial number of participants which ensured robust statistical power. Serum samples were simultaneously tested using a highly sensitive and specific fluorescent bead-based multiplex immunoassay[50] which has been shown to be the future of sero-diagnostics for surveillance and epidemiology[51]. Additionally, the dataset was derived from a series of cross-sectional surveys conducted over different years, providing a temporal perspective on the evolving seroprevalence of diphtheria, tetanus and pertussis across different age groups within the population. Overall, this dataset will significantly contribute to the understanding of immunity levels for these diseases in Kenya, serving as a valuable case study for LMICs.

5.7.6 Limitations

The main limitation in this study lies in the generalisability of these findings to diverse settings. The study was carried out in a rural area within the African region, where most VPDs including diphtheria, tetanus and pertussis are endemic. Although these results are largely representative of rural areas in DPT endemic settings with similar vaccination schedule and coverage, the estimates may exhibit significant variation across rural and urban settings primarily due to differences in vaccination coverage, levels of natural exposure, and distinct mixing patterns across various age groups.

5.7.7 Conclusion

It is imperative to intensify efforts aimed at improving the coverage of the three-dose vaccination which is crucial for increasing the levels of full seroprotection against tetanus and diphtheria in children post-primary vaccination. The swift decline in antibody titres with age, particularly for diphtheria indicates inadequate protection against disease outbreaks. This coupled with a substantial proportion of older children having minimal tetanus seroprotection, and even minimal evidence of pertussis circulating in the population, emphasizes the potential need for prolonged protection through booster pentavalent doses—an element currently absent from the Kenyan vaccination schedule.

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6. Chapter 6: Estimating tetanus immunisation coverage from vaccination records and cross-sectional serological surveys in Kilifi

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6.1 Research paper cover sheet

RESEARCH PAPER COVER SHEET

Please note that a cover sheet must be completed for each research paper included within a thesis.

SECTION A – Student Details

Student ID Number	2003951	**Title**	Mrs.
First Name(s)	Caroline		
Surname/Family Name	Mburu		
Thesis Title	The utility of mathematical modelling of serological data in assessing the impact of vaccination programmes in Kenya		
Primary Supervisor	Stefan Flasche		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

SECTION B – Paper already published

Where was the work published?	
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Where is the work intended to be published?	To be determined
Please list the paper's authors in the intended authorship order:	Mburu CN, Ojal J, Chebet R, Ombati R , Akech D, Karia B, Tuju J, Sigilai A, Smits G, van Gageldonk PGM, van der Klis FRM, Scott JAG , Adetifa IMO, Flasche S
Stage of publication	**Prepared to be submitted**

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	Design, data acquisition, data analysis, write up, and submission

SECTION E

Student Signature	[redacted]
Date	

Supervisor Signature	[redacted]	
Date		

6.2 Bridging section

Currently in preparation for submission, this paper is dedicated to addressing objective two, part b. The study utilizes tetanus immunity profiles obtained in objective two, part a, along with vaccination coverage estimates obtained from vaccine records. The goal is to assess the effectiveness of tetanus serological data in identifying gaps in vaccination, particularly in scenarios where vaccination records are unavailable.

The estimates generated through this research are anticipated to play a crucial role in monitoring the Kenyan immunisation program. This is especially significant as organizations like Gavi and other international agencies closely examine how countries evaluate the effectiveness of their immunisation services. Providing credible data to justify financial investments becomes essential, and this paper aims to contribute valuable insights in this regard.

I conducted the analysis using a series of statistical test and wrote all the code. I also wrote the paper and generated the figures. Throughout the process I received input/suggestions/edits from my supervisors.

The supplementary material of the paper is included as Appendix F in this thesis, so any references to the supplementary material can be found there.

6.3 Abstract

Background: Vaccination coverage estimates, such as from administrative records or household surveys, rely on record keeping that is not always implemented sufficiently well and do not necessarily reflect the proportion of the population who was successfully immunised. We evaluate the utility of tetanus serological data in identifying vaccination gaps, particularly in situations where vaccination records are absent.

Methods: The study population consisted of randomly selected children from cross-sectional surveys conducted in Kilifi Health Demographic Surveillance System (KHDSS). IgG antibodies against tetanus were measured using a fluorescent bead-based multiplex immunoassay. Vaccination records were obtained from the Kilifi Vaccine Monitoring System (KVMS). We linked tetanus serological data and vaccination records and explored their association using logistic regression models, controlling for age and sex.

Results: The serological study included 2,757 children, 2426 (88%) had records on their vaccination history. We found a clear association between tetanus IgG levels and the number of vaccine doses, with seroprevalence decreasing from 97% (95% CI: 96 to 98) for three doses to 92% (95% CI: 84 to 96) for two doses, and 79% (95% CI: 59 to 90) for one dose. Remarkably, children with only one documented dose exhibited a high seroconversion rate, while those with no documented doses also showed a high seroprevalence of 72% (95% CI: 49 to 87). 12% of the samples lacked vaccine records yet demonstrated a seroprevalence of 93% (95% CI: 89 to 95). Our research further revealed a decrease in antibody levels with age, estimating a half-life of 14 years (95% CI, 12–17). Among all predictive models, IgG levels appear to be associated with documented vaccination status, although the model performance varied based on the outcome structure, with AUC scores ranging from 68% to 82%.

Conclusion: Our findings illustrate the utility of serological surveys to identify tetanus immunity gaps associated with under-vaccinated individuals or communities.

6.4 Background

Vaccination stands as one of the most crucial and cost-effective public health interventions for the prevention and control of vaccine preventable diseases (VPDs). At present, about 3 million deaths are averted annually while 1.5 million deaths stand to be avoided if global vaccination coverage can be improved [1]. Vaccine coverage estimates are often used as a measure of effectiveness of vaccination programs, as a correlate of population immunity to VPDs and to infer the residual risk of disease[2] . Additionally, these estimates provide evidence on whether vaccination targets have been met which may inform and guide decisions for performance-based funding [3].

Coverage of the 3rd dose of a diphtheria-pertussis-tetanus (DPT3)-containing vaccine at age 12 months in a sample of children aged 12-23 months is frequently used as a benchmark of a country's vaccination programme as a whole[4]. Vaccine coverage is commonly measured using administrative records or household surveys of vaccination cards and maternal recall [5]. However, data from these sources that should pinpoint areas needing improved immunisation services are often incomplete due to challenges with record keeping. In addition, vaccine receipt may only offer limited insights into population immunity as vaccination does not always guarantee immunisation and immunity may wane over time.

Serological surveys offer a complementary approach to quantify immunisation coverage as they directly estimate the levels of population protection, indicating effective coverage against VPDs [6]. Serological markers selected with respect to age, can be used to gauge the effectiveness of immunisation services by identifying vaccination gaps particularly in regions with inadequate record-keeping systems. Despite their many advantages, serosurveys are resource intensive and require technical expertise to conduct. Their utility hinges on the availability of reliable correlates of protection, the ability to distinguish vaccine-induced immunity from natural infection, and the capacity to account for waning immunity[7].

Tetanus does not transmit from person to person, whether directly or indirectly, and natural infection does not provide any immunity: a small amount of tetanus toxin has been shown to be enough to cause an infection but insufficient to generate protective antibody levels [8-10]. Some early studies have suggested the possibility of natural immunity developing after asymptomatic colonization of the intestinal tract[11-13] although the role of this immunity in preventing tetanus has not been thoroughly investigated. Tetanus toxoid vaccine is one of the most immunogenic and

highly stable antigens in normal room temperatures with very low reported rates of primary vaccine failure[8]. Seroconversion rate after primary immunisation has been shown to range between 98% to 100% in children who receive two or more vaccine doses and above[14-16]. The correlate of protection against infection for tetanus immunity is assay-specific, with concentrations above or equal to ≥0.011 IU/ml for modified ELISAs or bead-based immunofluorescence assays considered protective against disease[17]. Consequently, assessment of tetanus antibodies with a highly sensitive assay has been suggested as having potential value in evaluating the prevalence of immunised children thereby identifying vaccination gaps in absence of vaccination records [7, 10].

In Kilifi, where health and demographic surveillance was established in 2000[18], regular serological surveys have been conducted since 2009 alongside monitoring for vaccine uptake. We use this unique data to compare vaccination coverage from vaccination records against tetanus antibody concentrations and explore the utility of tetanus serology in identifying vaccination gaps, especially in regions where vaccination status is uncertain, or record-keeping is deficient.

6.5 Methods

6.5.1 Data sources

The serological data from 2009 to 2019 originated from two cross-sectional surveys: the Malaria Cross-Sectional Survey [19, 20] which is in part longitudinal(thesis chapter 2) and the Pneumococcal Conjugate Vaccine Impact Study (PCVIS)[21] which is primarily cross-sectional in nature. These surveys were conducted within the Kilifi Health Demographic Surveillance System (KHDSS)[18] as part of an ongoing effort to actively monitor malaria and pneumonia infections in children under 15 years of age. The data in 2021 was from participants who were recruited from COVID-19 serosurveillance in Kenya conducted as part of the pandemic response [22].The comprehensive details on study population and sample testing procedures have been described elsewhere (thesis chapter 2 and chapter 5). In brief, data from 2009 to 2013 was part of a longitudinal cohort of age-stratified random samples in different areas in the Kilifi HDSS in 10 age strata (aged 0, 1, 2, 3, 4, 5, 6, 7, 8-9 and 10-14 years) with 50 children in each stratum. Data from 2015 to 2019 was from independent age-stratified cross-sectional random samples of the Kilifi HDSS in similar age strata as above with 50 children in each stratum. The 2021 data included 100 individuals randomly selected in each 5-year age band from 0-14 years in Kilifi HDSS. In all

instances blood samples <2ml were collected from participants and IgG antibodies against tetanus were determined using a fluorescent bead-based multiplex immunoassay[23].

The vaccination coverage data was sourced from the Kilifi Vaccine Monitoring System(KVMS), which was established in 2009 within vaccine clinics located in KHDSS area[24]. This system involves the electronic matching of children residing in the KHDSS to the population register, ensuring that all vaccinations administered during clinic visits are promptly and accurately recorded in real time. For older children who were not age-eligible to be part of KVMS, we cross-referenced immunisation records from vaccine cards.

6.5.2 Descriptive statistics

Participants with IgG concentrations above or equal to 0.011 IU/ml were considered protected against infection and these were further grouped into three levels of seroprotection; minimal-seroprotection ($0.011 \leq IgG < 0.1$IU/ml), full-seroprotection ($0.1 \leq IgG < 1$ IU/ml) and long-term protection ($IgG \geq 1$ IU/ml). We explored the association between tetanus IgG antibodies, age and vaccination status.

To assess the durability of tetanus-specific antibodies in fully vaccinated children, we used a linear regression model with log of tetanus antibodies as the outcome and time elapsed since vaccination, as the independent variable for children with a documented history of receiving three vaccine doses with available dates indicating when the vaccines were administered. We conducted a sensitivity analysis by restricting the data to only those children included in at least 2 surveys while accounting for individual level random effects.

6.5.3 Statistical model

In the primary model, we excluded all children with missing vaccine records, fitting the model exclusively to the dataset with complete information on vaccination status. A Bayesian logistic regression model was used to examine the association between the receipt of tetanus vaccination and seroconversion. A single dose of vaccine in the absence of priming has been shown to induce little if any protection[8]. We therefore structured the outcome as a binary variable of probability of having received at least two doses of tetanus containing vaccine. The primary predictor in all the models was the logarithm of tetanus IgG levels while gender was also included as a covariate. The model was fitted using brm function available in the brms package and uninformative priors

were used for the model parameters [25]. We obtained posterior distributions of model parameters through the Markov Chain Monte Carlo (MCMC) sampling and calculated the odds ratio along with 95% credible intervals to quantify uncertainty. We internally validated the model by calculating an optimism-corrected area under the receiver operating characteristic (AUROC) which allowed us to assess the performance of the predictive model while accounting for overfitting. This was done by creating 1000 bootstrap samples from the development dataset and comparing the refitted model's performance in each of these samples with the original model's performance. (Note s6.1). A value of 0·5 indicates no predictive ability, 0.8 is indicative of good predictive performance, and a perfect prediction is indicated by a value of 1. Additionally, we assessed goodness-of-fit using the Brier score which was calculated as the mean squared difference between the predicted probabilities and actual outcomes.

6.5.4 Sensitivity Analyses

We conducted a series of sensitivity analysis to ensure the robustness of our findings. First, we fitted the model to the full dataset by assuming a missing at random (MAR) mechanism for the observed missing vaccination records. Multiple imputation, utilising vaccination status, log of tetanus IgG and sex was implemented using the chained equation approach. A logistic model similar to the primary one was developed and validated based on 12 imputed datasets, each subjected to 100 iterations. The results were then combined to generate a unified set of statistics, employing Rubin's rules[26].

Second, we restructured the outcome to a binary variable of probability of being vaccinated with either 1,2 or 3 doses vs probability of not being vaccinated and refitted the model.

Third, we fitted the same model but categorised the tetanus IgG into four levels of seroprotection; unprotected(IgG<0.1IU/ml), minimal-seroprotection(0.011≤IgG<0.1IU/ml), full-seroprotection (0.1≤IgG<1 IU/ml) and long-term protection (IgG≥1 IU/ml).

Fourth, we fitted a similar model to the primary model but included an interaction term between age and log of tetanus IgG. This allowed us to investigate whether the association between vaccination status and the log of tetanus IgG varies based on age.

Fifth, we excluded children with a record of having received only one dose from the analysis, restructured the outcome to a binary variable of probability of being vaccinated with either 2 or 3 doses vs probability of not being vaccinated and refitted the model.

Finally, to mitigate any potential influence of data dependencies from earlier years on the outcomes, we limited the model fitting to single observations. Specifically, for individuals with multiple observations, we randomly selected only one observation and applied a model akin to the primary one.

6.5.5 Association of serology and vaccine record

We conducted a binary logistic regression analysis to assess the association between having a vaccine record and a serological outcome. Our objective was to determine whether individuals with and without vaccine records exhibited similar probabilities of being vaccinated as inferred by the serological data. The dependent variable was a binary outcome indicating seropositivity or seronegativity based on the tetanus IgG while the independent variable was a binary variable representing the presence or absence of vaccine records.

6.6 Results

6.6.1 Characteristics of study participants

The data comprised of serological information on 2757 children, of which 2426 (88%) had information on vaccination status. All these children were age eligible to have received 3 doses of DTP vaccine; 2305 (96%) were fully vaccinated with the expected number of doses for their age. Males slightly outnumbered females (51% vs. 49%). The majority of samples exhibited full seroprotection, as defined by tetanus IgG levels ranging from >0.1 to <1 IU/ml. The ages were quite well distributed across 0-15 yrs. (Table 6:1).

Table 6:1 Characteristics of study participants

	n	**%**
Total	2757	100
Sex		
Male	1417	51
Female	1340	49
Age		
<1yr	148	5

1-4yrs	979	36
5-9yrs	1146	42
10-14yrs	484	18
Tetanus IgG		
Unprotected (IgG<0.01)	104	4
Minimal seroprotection (0.011≤IgG<0.1 IU/ml)	699	25
Full seroprotection (0.1≤IgG<1 IU/ml)	1410	51
Long-term seroprotection (IgG≥1 IU/ml)	544	20
Vaccine doses		
0	18	1
1	24	1
2	79	3
3	2305	83
missing information	331	12

6.6.2 Vaccination status from vaccine records

Overall, 2653 out of 2757 (96%) children had some evidence of protection based on the serological data, compared to 2384 (98%) with records indicating receipt of at least 2 doses out of the 2426 children with records documenting vaccination histories. Of the children surveyed, 331 (12%) had no vaccine records. The average age among these children was 8 yrs. while the average age among those with 3 doses was 5 yrs. The seroprevalence among children with missing vaccine records was 93% (95% CI: 89 to 95). There was a clear dose-response association between the prevalence of protective tetanus IgG and the number of vaccine doses received whereby children with three vaccine doses had a seroprevalence of 97% (95% CI: 96 to 98), those with two doses had a seroprevalence of 92% (95% CI: 84 to 96), and children who had received only one dose exhibited a seroprevalence of 79% (95% CI: 59 to 90). This trend was statistically significant (χ=28.56, p<0.001) (Fig. 6:1)

Children with a documented zero-dose history had a seroprevalence rate of 72% (95% CI: 49 to 87). Within this group, there were 8 children, 6 of whom were sampled more than once bringing the total samples to 18. Among the 6 children with repeated measures, 5 displayed a general decline in tetanus antibody levels over time while one showed an unusual spike in tetanus IgG between surveys, occurring outside the typical vaccination timeframe (Table s6:1).

6.6.3 Association of serology and vaccine records

In the binary logistic model, we observed that children with available vaccine records had a 2.25-fold higher likelihood (95% CI 1.37-3.55) of achieving a positive serological outcome compared to those without vaccine records.

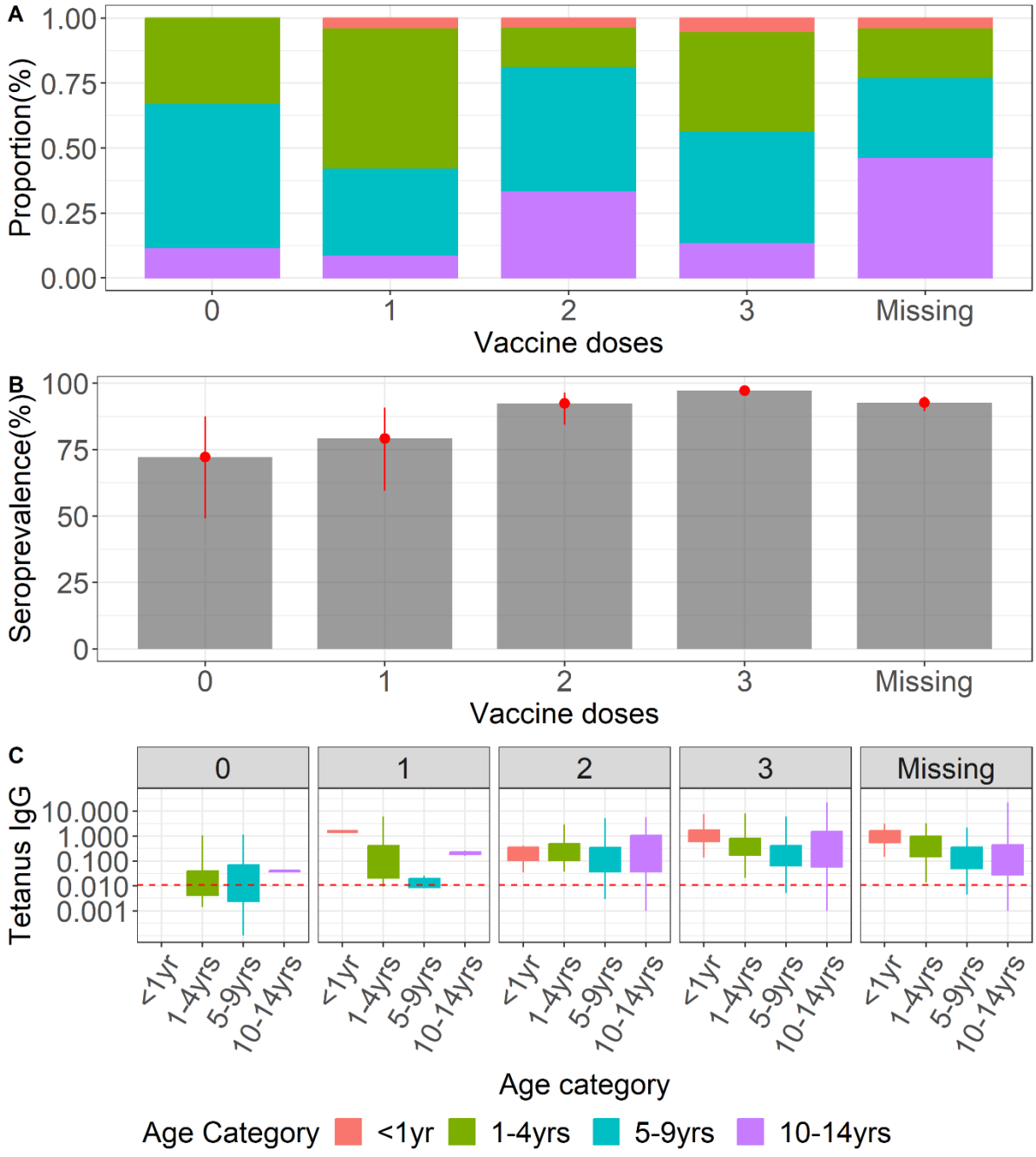

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Figure 6:1 a) displays the distribution of vaccine doses from vaccine records among children categorized by age group, b) illustrates tetanus antibody seroprevalence, and c) presents the log of tetanus IgG

categorized by different age groups. Confidence bounds in b) and c) are the 95% binomial confidence intervals.

6.6.4 Antibody titres in relation to age

Among the 2,030 fully vaccinated children with documented vaccine administration dates, the levels of tetanus antibodies declined with increasing time since vaccination. Our regression model yielded an estimated rate of tetanus antibody waning of 5.09% (95% CI 4.08 to 6.12%) per year. This corresponds to a half-life of tetanus-specific antibodies of 14 years (95% CI, 12–17) (Fig. 6:2)

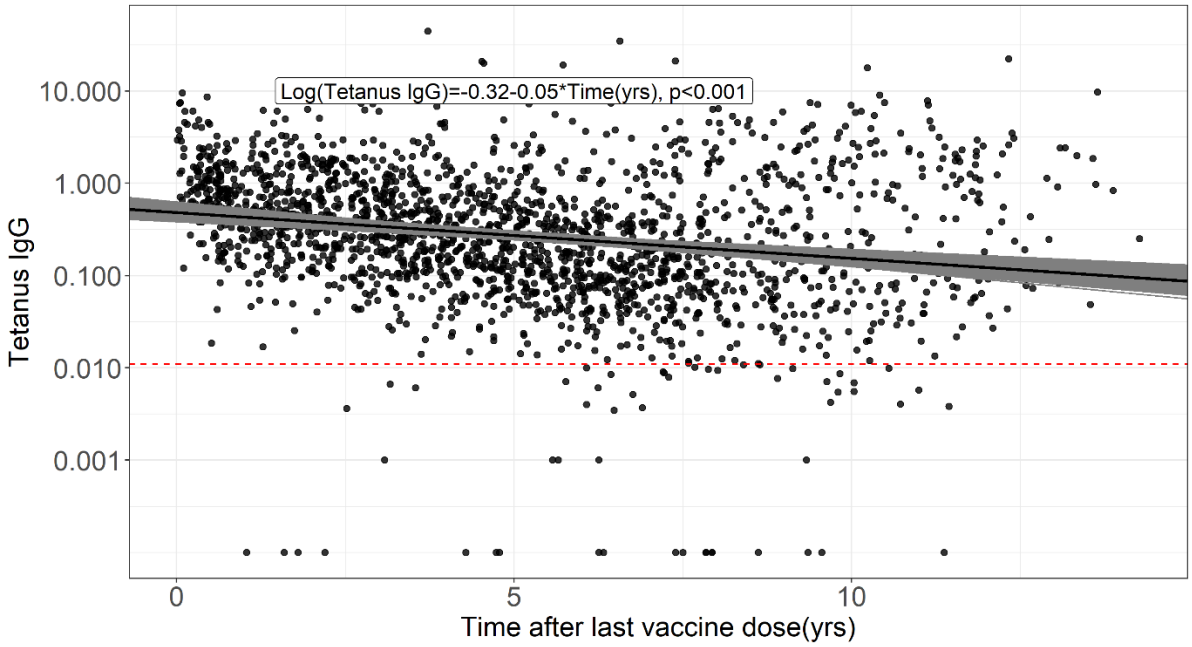

Figure 6:2 Log of tetanus antibodies in fully vaccinated children 0-14 yrs. with available vaccine administration dates plotted versus age in months (n=2305). The solid black line is the fitted regression line representing the antibody half-life decay rate, and the shaded grey region represents the upper and lower bound of 95% confidence interval (CI) for the cross-sectional antibody half-life estimation. The decay rate is 5.09% (95% CI 4.08 to 6.12%) per year which corresponds to an estimated half-life of 14yrs (12-17yrs). The dashed red line indicates the minimum protective threshold against tetanus infection of 0.01 IU/ml

In the sensitivity analysis, a total of 338 children met the criteria of full vaccination, had documented vaccine receipt dates, and had measurements of tetanus IgG on at least two occasions. We applied a random effects model to this subset of children and determined a tetanus antibody

waning rate of 3.77% per year (95% CI 1.82 to 5.87). This equates to a tetanus-specific antibody half-life of approximately 19 years (95% CI, 12–39).

6.6.5 Association of vaccine status and serology

In the primary model, a 1 IU/ml increase in the log of tetanus IgG was associated with a 2.41-fold higher likelihood (95% CI 1.80-3.19) of children receiving at least 2 vaccine doses. However, there were no strong evidence of a gender difference in the likelihood of receiving at least 2 vaccine doses (see Table 6:2).

In the sensitivity analysis, the multiple imputation model yielded similar results, indicating that for every 1 IU/ml increase in log of tetanus IgG, children were 2.56 times (95% CI 1.89-3.47) more likely to be vaccinated with 2 or 3 vaccine doses, while there was no strong evidence of a gender difference in the likelihood of receiving at least 2 vaccine doses.

In the second model where the outcome was probability of having received any vaccination, children were 2.55 times (95% CI 1.70-3.82) more likely to be vaccinated with either 1,2 or 3 vaccine doses for every 1 IU/ml increase in log of tetanus IgG while males were more likely to have received any vaccination compared to females.

In the third model, where we restructured the tetanus IgG predictor into a categorical variable based on varying levels of tetanus IgG, the results indicated that children with minimal tetanus seroprotection were 5.29 times more likely (95% CI 2.25-12.12) to receive 2 or 3 vaccine doses compared to those without seroprotection. Furthermore, those with full tetanus seroprotection had a 13-fold higher likelihood (95% CI 5.57-32.02) of having documented receipt of at least 2 vaccine doses, while those with long-term seroprotection were 18.10 times more likely (95% CI 5.71-67.24) to have received at least 2 vaccine doses compared to those without seroprotection.

In the fourth model, where children with one dose were excluded from the analysis, children were 2.60 times more likely (95% CI 1.69-3.80) to have received 2 or 3 vaccine doses compared to those who had not received any vaccines while males were more likely to have received 2 or 3 vaccine doses compared to females.

In the fifth model where we restricted the model fitting to single observations, children were 2.39 times (95% CI 1.64-3.44) more likely to be vaccinated with 2 or 3 vaccine doses for every 1 IU/ml increase in log tetanus IgG. In the final sensitivity analysis model, there was no credible evidence

of association between the interaction of age and the log of tetanus IgG and the probability of having received 2 or 3 vaccine doses; OR=0.95 (95% CI 0.87-1.04).

Table 6:2 Summary estimates from the predictive models

Model	Dataset	Response	Predictors	Odds	lower CI	Upper CI
Primary model	Complete vaccination history(n=2426)	Vaccinated (2,3 doses, n=2384) and not vaccinated/partially vaccinated (0,1 n=42)	Log of tetanus IgG	2.410	1.798	3.191
			Sex(male)	1.664	0.897	3.251
Sensitivity analysis	Imputed vaccination history(n=2757)	Vaccinated (2,3 doses, n=2706) and not vaccinated/partially vaccinated (0,1 n=51)	Log of tetanus IgG	2.564	1.885	3.468
			Sex(male)	1.529	0.828	2.826
	Complete vaccination history(Restructured outcome)(n=2426)	Vaccinated (1,2,3 doses, n=2408) and not vaccinated (0 doses n=18)	Log of tetanus IgG	2.554	1.695	3.815
			Sex(male)	3.521	1.654	5.646
	Complete vaccination history(Restructured predictor)(n=2426)	Vaccinated (2,3 doses, n=2384) and not vaccinated/partially vaccinated (0,1 n=42)	Tetanus IgG(Minimal)	5.286	2.248	12.123
			Tetanus IgG(Full)	13.312	5.571	32.022
			Tetanus IgG(Long-term)	18.104	5.705	67.243
			Sex(male)	1.632	0.881	3.122
	Complete vaccination history(without 1 dose children)(n=2402)	Vaccinated (2,3 doses, n=2384) and not vaccinated (0,n=18)	Log of tetanus IgG	2.596	1.691	3.802
			Sex(male)	3.322	1.651	5.086
	Complete vaccination history(only unique IDs=1776)	Vaccinated (2,3 doses, n=1748) and not vaccinated/partially vaccinated (0,1 n=28)	Log of tetanus IgG	2.392	1.638	3.438
			Sex(male)	1.401	0.645	3.032
	Complete vaccination history(with interaction term)(n=2426)	Vaccinated (2,3 doses, n=2384) and not vaccinated/partially vaccinated (0,1 n=42)	Log of tetanus IgG	3.221	1.850	5.608
			Log of tetanus IgG*Age(yrs)	0.954	0.869	1.044
			Sex(male)	1.738	0.921	3.376

6.6.6 Internal validation

In the primary model, the optimism-corrected AUC score after bootstrapping was 71.7%. Consequently, in 72% of all instances the modelled probability for vaccination of a randomly selected child vaccinated with at least 2 vaccine doses was higher than that of a child vaccinated with no more than 1 dose. Out of the 2384 children with a record of at least 2 doses, the model estimated 1788(75%) to be vaccinated. Of the 42 children with a record of no more than one dose, the model estimated 28(66%) to be unvaccinated. The model had a low mean squared error

between actual outcomes and predictions shown by the Brier score of 1.7%. The difference between the apparent AUC and the optimism-corrected AUC after bootstrap resampling was marginal, indicating minimal overfitting (Table 6:3 and Fig. 6:3).

Across various model variations, the optimism-corrected AUC ranged from 68% to 82%. Notably, the model assessing the probability of having received at least two vaccine doses versus not having received any vaccine dose achieved the highest AUC score and the lowest Brier score. This model effectively differentiated between children who received either 2 or 3 vaccine doses and those who were not vaccinated 82% of the time. Out of the 2384 children with a record of at least 2 doses, the model estimated 2146(90%) to be vaccinated. Of the 18 children with a record of zero dose, the model estimated 12(66%) to be unvaccinated. Conversely, the model fitted to the single observations, examining the probability of having received at least two vaccine doses, recorded the lowest AUC score of 68% (Table 6:3).

Table 6:3 Model performance measures during internal validation

Model	**Dataset**	**Response**	**Sensitivity(%)**	**Specificity(%)**	**Brier score(%)**	**Original Auc (%)**	**Optimism adjusted Auc(%)**
Primary model	Complete vaccination history(n=2426)	Vaccinated (2,3 doses, n=2384) and not vaccinated/partially vaccinated (0,1 n=42)	74.58	69.05	1.7	73.53	71.75
Sensitivity analysis	Imputed vaccination history(n=2757)	Vaccinated (2,3 doses, n=2703) and not vaccinated/partially vaccinated (0,1 n=54)	84.35	62.96	1.88	75.16	74.04
	Complete vaccination history(Restructured outcome)(n=2426)	Vaccinated (1,2,3 doses, n=2408) and not vaccinated (0 doses n=18)	89.41	66.67	0.73	83.71	81.38
	Complete vaccination history(Restructured predictor)(n=2426)	Vaccinated (2,3 doses, n=2384) and not vaccinated/partially vaccinated (0,1 n=42)	72.65	66.67	1.7	72.41	69.28
	Complete vaccination history(without 1 dose children)(n=2402)	Vaccinated (2,3 doses, n=2384) and not vaccinated (0,n=18)	89.97	66.67	0.71	83.86	81.53
	Complete vaccination history(only unique IDs, n=1776)	Vaccinated (2,3 doses, n=1748) and not vaccinated/partially vaccinated (0,1 n=28)	72.25	76.47	1.53	69.21	67.52
	Complete vaccination history(with	Vaccinated (2,3 doses, n=2384) and not	80.49	61.92	1.7	73.83	71.51

	interaction term)(n=2426)	vaccinated/partially vaccinated (0,1 n=42)					

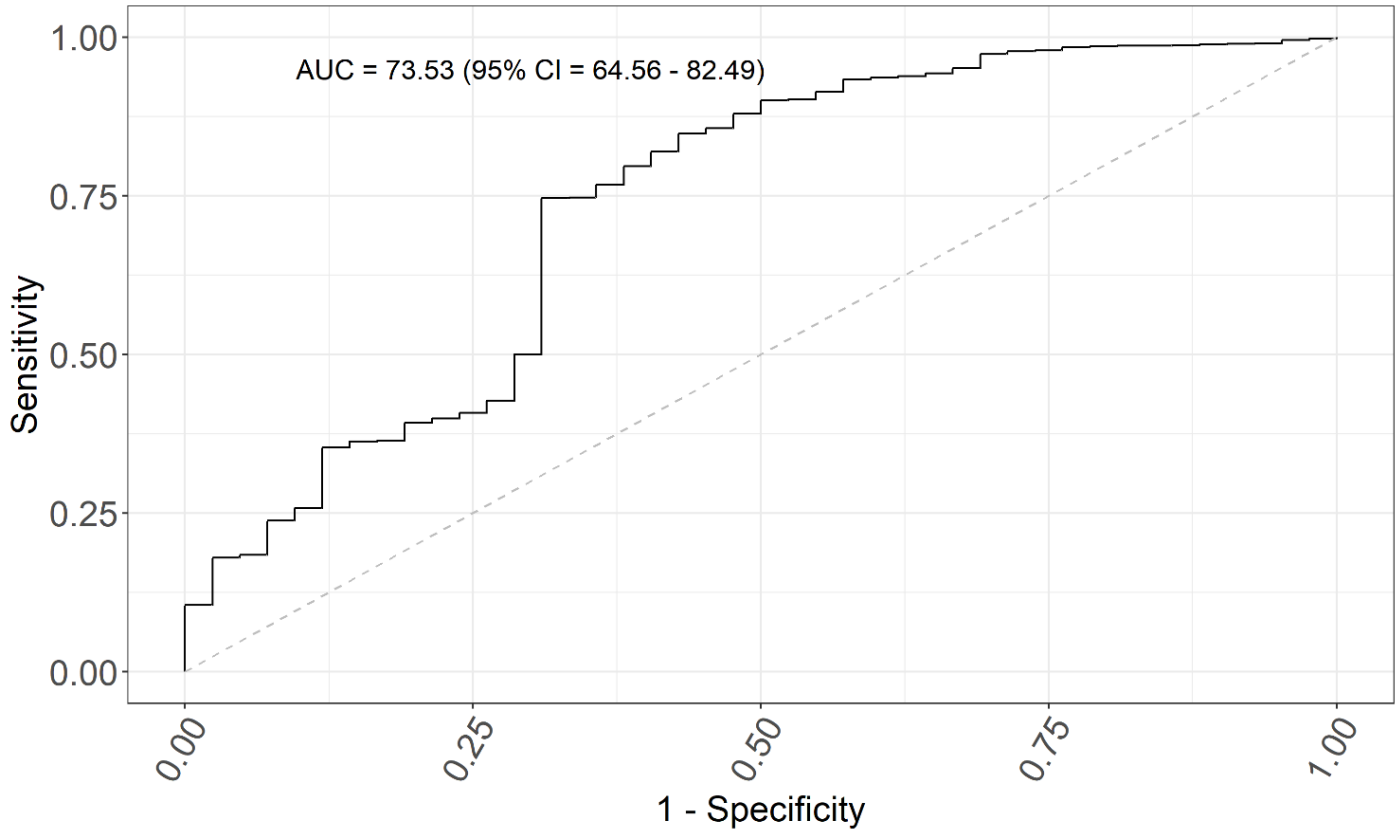

Figure 6:3 Roc curve based on the primary model.

6.7 Discussion

In our study, we integrated data from a series of population-based cross-sectional surveys, combined with vaccination coverage estimates obtained from vaccine records to determine the prevalence of immunisation among children, estimate the decay of tetanus antibody levels, compare the association between serological data and documented vaccination and evaluate the utility of tetanus serological data in identifying vaccination gaps. We demonstrated a strong correlation between tetanus IgG titres and the recorded number of vaccine doses, with seroprevalence dropping from 97% (3 doses) and 92% (2 doses) to 79% (1 dose) and 72% (0 dose); the latter demonstrating a likely underreporting of DTP receipt in vaccination records. Approximately 12% of study participants had no vaccine records and yet 93% had evidence of protection through seroconversion; indicating that most of them had likely been vaccinated. Our study also revealed a decline in antibody levels with age, with an estimated half-life of 14 years. In the predictive model, IgG levels appear to exhibit some correlation with documented vaccination status, as individuals with no or limited vaccination records tend to have lower IgG titres. These results underscore the importance of meticulous record-keeping and highlight the utility of serological data in identifying potential gaps in vaccination coverage, particularly in the absence of comprehensive vaccination records.

Our findings uncovered several insights related to vaccination records within our population. Firstly, we identified a problem with poor record-keeping, evident from the incomplete vaccination records. About 12% of the children in our study had missing vaccination information. However, most of these children were older, suggesting improved record-keeping in recent years and particularly since the establishment of KVMS [20]. Also, 93% these children had high tetanus seroprevalence, suggesting that most had in fact been vaccinated. This has also been observed in other regions, such as a study in three districts in Ethiopia, where 41% of toddlers without vaccination records exhibited protective tetanus antitoxin biomarkers[27] .

In addition to the issue of missing vaccination records, our analysis highlights concern about the accuracy of vaccine information. Children with a documented history of zero vaccine doses exhibited a surprisingly high seroprevalence of 72%. Upon closer examination of these samples, most of those participants had IgG antibodies similar to age matched fully vaccinated children and also similarly their IgG declined over time, providing further support for the argument that tetanus

antibodies were primarily a result of vaccination[17]. Only in one participant with a record of no vaccination we found a substantial rise in titre at an age well beyond the recommended age of vaccination. It's worth noting that tetanus toxoid is recommended for use in adults and children who are 4 years or older, especially those presenting with wounds or injuries, particularly if they have had fewer than 3 primary doses of TT or unknown information on prior doses[28]. This could be a contributing factor for this observation. These results suggest caution in interpreting analyses that aim to understand individual-level factors associated with a lack of vaccination based solely on vaccine records in Kilifi.

Interestingly, children with just one documented vaccine dose exhibited a relatively high seroprevalence of 79%, even though a single dose without prior priming is typically known to provide little or no protection[16]. There are several potential factors contributing to this discrepancy. Firstly, it's plausible that similarly to the seemingly unvaccinated, those with a documented history of only a single vaccine dose may have actually received multiple doses, a common challenge in record-keeping, especially when it involves multiple doses[29]. Secondly, it's possible that the children received only one documented dose and seroconverted, but they may not maintain protective antibody levels over an extended period. Our findings align with those from a study in Ethiopia, where they similarly identified protective antibody levels in a substantial proportion of infants in three districts in Ethiopia, including those with only one documented dose: 80 to 95% in those with three vaccine doses, 67 to 94% in those with two doses, and seroprevalence ranging from 40 to 80% in infants who had received just one documented dose across the three districts[27]. Third, while the likelihood is low, considering the high assay specificity of 93%, these discrepancies could also be attributed to assay specificity [19].

Another plausible explanation for this observed high seroprevalence among children with one vaccine dose and those with missing vaccine records could potentially be attributed to natural exposure. Limited studies have explored the role of natural exposure in tetanus. Early research in the 1980s demonstrated evidence of natural immunity to tetanus, indicated by measurable tetanus antitoxin in individuals who had not received tetanus toxoid[11, 12]including animals [13]. More recently, a study in Taiwan revealed that individuals born before the initiation of childhood tetanus vaccination, who never received tetanus toxoid, still exhibited protective antitoxin levels [30]. It has been proposed that natural tetanus immunity may be induced by fragments released from

Clostridial tetani in the digestive tract, potentially originating from ingesting tetanus spores [11]. Some authors argue that serum to antitoxin responses induced by natural infection can assist in diagnosing tetanus and assessing infection extent within a population, but they may not be the specific mechanisms of protective immunity[31]. Overall, the clinical implications of natural immunity to tetanus remains controversial.

Tetanus antibodies declined with increasing time since vaccination, with an estimated half-life of 14 years in children who had evidence of receiving three vaccine doses. In a sensitivity analysis of children with repeated measures from the longitudinal section of the study, the estimated half-life was slightly longer, at 19 years. This finding is consistent with prior research, where a cross-sectional analysis of 546 subjects estimated a tetanus antibody half-life of 14 years [29] while a longitudinal study estimated the half-life of tetanus immunity to be 11 years [30]. These results suggest that while serology can effectively serve to objectively monitor the proportion of toddlers who have received pentavalent vaccines, it still remains reliable as a marker of vaccination in older children, with only a marginal decrease in sensitivity due to the long-term persistence.

The results of the predictive models reaffirmed the observations previously discussed regarding the challenges in record-keeping. Notably, there was a significant association between serological data and the likelihood of having received vaccinations, although the model performance exhibited variability depending on the specific outcome structure. Models that aimed to assess the probability of having received at least two vaccine doses compared to either receiving just one dose or not receiving any displayed moderate performance, with AUC scores falling below the recommended 80% threshold for good discrimination[32]. This was not surprising given the high proportion of children with one dose who seroconverted in our study. Reconfiguring the outcome to distinguish between having received any vaccination versus not receiving any improved the model's discrimination ability to 81% and excluding the one-dose children enhanced it further to 82%. Furthermore, this last model demonstrated the highest sensitivity and specificity, correctly identifying 90% of children who received at least 2 doses and 67% of unvaccinated children. It also exhibited the lowest mean squared error between actual outcomes and predictions, as indicated by the Brier score of 0.7%, aligning with a desirable model performance[33].

If tetanus antibodies are predominantly generated through vaccination, the optimal model should ideally demonstrate superior discrimination between vaccinated and non-vaccinated children. The

observed outcomes indicate the need for a more nuanced comprehension of the role of natural immunity in tetanus as it has implications for the reliability of utilizing serology estimates to assess immunisation coverage.

A key strength of our study was the availability of serological data from a population-based study spanning over a decade. This data was analysed using a highly sensitive assay[23] which offered an exceptional means of directly measuring tetanus antibody levels. Additionally, the KHDSS population registry played a pivotal role in providing information on the number of vaccine doses received [18]. Furthermore, the absence of DPT campaigns within the Kenyan vaccination program ensured that the vaccination doses received by the study participants were exclusively from routine vaccination.

Our study had several limitations, with the primary constraint being the limited number of children who were not vaccinated yet exhibited high seroprevalence. This limitation may have influenced the suboptimal performance of our predictive models. While we conducted a sensitivity analysis to explore the potential role of natural immunity by assessing IgG antibody titers in children with repeated measurements, there remains a need for further sensitivity analyses that we were unable to adequately conduct due to the limited sample size. One such sensitivity analysis that could have been valuable is the exclusion of children with zero-dose vaccination records and high seroprevalence from the predictive models. However, the small number of children meeting these criteria restricted our ability to conduct robust analyses. Additionally, we could not definitively rule out the role of tetanus toxoid vaccination in these children, as such vaccinations are not consistently documented. It is noteworthy that tetanus toxoid is recommended for children older than 5 years, particularly following wounds or injuries, even for those not vaccinated during the Expanded Program on Immunization (EPI).

Secondly, we had missing records of vaccination for 12% of the participants in the study which further led to a low EPV in the models. This limitation was addressed by multiply imputing the missing data which resulted to a much larger sample size. Another limitation was lack of sufficient data to externally validate the model. It is essential for future research to validate the model using data from diverse populations to ensure its broader applicability and reliability.

In conclusion, our findings underscore the value of serosurveys as a means to monitor the proportion of children who have received the pentavalent vaccine, providing valuable insights for public health officials evaluating immunisation services. While they can serve as standalone tools

to identify vaccination gaps that can be filled during immunisation campaigns, linking them with vaccine records is also essential for addressing record-keeping challenges. Nonetheless, it's worth noting that serosurveys can be expensive, and to maximize their utility, especially in developing nations, exploring alternatives to blood collection should be considered to enhance practicality and cost-effectiveness.

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7. Chapter 7: HBV seroprevalence in Kenyan children and the effectiveness of the current vaccination program against HBV infection

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7.1 Research paper cover sheet

RESEARCH PAPER COVER SHEET

Please note that a cover sheet must be completed for each research paper included within a thesis.

SECTION A – Student Details

Student ID Number	2003951	**Title**	Mrs.
First Name(s)	Caroline		
Surname/Family Name	Mburu		
Thesis Title	The utility of mathematical modelling of serological data in assessing the impact of vaccination programmes in Kenya		
Primary Supervisor	Stefan Flasche		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

SECTION B – Paper already published

Where was the work published?	
When was the work published?	

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Where is the work intended to be published?	To be determined
Please list the paper's authors in the intended authorship order:	Mburu CN, Ojal J, Chebet R, Ombati R, Akech D, Karia B, Tuju J, Sigilai A, Smits, G, van Gageldonk PGM, van der Klis FRM, EW Kagucia1, Scott JAG, Adetifa IMO, Flasche S
Stage of publication	**Prepared to be submitted**

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	Design, data acquisition, data analysis, write up, and submission

SECTION E

Student Signature	[redacted]
Date	

Supervisor Signature	[redacted]	
Date		

7.2 Bridging section

This paper is devoted to fulfilling objective three. The aim is to estimate of Hepatitis B virus (HBV) seroprevalence using a panel test of various HBV markers. Subsequently, I have integrated data from these markers with vaccination records retrieved from a vaccine registry in the KHDSS. This integration aimed to update vaccine information for participants with missing records by utilizing insights derived from serological data. Following this, I have combined the datasets of active HBV infections and vaccination information and employed two distinct approaches to estimate HBV vaccine effectiveness (VE) against infection: a catalytic model, utilizing population-level HBsAg seroprevalence, and a regression model utilizing individual-level data on the presence or absence of an active HBV infection.

This work is particularly significant, especially considering the World Health Assembly's 2016 call to combat and eradicate HBV globally. It is essential to assess the current performance of the vaccination program and comprehend the present profile and distribution of HBV prevalence for informed public health policy decisions.

I conducted the analysis using a series of statistical tests and a catalytic model. I developed the model and wrote all the code. I also wrote the paper and generated the figures. Throughout the process I received input/suggestions/edits from my supervisors.

The supplementary material of the paper is included as Appendix G in this thesis, so any references to the supplementary material can be found there.

7.3 Abstract

Background: The global initiative to combat and eradicate Hepatitis B Virus (HBV), as endorsed by the World Health Assembly in 2016, emphasizes the need to assess vaccination programs and understand current HBV prevalence for informed public health decision-making. This study aims to fill this research gap by estimating HBV prevalence in children in Kilifi Health Demographic Surveillance System (KHDSS) and evaluating the effectiveness of the vaccination program against HBV infection.

Methods: Utilising cross-sectional surveys conducted in KHDSS from 2007 to 2021, we estimated HBV seroprevalence using a combination of HBV markers. We integrated serological data with vaccination records from a KHDSS vaccine registry to update vaccine information. We then employed two distinct approaches to estimate HBV vaccine effectiveness(VE) against infection: a catalytic model based on population-level HBsAg seroprevalence and a regression model using individual-level data on active HBV infections.

Results: HBsAg prevalence in children within the KHDSS was 5.0% (CI: 4.2%-5.9%). Chronic HBV was present in 21% CI: (13-22%) of the children in the longitudinal subset. 2055(83%) were classified as vaccinated, 230(9%) were unvaccinated while 181 children (7%) remained unclassified. The proportion of vaccinated children revealed a statistically significant increasing trend (χ=236.3, $p<0.001$). The annual FOI was high between 2007 and 2011 ranging between 0.79(95% Cr 0.48-0.99) to 0.91(95% Cr 0.62-0.99) and later declined across all subsequent periods to below 0.02. The estimated VE against active HBV infection was 67.3% (95% Cr: 50.3%-78.8%) in the catalytic model with a mean duration of 0.37 years (95% Cr: 0.24-0.61years) suggesting a substantial degree of independence in observed seroprevalence between consecutive surveys while the statistical model estimated a slightly higher VE of 78.6% (95% Cr 65.5-86.8).

Conclusion: The vaccination program has demonstrated a positive impact, evidenced by declining HBV seroprevalence, reduced FOI, and substantial VE estimates. Introducing a birth dose and enhancing third-dose coverage could significantly contribute to HBV transmission reduction, aligning with the global goal of HBV elimination by 2030.

7.4 Background

Hepatitis B virus (HBV) infection is a global public health problem impacting an estimated 257 million individuals worldwide and leading to approximately 887,000 deaths annually [1, 2]. A substantial burden of HBV is in the LMICs with the majority of the countries classified as having high or highly intermediate prevalence, characterized by serologic prevalence of hepatitis B surface antigen (HBsAg) above 8% and 5%, respectively, within the general population [3].HBV transmission in these settings has been shown to primarily occur perinatally and through horizontal transmission among children under 5 years old, elevating the risk of chronic HBV infection in adults and ultimately the risk of liver cirrhosis and hepatocellular carcinoma(HCC)[4].

Although a cure for HBV infection remains elusive, effective prevention can be achieved through infant vaccination. Recombinant hepatitis B vaccines were developed in the 1980s, and in 1991, the World Health Assembly (WHA) recommended their inclusion in national immunisation programs. With support from GAVI, Kenya introduced hepatitis B vaccination in November 2001, following a three-dose schedule administered at 6-10-14 weeks as part of the Expanded Programme on Immunisation (EPI)[5]. However, the coverage of all three doses, which has fluctuated between 75% and 81% since 2002 to 2021 [6] falls short of the WHA's recommended 90% coverage target outlined in their global strategy to eliminate HBV infection [2, 7]. In addition to the three-dose schedule, WHO recommends one dose of hepatitis B vaccine at birth, (Hep-BD), to eliminate perinatal transmission, which poses the highest risk of chronic infection. Regrettably, the uptake of this birth dose, introduced in only 13 out of 47 countries in the African region (Kenya excluded), remains low at approximately 6%, in contrast to the 43% global uptake [8].

The epidemiological context of HBV infection in Kenya is characterized by a paucity of data in the prevalence of HBV infection particularly in children. Existing studies have mainly centered on adults and specific high-risk groups [9-12]. A recent systematic review of HBV in Kenya uncovered a wide-ranging prevalence of HBV, from 3.4% to 29.2%, across various risk profiles. However, it highlighted the overall low quality of the studies and the pressing need for high-quality seroprevalence data[13]. Furthermore, there has been no evaluation of HBV prevalence among infants and children since the introduction of the hepatitis B vaccine in Kenya. Thus, the vaccine's effectiveness in this population remains uncertain.

Given the World Health Assembly's 2016 call to combat and eradicate HBV globally, it is essential to assess the current vaccination program's performance and understand the current profile and distribution of HBV prevalence for informed public health policy decisions. Our study aims to address this research gap by estimating HBV prevalence in children and evaluating the vaccination program's effectiveness against HBV infection.

7.5 Methods

7.5.1 Study population and laboratory testing

The data spanning from 2009 to 2019 originated from two surveys: the Malaria Cross-Sectional Survey [14, 15] which is in part longitudinal and the Pneumococcal Conjugate Vaccine Impact Study (PCVIS)[16] which is primarily cross-sectional in nature. These surveys were conducted within the Kilifi Health Demographic Surveillance System (KHDSS)[17] as part of an ongoing effort to actively monitor malaria and pneumonia infections in children under 15 years of age. Data in 2021 was from participants who were recruited from COVID-19 serosurveillance in Kenya conducted as part of the pandemic response [18].The comprehensive details on study design and population have been described elsewhere (thesis chapter 2 and chapter 5). HBV data comprised results of tests for hepatitis B surface antigen (HBsAg), antibody to hepatitis B (anti-HBs),antibody to hepatitis B core antigen (anti-HBc) and IgM antibody to hepatitis B core antigen (anti-HBc IgM) all tested using WHO pre-qualified assays [19]. Both the sensitivity and specificity of the HBsAg and HBcAb assays after in-house validation was100% while sensitivity of HBsAb was also 100% and specificity was 98.8%.

The vaccination coverage data was sourced from the Kilifi Vaccine Monitoring System(KVMS), which was established in 2009 within vaccine clinics located in KHDSS area[20]. This system involves the electronic matching of children residing in the KHDSS to the population register, ensuring that all vaccinations administered during clinic visits are promptly and accurately recorded in real time. For older children who were not age-eligible to be part of KVMS, we cross-referenced immunisation records from vaccine cards.

7.5.2 HBV infection history classification

The complex serology and natural history associated with HBV infection created a challenge for us to separate chronic and acute HBV infections using the available data. In addition, the positive predictive value for IgM to anti-HBc which we were hoping to utilise to do this has been shown to be low as asymptomatic persons with liver flares from chronic infection can also test positive for IgM anti-HBc. Consequently, use of the test for diagnosis of acute hepatitis B is recommended to be limited to persons with clinical evidence of acute hepatitis[21, 22]. Based on this, we decided to simply classify all HBsAg positive samples as active infections and retained the CDC definitions of susceptible, immune from vaccination and immune from a resolved infection(Fig. 7:1)[23].

7.5.3 Vaccine history classification

Children born in Kenya after the introduction of the vaccine, who were classified as immune from vaccination based on serological markers (defined as a positive test for anti-HBs marker and negative for both anti-HBc and HBsAg), were categorized as vaccinated. For all other children we relied on vaccine records and date of birth to update the vaccination status. Because the vaccine is co-administered with other vaccines, children born before November 2001 were all classified as unvaccinated irrespective of the vaccine record i.e. vaccinated, unvaccinated and unknown. Children with unknown records born after November 2001 could not be classified further and their status remained unknown.

7.5.4 Descriptive statistics

Cumulative prevalence of HBV infection was calculated by dividing the number of children with a positive HBsAg test result by the number of children with a HBsAg test result at any time between 2009 and 2021.Changes in HBV prevalence were also calculated for the different survey years.

An alternative definition of chronic HBV infection is the presence of HBsAg in the serum for at least 6 months [23]. Using the longitudinal cohort among children with a positive HBsAg test result indicating an active infection, we reviewed subsequent HBsAg tests to further define their HBV status. Children were defined as having chronic infection if they had a positive HBsAg test result at least 2 years (time period between two surveys) after their first positive HBsAg test result, with no intervening negative result. Children without chronic HBV and with at least one negative

HBsAg test result after their first positive HBsAg test result were defined as having had recent infection which resolved. Children that did not meet either criteria were defined as infected with no further classification.

7.5.5 Vaccine effectiveness

We employed two distinct approaches to estimate HBV vaccine effectiveness against infection: a catalytic model and a regression model. The catalytic model used population-level HBsAg seroprevalence information, categorized into seropositive (indicating the proportion with an active infection) and seronegative (indicating the proportion without an active infection). In addition to calculating vaccine effectiveness (VE), the catalytic model enabled us to estimate two additional parameters: the mean duration of HBsAg and the Force of Infection (FOI) between different survey years. The regression model used individual-level data on the presence or absence of an active HBV infection and also controlled for covariates including age and sex.

7.5.6 Catalytic model

To assess the effectiveness of the present vaccination program against active HBV infections, we developed a joint reverse catalytic model. The fundamental reverse catalytic model follows individuals from birth and assumes a constant force of infection (FOI) denoted as λ, independent of age (a) or calendar year. The model also allows previously infected individuals to become susceptible again. This was necessitated by the fact that there are various outcomes depending on the type of HBV infection; A resolved HBV infection can confer lifelong protection against recurrent infections and development of disease, a resolved infection might still leave an individual susceptible to reinfection, particularly if it was a mild infection and chronic infections can persist indefinitely resulting to development of disease. From the consistency checks of the HBV markers, active infections defined by HBsAg-positive samples in our study encompassed a combination of all three potential scenarios (Fig s7:3). The model did not account for HBV related mortality and assumed a similar mortality rate for susceptible and infected individuals.

We then expanded the reverse catalytic model to allow a time-dependent FOI which was structured according to the temporal gap between successive survey points and used it to estimate and compare the overall FOI for the participants in the different surveys. The model was based on the equations below (full equations in supplement, Note s7:1);

$$Z_y(a) = \frac{\lambda_1}{\lambda_1+\omega}(1 - e^{-a(\lambda_1+\omega)}) \qquad \text{for } a < a_0$$

$$Z_y(a) = \frac{\lambda_2}{\lambda_2+\omega}\left(1 - e^{-(a-a_0)(\lambda_2+\omega)} - \frac{\lambda_1}{\lambda_1+\omega}\right)\left(e^{-a_0(\lambda_1+\omega)}\right)\frac{\lambda_1}{\lambda_1+\omega} \quad \text{for } a \geq a_0$$

where $Z_y(a)$ represents the proportion of individuals with an active infection defined by a positive HBsAg marker in year *y* at age *a*. The parameter λ_1 is the force of infection (FOI) within the younger age brackets, while λ_2 pertains to the FOI within the older age group. The variable ω corresponds to the rate at which seroconversion wanes. a is age of individuals and a_0 is the threshold of 2 years, established based on the time elapsed between consecutive survey points.

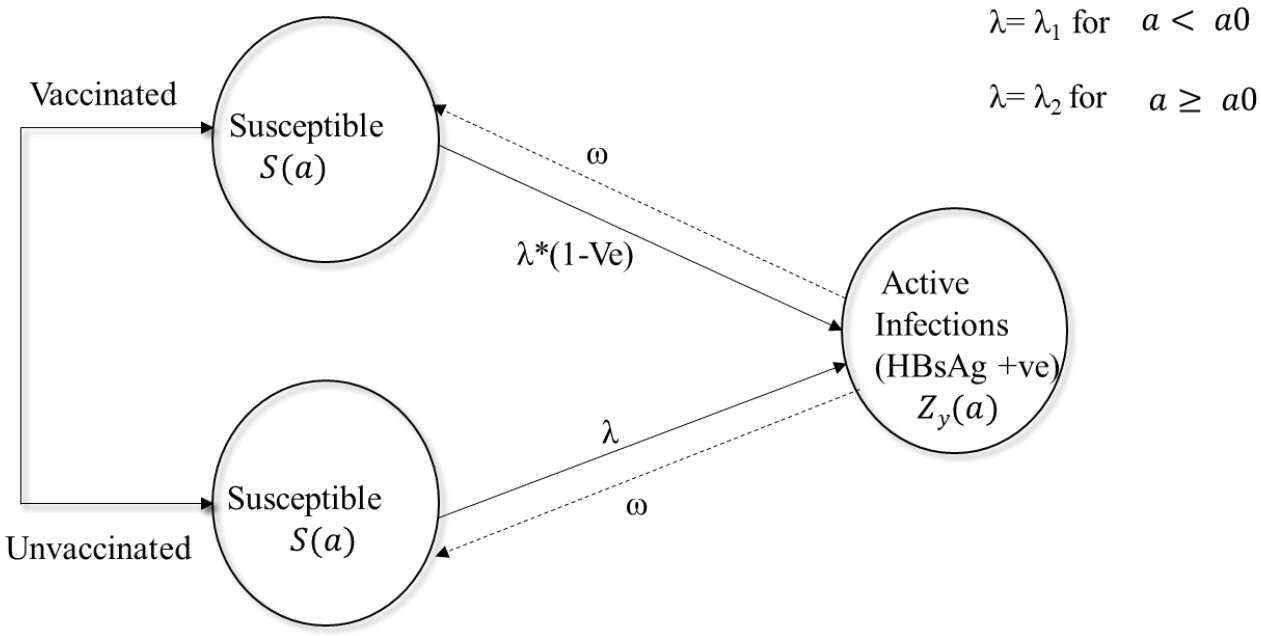

Figure 7:1 Schematic of the reverse catalytic model used to estimate the effectiveness of the current vaccination program against HBV infections. λ1 is the force of infection (FOI) within the younger age brackets, while λ2 pertains to the FOI within the older age group. a is age of individuals and a0 is the threshold of 2 years, established based on the time elapsed between consecutive survey points. Ve is vaccine effectiveness and ω is the mean duration of HBsAg

The model was fitted to HBV seroprevalence indicated by the presence of positive HBsAg which served as a marker for active (acute or chronic) HBV infection. We estimated vaccine effectiveness(VE) defined as a reduced probability of seroconversion upon exposure by fitting the model to both vaccinated and unvaccinated cohorts and multiplying the FOI within the vaccinated group with a rate equivalent to 1 minus VE. We assumed a constant FOI before the initial 2007 survey and estimated the relative alterations in FOI across subsequent surveys. The model was jointly fitted across the years and parameters VE and waning collectively estimated across all survey instances.

We conducted a series of sensitivity analyses in order to evaluate the robustness of these results. Firstly, we employed a basic catalytic model, assuming persistent lifelong protection after a resolved infection. This choice was influenced by the observation that certain resolved active HBV infections in our study could provide lasting protection against future infections, a conclusion reinforced by the consistency checks conducted on the HBV markers (Fig. s7:3). Secondly, we restricted the model fitting to the most recent cross-sectional years of the survey (2015-2021), owing to variations in study design between the earlier and later years of the study. The different models explored are summarized in table 7:1 below.

Table 7:1 Description of the different variations of catalytic model that were explored

Model	**Priors**	**Estimate**
Main model: Reverse catalytic model with time varying FOI jointly estimated between consecutive surveys for all surveys(2007-2021)	FOI~uniform(0,1) Waning~uniform (0,5) 1-VE~uniform(0,1)	Waning, VE
Reverse catalytic model with time varying FOI jointly estimated between consecutive surveys(2015-2021)	FOI~uniform(0,1) Waning~uniform (0,5) 1-VE~uniform(0,1)	Waning, VE
Simple catalytic model with time varying FOI jointly estimated between consecutive surveys for all surveys(2007-2021)	FOI~uniform (0,1) 1-VE~uniform(0,1)	VE
Simple catalytic model with time varying FOI jointly estimated between consecutive surveys(2015-2021)	FOI~uniform (0,1) 1-VE~uniform(0,1)	VE

The models were fitted within the rjags framework [24] with parameter estimation conducted by use of a Markov Chain Monte Carlo (MCMC) approach employing the Gibbs sampling algorithm. For modeling the data likelihood, a binomial distribution was adopted, alongside uninformative priors for waning rate, VE and FOI. Convergence of the MCMC process was assessed utilizing the Gelman-Rubin statistic, where a threshold of less than 1.1 was adopted as a criterion for satisfactory convergence. Additionally, we assessed the effective sample size (ESS), an estimation of independent samples accounting for autocorrelations generated by the MCMC. An ESS exceeding 200 was deemed acceptable.

7.5.7 Bayesian binary logistic regression with non-informative priors

We also evaluated the effectiveness of the HBV vaccination program by employing a statistical regression model which was fitted using Bayesian inference method. Similar to the catalytic model, the model was fitted to active HBV infections which was a binary outcome of presence or absence of infection. The model incorporated three predictors, each assigned non-informative priors. The primary predictor was vaccination status, categorized as vaccinated or unvaccinated according to the criteria outlined above. Age in years and sex were also included as covariates. The Bayesian model was formulated as follows;

$$Z_Y(a) = \beta 0 + \beta 1 * Vaccination\ status + \beta 2 * Age + \beta 3 * Sex$$

Where $Z_Y(a)$ is the probability of having an active infection and age,sex and vaccination status are the predictors.

We used Markov Chain Monte Carlo (MCMC) sampling using the brm function available in the brms package[25]. Our sampling comprised a total of 2000 iterations, of which 500 were designated as a burn-in. Posterior distributions of model parameters were obtained through the MCMC process and posterior means and 95% credible intervals were calculated to quantify uncertainty. Convergence of the MCMC process was assessed utilizing the Gelman-Rubin statistic, where a threshold of less than 1.1 was adopted as a criterion for convergence and ESS>200 considered adequate. We employed the correct classification rate and the area under the curve

(AUC) measures to evaluate the model performance. The analysis was conducted using the R programming language [26] and Brms package[25], and the detailed code can be accessed on GitHub via the provided link(https://github.com/CarolineNM/HBV).

7.6 Results

7.6.1 Characteristics of the study population

In total, there were 1983 children included,127 of whom were sampled more than once in the first four years of the study bringing the total samples tested to 2741. The least number of study participants was 224 recorded in 2007 while the highest number of participants was 491 in 2019. In all the survey years, the majority of the samples were in the 1-4yrs and 5-9yrs age categories while the number of samples in both males and females was comparable (Table 7:2).

Table 7:2 Total number of participants per survey year stratified by age strata, sex, number tested for the different HBV markers and seropositivity

Survey Year	**2007**		**2009**		**2011**		**2013**		**2015**		**2017**		**2019**		**2021**	
Age category	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Total	224	100	368	100	282	100	396	100	278	100	408	100	491	100	294	100
<1yr	8	4	0	0	0	0	0	0	4	1	8	2	7	1	6	2
1-4yrs	118	53	132	36	89	32	106	27	123	44	143	35	209	43	80	27
5-9yrs	96	43	202	55	115	41	141	36	114	41	193	47	214	44	98	33
10-14yrs	2	1	34	9	78	28	149	38	37	13	64	16	61	12	110	37
Sex																
Female	109	49	179	49	136	48	192	48	130	47	203	50	245	50	136	46
Male	115	51	189	51	146	52	204	52	148	53	205	50	246	50	158	52
HBV markers																
HBsAg	224	5	368	13	282	16	396	5	278	0	408	0	491	1	292	3
Anti-HBc	219	3	365	6	282	6	270	6	270	1	363	2	489	1	289	4
Anti-HBs	208	56	331	57	248	49	251	47	257	64	347	60	469	54	281	51

7.6.2 Immunological markers

All the 2741 samples were tested for HBsAg, 2605 of which tested negative.2547 of these had sufficient volume to allow further testing of anti-HBc in order to assess evidence of previous exposure. A total of 63(2.5%) had evidence of previous HBV exposure indicated by positive anti-HBc,4(0.1%)were equivocal results while 2346(92%) had no evidence of exposure to HBV. Samples with sufficient volume were further tested for anti-HBs to assess immunity status.54(2%) had evidence of immunity from a resolved infection,9(<1%) had an isolated anti-HBc,1230(45%) had immunity from vaccination while 1045(38%) were susceptible. 136 (5%) samples had an active infection as evidenced by presence of HBsAg with 12 of these being false positives based on simultaneous positive HBsAg and anti-HBs without evidence of vaccination (Fig. 7:2a).

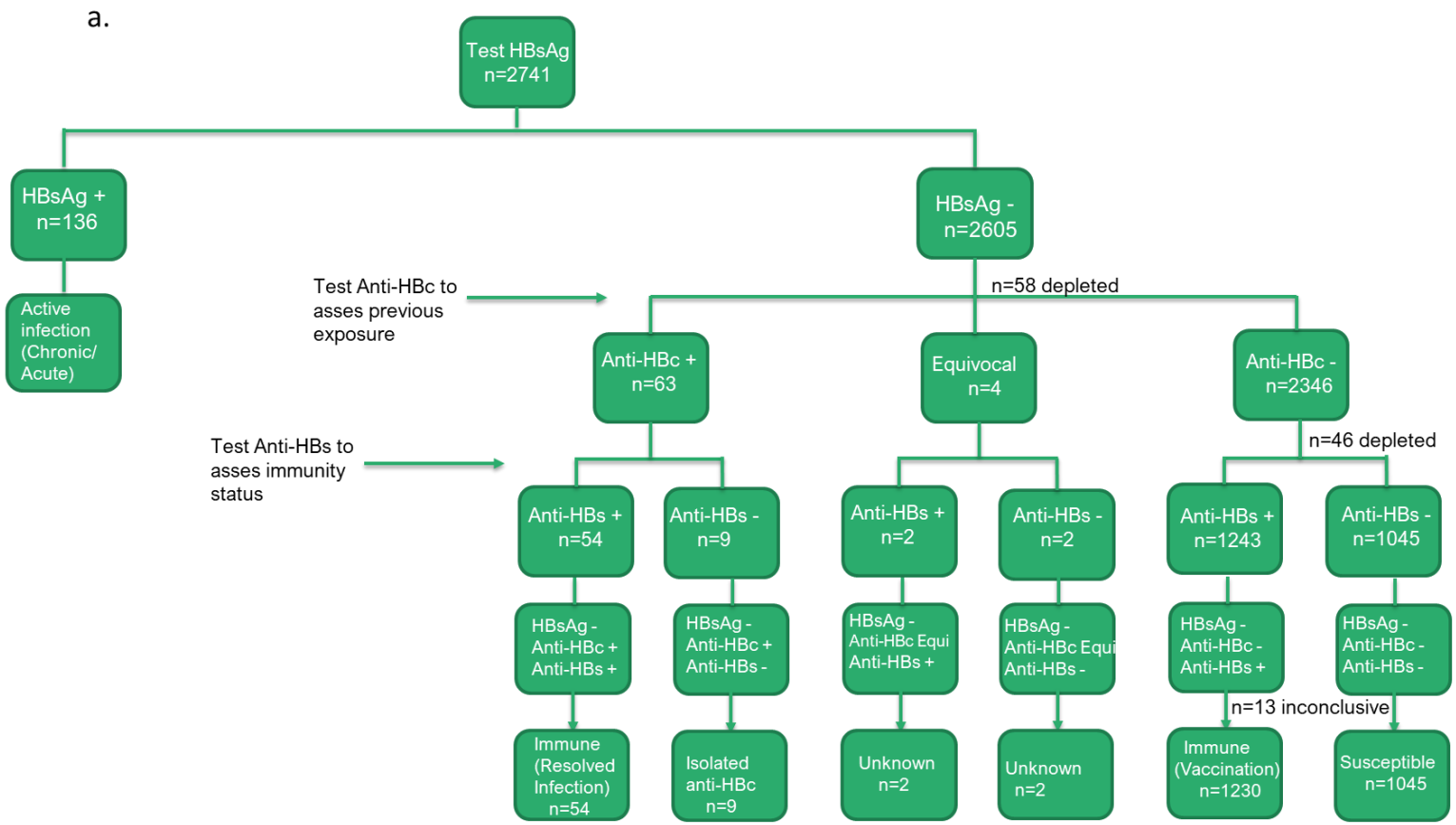

Figure 7:2 a) is the final algorithm for hepatitis B serology testing. HBsAg, hepatitis B surface antigen; Anti-HBc, antibody to hepatitis B core antigen; Anti-HBs, antibody to hepatitis B surface antibody.

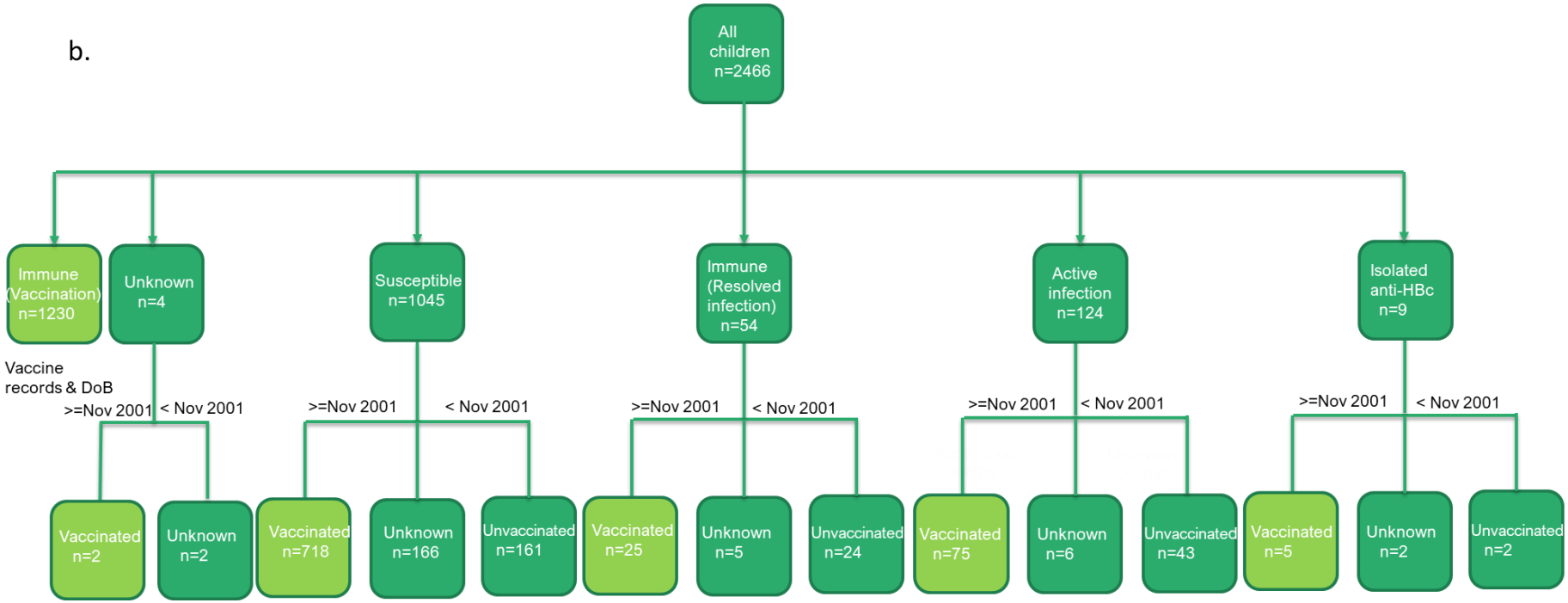

Figure 7:3 b) is the algorithm used to update vaccination status using information from serological markers, vaccination records and date of vaccine introduction. Any child with both a vaccinated record and born after November 2001 was considered vaccinated while those born before November 2001 were considered unvaccinated irrespective of the vaccine record

7.6.3 Serological profile and vaccination records

Among the 1230 individuals who tested positive for anti-HBs, indicating vaccination, 4 (0.3%) were initially classified as unvaccinated based on vaccine records, and 124 (10%) lacked available information in the vaccine records (Table s7:1). After updating the vaccination information, 2055(83%) were classified as vaccinated, 230(9%) were unvaccinated while the status of 181 children (7%) could not be further classified based on the available information, leaving their vaccination status unknown (Fig. 7:2b). Examining the coverage rates associated with the revised vaccination status revealed a statistically significant increasing trend (χ=236.3, $p<0.001$) in vaccination rates over the years (Table s7:2).

7.6.4 Cumulative prevalence of HBsAg in children

Among 1983 children with at least one HBsAg test result, 102 tested positive at least once, resulting in a cumulative HBsAg prevalence of 5.1% (CI: 4.2%-6.2%). The non-cumulative prevalence, calculated based on the total number of positive HBsAg tests (136) and the total population of 2741, was 5.0% (CI: 4.2%-5.9%).

7.6.5 Chronic infection using longitudinal data HBsAg data

Out of 102 children who had ever had a positive HBsAg test result at least once, 39 children could not be further classified as having acute or chronic infection from the data available as there was no subsequent positive HBsAg. Of the remaining 63 children, 21% CI: (13-22%) had chronic hepatitis B, 67% CI: (54-77%) had a resolved infection and 13% CI: (7-23%) had inconclusive results (Pos-Pos-Neg or Pos-Pos-Pos-Neg or Neg-Pos-Pos-Neg)

7.6.6 Changes in HBV prevalence across the years

The prevalence of active HBV infections among children in Kilifi as indicated by the presence of HBsAg showed a fluctuating pattern, with the highest prevalence observed in the early years of the study and notably lower or negligible prevalence in the later years (Table s7:3 and Fig. 7:3). HBV infection prevalence was at its peak in 2011 at 17% CI: (6-33%), followed by 2007 and 2009 with a prevalence of 9% CI: (2-30%) and 9% CI: (4-27%) respectively. In contrast, no active HBV

infections were observed in 2015 and 2017 while prevalence was less than 1% in 2019 and 3% (CI: 1-22%) in 2021.Active HBV infection prevalence was higher in the older ages resulting to a significant heterogeneity of prevalence across ages in some of the years (Table s7:3).

The proportion of children with markers of immunity due to prior infections i.e. coexistence of both anti-HBc and anti-HBs was notably lower, and this pattern was distributed across various years and age groups. This proportion was less than 1% in both 2007 and 2019 and ranged between 1% and 4% in the remaining survey years. Similarly, the proportion of children with isolated anti-HBc remained consistently below 1% across all the years (Fig. 7:3).

Combining the three estimates gives an overall proportion of children with evidence of ever having been infected with HBV which reached its peak in 2011 at 20% CI: (7-37%), followed by 14% CI: (6-31%) in 2009 and 10% CI: (3-31%) in 2007.Less than 5% of the children had evidence of ever having been infected with HBV between 2015 to 2019 while 6% CI: (2-27%) were ever infected in 2021(Fig. s7:4).

7.6.7 Susceptible and vaccinated groups

The proportion of susceptible children increased significantly across the different age categories in all the years($p<0.001$) (Fig. 7:4 and Table s7:4). HBV susceptibility was at its peak in 2019 at 46% CI: (30-74%) and lowest in 2015 at 29% CI: (18-53%).Conversely, the proportion immune from vaccination was high in the young age groups and declined significantly across the different ages($P<0.001$). This proportion was also higher in the last years of study from 2015 onwards and reached its peak in 2021 at 51% CI: (31-70%).2015 had the least proportion of children who were immune from vaccination at 26% CI: (15-42%).The inconclusive group which included children negative for HBsAg who could not be classified further due to insufficient volume had fluctuating patterns across the different years (Fig. 7:4).

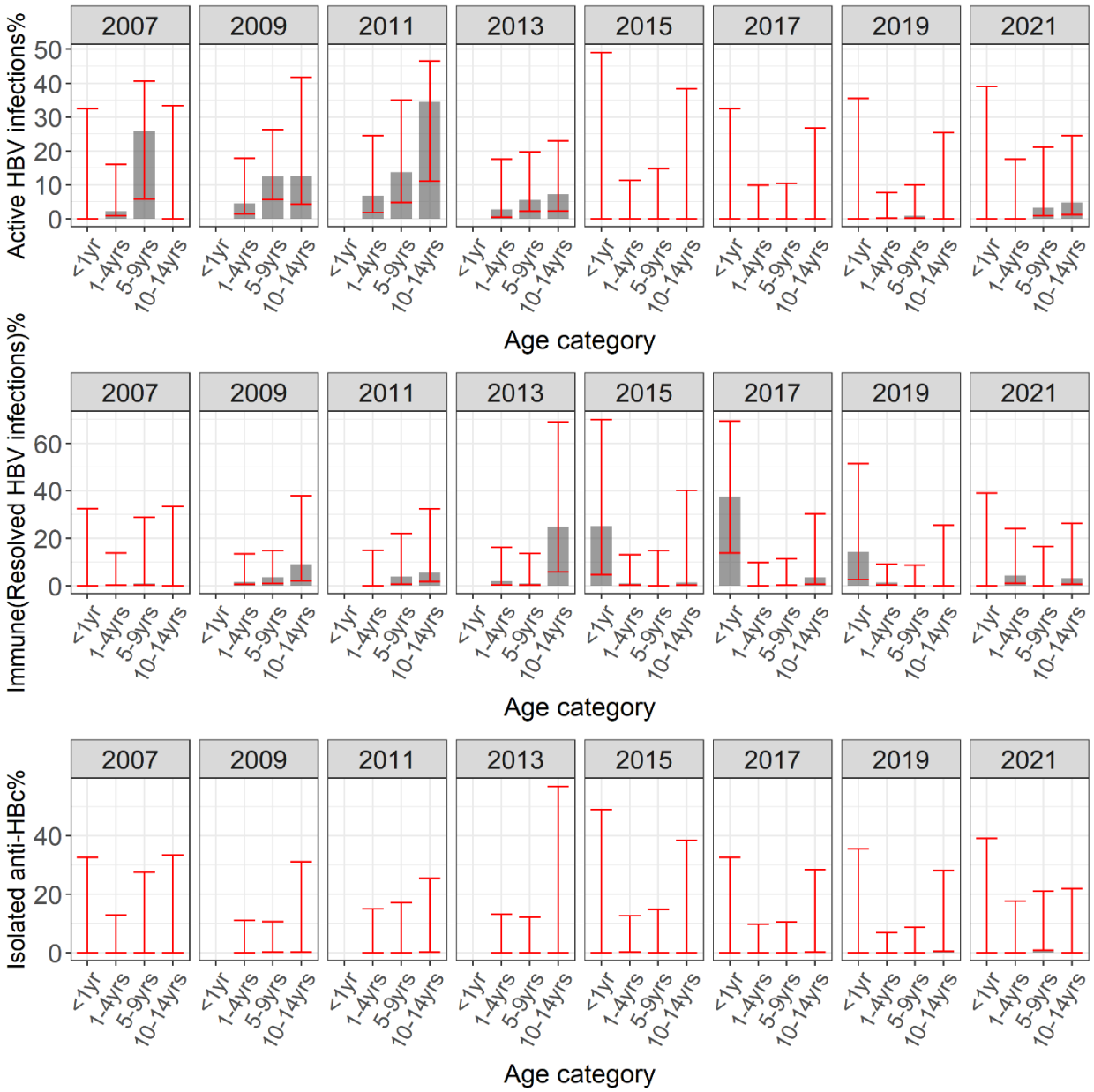

Figure 7:4 Proportion of children in Kilifi with active HBV infection, Immunity from a resolved infection as shown by positive test of both anti-HBc and anti-HBs and isolated anti-HBc adjusted for underlying population.

Figure 7:5 Proportion of children with current or resolved HBV infection, never exposed to HBV, immune from vaccination and with unclassifiable (inconclusive)HBV status, stratified by age and survey year

7.6.8 Vaccine effectiveness using a catalytic model

The joint reverse catalytic model successfully converged (Fig. s7:5) and mostly captured the overarching trends within both vaccinated and unvaccinated groups (Fig. 7:5). The estimated VE against active HBV infection was 67% (95% Cr: 50%-79%) and the duration of anti-HBs antibodies after an active HBV infection was 0.37 years (95% Cr: 0.24-0.61years) suggesting mostly independence in observed seroprevalence between consecutive surveys. The annual FOI before 2007 was evaluated to be 0.35(95% Cr 0.17-0.66). This escalated to 0.79(95% Cr 0.48-0.99) between 2007 and 2009 and further surged to 0.91(95% Cr 0.62-0.99) from 2009 to 2011(Table 7:3). A decline in FOI was observed across all subsequent periods, reaching its zenith between 2015 and 2017 at 0.02 (95% Cr: 0.00 - 0.08), followed by a resurgence in the last two study periods.

The simple catalytic model had a relatively poor fit (Fig. s7:5) and resulted into a lower VE estimate of 58% (95% Cr: 37%-72%) (Table s7:4). Similarly, both the reverse and simple catalytic models fitted to the recent years of study (2015-2021) also had a poor fit (Fig. s7:7 and Fig. s7:8)

and resulted to a lower estimate of VE, 50% (95% Cr: 25%-98%) and 51% (95% Cr: 24%-98%) respectively (Table s7:5).

Table 7:3 Parameter estimates from the joint reverse catalytic model

Model	Period	FOI (95% Cr)	Waning	1-VE	VE(%)
Reverse catalytic model with time varying FOI jointly estimated between consecutive surveys for all surveys(2007-2021)	Pre_2007	0.352 (0.169 - 0.658)	2.676 (1.639 - 4.103)	0.327 (0.215 - 0.497)	67(50-79)
	2007-2009	0.792 (0.479 - 0.987)			
	2009-2011	0.906 (0.62 - 0.996)			
	2011-2013	0.391 (0.204 - 0.663)			
	2013-2015	0.024 (0.001 - 0.136)			
	2015-2017	0.015 (0.001 - 0.084)			
	2017-2019	0.068 (0.019 - 0.177)			
	2019-2021	0.092 (0.02 - 0.274)			

7.6.9 Vaccine effectiveness using a statistical model(BRM)

The model was a good fit and achieved successful convergence, as demonstrated by the trace plots (Fig. s7:9) with no indications of autocorrelation across all model variables.
Specifically, assuming all factors are held constant, an increment of one year in age corresponded to a 9.8% increase in the odds of having an active HBV infection (95% Cr 3.2-17.0). In contrast, having received vaccination was linked to a 78.6% (95% Cr 65.5-86.8) reduction in the odds of having an active HBV infection. The model was able to correctly classify 95.2% of all observations, with an accompanying area under the curve (AUC) score of 0.72 (Table 7:4).

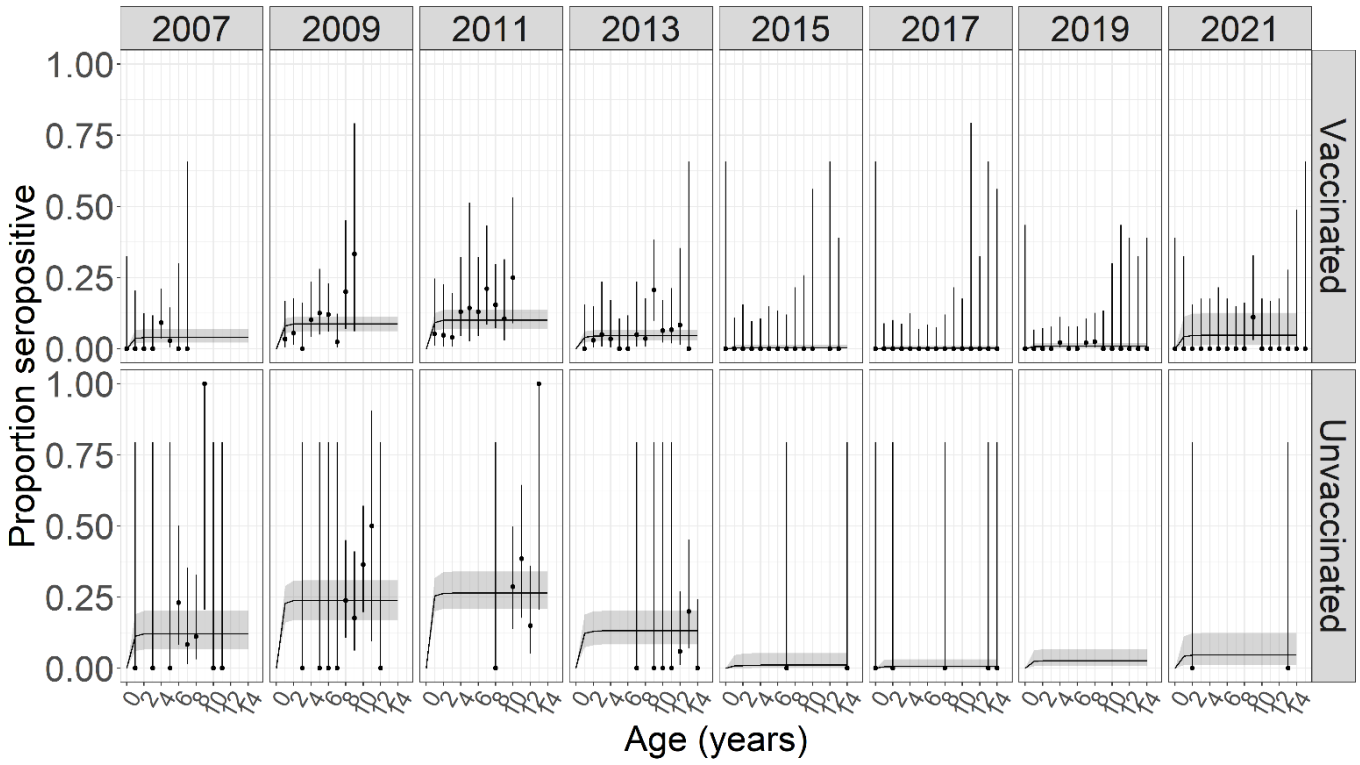

Figure 7:6 Joint reverse catalytic model .The points are the observed proportion of HBsAg seropositive individuals. Lines are the seroprevalence curves, sampled from the fitted model where shaded region represents 95% credible interval of the predictive posterior distribution.

Table 7:4 Summary estimates from the binary logistic model.

Predictors	**Estimate**	**95% Cr**	**VE**	**Correct classification rate**	**AUC measure (Discrimination)**
Intercept	0.078	(0.037-0.161)	79(66-87)%	0.952	0.72
Vaccination status (Vaccinated)	0.214	(0.132-0.345)			
Age in years	1.098	(1.032-1.170)			
Sex (Male)	1.376	(0.928-2.048)			

7.7 Discussion

We identified HBsAg prevalence of 5.0% (CI: 4.2%-5.9%) indicating active HBV infections, in children within the KHDSS. This aligns with high intermediate endemicity, which is characterized by a HBsAg prevalence of 5% to 7%[27]. The prevalence exhibited heterogeneity across different years, peaking in the early study years and diminishing to nearly non-existent levels in later years. This pattern was mirrored by the Force of Infection (FOI), ranging from 0.79 to 0.91 between 2007 and 2011 and subsequently declining in later period. Chronic HBV, defined as the persistence of HBsAg in the serum for at least 6 months, was present in 21% of the children in the longitudinal subset two years after their first HBsAg positive tests. The majority of the children (83%) were classified as vaccinated based on the combination of the sole presence of the anti-HBs immune marker and vaccination records. Proportion of vaccinated children based on this updated status showed a statistically significant upward trend over the years. The estimated VE against active HBV infection was determined as 67% (95% Cr: 50%-79%) in the catalytic model with a seroreversion rate of 0.37 yrs suggesting substantial degree of independence in observed seroprevalence between consecutive surveys while the statistical model estimated a slightly higher VE of 78.6% (95% Cr 65.5-86.8).

The HBsAg seroprevalence among children in Kilifi was notably high during the initial three years (HBsAg > 8%), indicating a state of high endemicity. In contrast, in the subsequent years, there was a decline in seroprevalence (HBsAg < 4%), signifying a shift to low intermediate endemicity. This pattern was complemented by the FOI, which was initially high, estimated to range between 0.79 and 0.91 in the early years. The later surveys indicated a decrease in transmission intensity with the most significant decline observed between 2015 and 2017, where the estimated FOI dropped to 0.01. The elevated seroprevalence and FOI at the study's outset could be attributed to various factors. Firstly, the initial survey conducted in 2007, approximately five years after vaccine introduction, could strongly contribute to the observed high seroprevalence, particularly among older age groups that did not benefit directly from the vaccine. Although indirect benefits, such as herd immunity, might reduce the HBsAg-positive pool, the impact could take years to materialize [28]. Secondly, potential improvements in vaccine coverage rates over the years might explain the observed decline. In our study, we observed a statistically significant increasing rate in the proportion of vaccinated children over the years using the updated vaccination information. Our estimates ranged between 77% to 100% between 2007 and 2021. Comparing these estimates with

national administrative coverage rates for HepB3 coverage did not reveal a consistent pattern. Some years showed higher coverage than reported estimates, while in others, it was lower. Administrative coverage rates fluctuated over the years, ranging from 71% in 2017 to 87.5% in 2021, with no significant variations between 2007 and 2021[6].

The observed HBV high seroprevalence in children in the early years in our study markedly exceeded rates reported in other countries, such as Tunisia (0.1% in children under 20 years)[29], Taiwan (0.5% in children under 15 years)[30],two rural villages in Gambia (1.1% in children) [31]and Senegal (1.1% in children under 15 years) [32]reported 10 to 30 years after the initiation of their vaccination programs. A common characteristic among these countries is the inclusion of a birth dose (HepB-BD) in addition to achieving high 3-dose coverage. For instance, Gambia, which introduced HepB-BD in the early 1990s has consistently maintained excellent coverage rates for the 3rd dose of hepatitis B, reaching 93% in 2018, along with timely and high coverage of HepB-BD at 94% in the same year. Similarly, Senegal, which introduced HepB-BD in 2016, achieved a 96% coverage rate for the 3rd dose and 92% coverage for HepB-BD in 2018 [5]. However, the elevated HBV seroprevalence observed in our study aligns with the 11.4% HBV prevalence reported in children from a systematic review in Nigeria[33]. Nigeria, which introduced HepB-BD in 2004, has consistently maintained low coverage rates, reaching 32% in 2018, with a 58% coverage for the 3rd dose in the same year [5]. While other factors may also be in play, this underscores that the introduction of HepB-BD alone is insufficient for controlling HBV. Instead, a combination of achieving high coverage for the three doses, along with timely administration and extensive coverage of HepB-BD, is crucial for reducing the burden of HBV. These recommendations align with WHO guidelines, even as they advocate for the introduction of HepB birth dose in countries[34].

The use of serological data to monitor the effective coverage of immunisation programs is gaining increased attention, particularly in regions with inadequate record-keeping systems[35]. In the context of hepatitis B, serologic testing for multiple antigens allows differentiation between natural infection and vaccination[34, 36]. We utilised anti-HBs markers to update vaccine records for children with missing vaccination information. This approach enabled the reclassification of 124 out of 305 (41%) children with missing vaccine records who tested positive for the anti-HBs marker only, and also corrected the classification of approximately 4 misclassified children based on vaccine records. Similar to a prior tetanus analysis (chapter 6 of the thesis), these findings

provided insights into vaccination records within our population, revealing potential issues of poor record-keeping and incomplete vaccination records or even issues with the serology data. For example, during data consistency checks, we identified five children born before the vaccine was introduced who tested positive for anti-HBs only. Although these children were not included in further analysis, this highlights a scenario where serological markers could be misleading probably due to the sensitivity of the assay used or even misinterpretation of immune markers[37]. In rare instances, it could also signify chronic occult HBV, characterized by an isolated anti-HBs[38] .Additionally, the case of four children initially classified as unvaccinated may be attributed to inaccuracies in vaccine records[39].

We utilise both a catalytic model and a statistical model to estimate the VE against HBV infection. The decision to employ a catalytic model was driven by two main considerations. Firstly, there was a disproportionately low number of unvaccinated individuals compared to those vaccinated. The mechanistic approach of the catalytic model was anticipated to provide better insights into VE given the data limitations. Secondly, our interest extended beyond seroprevalence estimates; we aimed to estimate the rate at which children acquire infection and how this rate has evolved over the years. Despite these intentions, we encountered several challenges with the catalytic model approach. Uncertainties regarding immune markers and the serology of HBV complicated the analysis, as detailed in the supplementary section. To account for the occurrence of mild infections in our study we fitted a reverse catalytic model which allows for the protective antibody (anti-HBs) expected to develop after development of HBsAg (marker of infection) to decline overtime and for previously infected individuals to become susceptible again.

We calculated vaccine effectiveness (VE) estimates of 67% (95% Cr: 50%-79%) and 78.6% (95% Cr 65.5-86.8) against all HBV infections, in line with the intermediate endemicity observed in seroprevalence estimates. Although the credible intervals overlap, the distinctions between the catalytic model and logistic regression VE estimates are rooted in various factors. Notably, the catalytic model assumes a certain level of homogeneity in the relationship between vaccine status and infection risk across the population. In contrast, logistic regression permits a more explicit modeling of individual-level heterogeneity, resulting in slight differences in these estimates.

Our estimates of VE were lower than those reported in a study conducted in Gambia. One potential explanation for this variance lies in our specific focus on VE against all HBV infections, in contrast to the study, which separately assessed VE against infections and chronic carriage. The

observational long-term follow-up study in Gambia reported VE of 80% against HBV infection and 94% against HBV chronic carriage 14 years after vaccine introduction[40]. Age-specific VE estimates in that study were notably higher in younger age groups compared to older age groups. Our estimates were substantially higher compared to Iran, where VE was 29% against HBV infection and 51% against chronic carriage after 25 years of vaccine introduction. The authors in Iran attributed the low VE estimates to waning VE against infection over time[41].

The model that incorporates the waning component demonstrated a superior fit to the seroprevalence trends in the data when compared to the simple catalytic model, which assumed no waning. This implies that the only way the catalytic model could reconcile with the data is by assuming high waning rates. This estimate carries two implications. First, it suggests that the observed seroprevalence in different surveys is largely independent, which in turn relaxes the assumption in the model of a constant FOI before year 2007. Second, the estimate indicates that a substantial portion of the infections in our study could be acute or mild, a conclusion supported by HBV reinfections observed in the longitudinal sub cohort.

A plausible explanation for these results could be that the majority of the infections are mild in nature, and as a result, they may not elicit sufficient anti-HBs production. In our consistency checks, we found evidence of repeated mild HBV infections characterized by the presence of HBsAg only, without the development of anti-HBs and anti-HBc (transition from infected to susceptible). There is evidence indicating that mild HBV infections can occur, manifesting as positive HBsAg results only, and the marker clears after a certain duration[42].This could also account for the relatively high HBsAg positives in our data compared to the prevalence of anti-HBc, where, on average, we would expect more anti-HBc compared to HBsAg.

Evidence of HBsAg without the development of anti-HBc has been previously documented among infants in China[43], but this was associated with immune incompetency. This phenomenon has also been observed in infants and immunocompromised individuals in France[44]. Other studies show that certain HBV variants can cause infection with low-level production of anti-HBc. A study in Senegal attributed the absence of anti-HBc or the lack of development of anti-HBc and anti-HBs after the disappearance of HBsAg to a new virus, termed HBV2 [45]. In Uganda, this phenomenon was explained as either the early asymptomatic period of HBV infection or the late incubation period[46] while other studies have suggested double testing of anti-HBc using two different assays to rule out technical errors[44].

A key strength of our study lies in the availability of representative age-specific seroprevalence data in children in KHDSS which included a substantial number of participants. This dataset was collected through a series of cross-sectional surveys conducted over various years, providing a temporal perspective on HBV seroprevalence across diverse age groups within the population. The inclusion of multiple HBV markers enabled us to deduce vaccine status, susceptibility, and immunity status using serological data. Furthermore, data from KVMS and KHDSS population registry provided important information regarding the number of vaccine doses received by participants. The utilisation of both catalytic and statistical models allowed us to integrate these diverse datasets, enabling us to draw meaningful conclusions about the effectiveness of the current vaccination program against HBV infection in Kilifi.

Our study faced several limitations. Firstly, there was an insufficient volume of samples, leading to depletion in some cases. This limitation resulted in an inability to test certain samples for all markers, constraining the comprehensive interpretation of the data. Additionally, even in samples where all markers were present, we encountered cases where marker interpretations contradicted existing literature. We attribute this discrepancy to the heterogeneous nature of HBV infection expression among participants.

Secondly, the complexities arising from different marker interpretations compelled us to make assumptions in the catalytic model. The applicability of these assumptions may vary in different settings. Thirdly, a considerable number of participants had missing vaccine records and so we couldn't incorporate these samples in the assessment of VE. Lastly, the generalisability of our conclusions to other settings is limited. Our seroprevalence study focused on a rural area within the country, and the prevalence of HBV can significantly differ even between regions and countries due to differences in vaccination coverage, levels of natural exposure, and distinct mixing patterns across various age groups. As a result, the findings from our study may only be applicable within specific contexts.

The current vaccination program appears to have a positive impact on reducing HBV seroprevalence, as evidenced by the observed decline in seroprevalence over the years, a reduction in FOI and substantial VE estimate against HBV infection. However, there is still room for improvement. It is crucial to enhance efforts to ensure that children receive the recommended three-dose coverage, aiming for a reduction in breakthrough infections among vaccinated individuals. Additionally, increasing vaccination coverage for each dose is essential. Introducing

a birth dose and improving the coverage of the third dose could play a vital role in reducing HBV transmission, aligning with the goals set for HBV elimination by 2030.

7.8 References

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8. Chapter 8: Discussion

8.1 Introduction

The concept of employing serosurveillance to assess the presence of pathogen-specific antibodies within populations and define the infectious disease landscape is gaining rapid momentum[1, 2]. This approach is increasingly recognized as a potent tool that can complement conventional case-based disease surveillance and routine vaccination coverage estimates, supplying a substantial amount of information to shape and guide immunisation programs. Despite its prevalence in HICs[3-7], it is still underutilised in LMICs[8]. In this thesis, I have used a series of case studies in an LMICs setting across different pathogens to critically assess the added value of serosurveillance beyond vaccine coverage estimate and case-based surveillance data in enhancing our understanding and ability to control VPDs.

I will give a summary of each chapter, before discussing the strengths, limitations, and implications of this work.

8.2 Summary of key findings

8.2.1 Seroprevalence of antibodies against Measles and Rubella over a 12-year period (2009 – 2021) in Kilifi, Kenya and the impact of Measles Rubella (MR) campaign of 2016

In chapter 2, I estimated age-specific population immunity profiles for measles and rubella in children using cross-sectional survey data from KHDSS spanning 2009 to 2021 and in adults using cross-sectional survey data from KHDSS in 2021. Antibody titres were measured using a fluorescent bead-based multiplex immunoassay. I used Bayesian multilevel regression with poststratification to obtain seroprevalence estimates adjusted for underlying population, sensitivity and specificity of the assay and a mixed effects logistic regression to test for association of seropositivity with related factors.

The findings shed light on challenges in the current measles control program. Suboptimal measles immunity in MCV1 and MCV2 eligible children was evident, as indicated by lower measles seroprevalence than the herd immunity threshold in most survey years, signifying insufficient protection against outbreaks. The lower measles seroprevalence in MCV1 eligible children compared to reported vaccination coverage in the study period suggested a delayed MCV1

timeliness, vaccine failures and/or reporting inaccuracies in vaccination coverage. The Kenyan measles immunisation programme is still highly relying on SIAs for immunity as shown by significant waning of antibody titres with age, leading to immunity gaps in older children in absence of boosting. This could be avoided by enhancing uptake of MCV2 vaccination. The findings also revealed immunity gaps in MCV1 ineligible children with seroprevalence lower than 50% in most of the years suggesting there is an extended period of susceptibility in young infants probably as a consequence of rapid decay of maternally acquired antibody. While delaying infant vaccination aims to minimize interference from maternal antibodies and optimize vaccine efficacy, the variability in the rate of maternal antibody decline presents challenges in determining the optimal vaccination timing. Early vaccination has been suggested in high-risk settings, although this is an ongoing discussion as moderate evidence indicates potential negative impacts on seroconversion to subsequent measles vaccine doses[9, 10].

For rubella, the results demonstrated the success of the vaccination program, with seroprevalence increasing from 45% to 79% in the MR campaign target group. Presently, rubella seroprevalence in children ranges between 82-90% across the survey years following vaccine introduction, aligning with the rubella herd immunity threshold of 83-86[11].This signifies adequate protection against outbreaks. In 2021, rubella seroprevalence among adults was 92%. Sustaining robust coverage is crucial to preventing immunity gaps in women of reproductive age and CRS in infants. Overall, these findings offer critical insights into the current state of the measles-rubella control program.

8.2.2 The relative contribution of infection, routine vaccination and supplementary immunisation activities to measles seroconversion in Kenyan children: A modelling study

In chapter 3, I extended the findings from chapter 2 by developing a static birth cohort model to track the proportion of children who are either measles-naïve or have seroconverted due to natural infection or vaccination through MCV1, MCV2, or SIAs. I fitted the model to measles serological and case notification data and vaccination coverage estimates from the KHDSS to estimate the relative contributions of vaccination and infection to measles immunity in Kilifi. I also explored the impact of changes in the timing and coverage of the current RI program on reducing dependence on SIAs and measles susceptibility.

I found that MCV1 remains the most crucial of the immunisation opportunities in the current program for measles control, accounting more than 50% of all seroconversion. None routine immunisation pathways accounted for a substantial proportion of seroconversion with natural infection and SIAs contributing 24% and 16% to all seroconversions, respectively. Projection scenarios demonstrated that a 10% increase in timely MCV1 coverage could reduce susceptibility to infection and reliance on SIAs by approximately 50%, emphasizing the importance of optimising routine coverage timing to diminish reliance on SIAs and lower measles susceptibility.

8.2.3 The importance of supplementary immunisation activities to prevent measles outbreaks during the COVID-19 pandemic in Kenya

In chapter 4, I combined measles serological data, local contact patterns and vaccination coverage estimates into a cohort model to estimate the impact of reduced measles vaccination coverage and suspended SIAs due to COVID-19 on the risk of measles outbreaks. Given the considerable uncertainty in actual reduction of routine vaccination uptake, I assumed reductions of 15% based on reduction in vaccine clinic visits in Kilifi County,50% based on the range of reported disruption in vaccination services from WHO immunisation pulse poll and 100%. I also assumed a 50% reduction in measles transmissibility given that the COVID-19 mitigation measures were reported to have reduced social contacts by the same margin.

I found that depending on the extent to which routine vaccination coverage was reduced, a decline in population immunity during COVID-19 pandemic would result in an increased risk of a measles outbreak. In February 2020, at the time of the planned national SIA, I estimated that 90% (85-92) of the population were immune which was equivalent to a 34% (8-54) probability of a large outbreak suggesting that the SIA would have been timely in closing immunity gaps. This risk of an outbreak which was in part mitigated by the assumption that contact patterns had reduced was accelerated by immunity gaps arising in children who missed their routinely delivered MCV1 and MCV2. Our model predicted this risk would increase in subsequent months and by December 2020, the estimated risk had increased to 38% (19-54), 46% (30-59), 54% (43-64) assuming a 15%, 50% and 100% reduction in measles vaccination coverage respectively. Overall the results presented in this chapter showed that SIAs would be crucial for preventing measles outbreaks, especially once contact restrictions in Kenya were reduced. In line with these findings, a

subnational SIA targeting under-fives was conducted in July 2021 in areas with high risk of measles outbreaks[12].

8.2.4 Seroprevalence of antibodies against Diphtheria, Tetanus and Pertussis over a 12-year period in children in Kilifi, Kenya (2009-2021)

In Chapter 5, I generated age-specific population immunity profiles for diphtheria, pertussis, and tetanus using cross-sectional survey data from KHDSS spanning 2009 to 2021 and in adults using cross-sectional survey data from KHDSS in 2021. Antibody titres were measured using a fluorescent bead-based multiplex immunoassay. Tetanus and diphtheria antibody titers were categorized into distinct seroprotection levels, while pertussis antibody titers were classified based on the time since infection. I used Bayesian multilevel regression with poststratification to obtain seroprevalence estimates adjusted for underlying population, sensitivity and specificity of the assay and a mixed effect logistic regression to test for association of seropositivity with related factors.

I found insufficient diphtheria immunity levels, with the proportion of children demonstrating at least full protection levels falling below the 84-89% herd immunity threshold in all survey years, indicating inadequate protection against outbreaks. In contrast, immunity against tetanus remained consistently robust, with approximately 70-80% of children consistently exhibiting at least full protection levels across different years. A substantial proportion among older children however, showed minimal seroprotection against tetanus infection. The study also revealed low evidence of recent pertussis infection, as only 5% of children and less than 1% of adults had antibody titres equal to or above 62.5 IU/ml in the last year. The rapid decline in antibody titers with age, particularly for diphtheria coupled with a substantial proportion of older children having minimal tetanus seroprotection and evidence of pertussis circulating in the population, emphasizes the potential need for prolonged protection through booster pentavalent doses. This is currently absent from the Kenyan vaccination schedule.

8.2.5 Estimating tetanus immunisation coverage from vaccination records and cross-sectional serological surveys in Kilifi

In Chapter 6, I built on the findings of Chapter 5 by combining the tetanus immunity profiles with vaccination coverage estimates obtained from vaccine records. This enabled the estimation of

effective tetanus vaccination coverage and the rate at which tetanus antibody levels diminish over time. Additionally, I conducted a comparative analysis of serological data and documented vaccination information. The ultimate objective was to evaluate the utility of tetanus serological data in identifying gaps in vaccination, especially in scenarios where vaccination records were unavailable.

I demonstrated a strong correlation between tetanus IgG titres and the recorded number of vaccine doses, with seroprevalence dropping from 97% (3 doses) and 92% (2 doses) to 79% (1 dose) and 72% (0 dose); the latter demonstrating a likely underreporting of DTP receipt in vaccination records. Approximately 12% of study participants had no vaccine records and yet 93% had evidence of protection through seroconversion; indicating that most of them had likely been vaccinated. Our study also revealed a decline in antibody levels with age, with an estimated half-life of 14 years. In the predictive model, IgG levels appear to exhibit some correlation with documented vaccination status, as individuals with no or limited vaccination records tend to have lower average IgG levels. These results underscore the importance of meticulous record-keeping and highlight the utility of serological data in identifying potential gaps in vaccination coverage, particularly in the absence of comprehensive vaccination records.

8.2.6 HBV seroprevalence in Kenyan children and the effectiveness of the current vaccination program against HBV infection

In Chapter 7, I estimated HBV seroprevalence based on active HBV infections using a combination of various HBV markers. Subsequently, I integrated data from these markers with vaccination records retrieved from a vaccine registry in KHDSS. This integration aimed to update vaccine information for participants with missing records by leveraging information derived from serological data. Following this, I merged these two datasets—active HBV infections and vaccination information and utilised two distinct approaches to estimate HBV vaccine effectiveness(VE) against infection: A catalytic model, utilizing population-level HBsAg seroprevalence and a regression model employing individual-level data on presence or absence of an active HBV infection.

I found that 5.0% (CI: 4.2%-5.9) of children in KHDSS had an active HBV infection, aligning with high intermediate endemicity which is characterized by a 5% to 7% HBsAg prevalence. Both

seroprevalence and FOI exhibited temporal heterogeneity, peaking in early study years and diminishing later, likely due to improved vaccination coverage reflected in updated vaccine records. Approximately 83% of children were classified as vaccinated based on the combination of the anti-HBs immune marker and vaccination records, with the proportion of vaccinated children based on this updated status showing a statistically significant increasing trend over the years (χ=236.3, p<0.001). VE against active HBV infection was estimated at 67% (95% Cr: 50%-79%) in the catalytic model and slightly higher at 78.6% (95% Cr 65.5-86.8) in the statistical model, indicating a positive impact of the current vaccination program. Nonetheless, there is room for improvement, and the introduction of a birth dose and improvement of third dose coverage could be pivotal in curbing HBV transmission, aligning with the established goals for HBV elimination by 2030.

8.3 Contribution to the field and strengths of this research

8.3.1 Overall contribution to literature

One of the recognized uses of serological data is to provide a direct measure of population immunity. In this thesis, I generated age-stratified seroprevalence estimates for different pathogens: measles, rubella, tetanus, diphtheria, pertussis and tetanus which were simultaneously detected by the use of a fluorescent bead-based multiplex immunoassay[13]. Additionally, I determined seroprevalence for HBV through the analysis of different immune markers tested using WHO prequalified assays. These estimates have revealed some interesting insights in the existing vaccination programs for these diseases and provided a more nuanced understanding of the immunity landscape of the different VPDs compared to vaccination coverage estimates. Depending solely on vaccination coverage might overlook susceptibility pockets within the population, given their limited ability to depict variations in immunity across diverse age groups influenced by factors such as vaccine effectiveness and waning immunity [1].

Across most of the pathogens, the immunity levels in the age of recommended completion of the respective vaccine schedule were lower than the reported administrative coverage estimates. In part, this is because receiving a vaccine does not always result in immunisation against the targeted disease. Depending on the vaccine and the number of doses a minority of individuals may not develop an immune response post-vaccination[14]. For example, following measles vaccination,

the proportion of children who develop protective antibody levels are approximately 85% at 9 months of age and 95% at 12 months of age[15].

The seroprevalence estimates also brought to light immunity gaps arising from the waning of IgG in the absence of natural boosting, especially in older children. This phenomenon was particularly observed for measles, diphtheria, hepatitis B, and to some extent, tetanus. However, the decline of IgG antibodies can hold different implications for various diseases. Correlates of Protection (CoPs) for all these pathogens may vary both quantitatively and qualitatively, contingent upon whether the goal is to prevent clinical disease or inhibit infection and transmission within a population. For example, for measles, IgG levels <2 IU/ml after vaccination protect against infection, while titres between 0.12 and 2 IU/mL protect against clinical signs of disease but not against infection; titres <0.12 IU/mL are non-protective [16, 17].The immune system has also evolved and vaccines, like prior natural infection may protect through multiple mechanisms[18]. In measles, the traditional focus for CoPs has centred on humoral immunity. However, there is a growing acknowledgment recognizing the contribution of cellular immunity. Individuals exhibiting strong T cell responses, linked to long-term protection, may remain protected even without detectable antibody titres [19]. This challenges the conventional dependence solely on antibody titres for ascertaining immunity[18]. Diphtheria and tetanus, caused by toxin-producing bacteria, have extensively studied CoPs. Antibody levels of 0.01 IU/ml provide significant protection against disease, with 0.1 IU/ml associated with full protection [20, 21]. For diphtheria, a higher antibody titre >0.1 IU/ml has been suggested for enhanced protection[22]. Decreasing antibody titres below 0.01 IU/ml are consequently linked to the risk of disease transmission. However, instances of diphtheria and tetanus have been documented despite high antibody concentrations above 0.01 IU/ml, possibly due to inadequate diffusion into toxin production sites, a factor likely to vary among individuals. This variability makes it challenging to establish a definitive antibody level for protection [21, 23]. In the context of HBV cases of infection are generally associated with low or undetectable anti-HBs IgG levels. Anti-HBs antibodies are considered markers of immunity to HBV, and their presence indicates protection against both disease and infection upon exposure to the virus[24]. A decline in anti-HBs antibody levels, especially below the protective threshold of 10 mIU/ml may suggest a reduced immune response and could be associated with an increased risk of HBV infection. However, the relationship between anti-HBs antibody levels and protection is not absolute[18]. Some individuals with low

or undetectable anti-HBs levels may still have protection due to the presence of cellular immunity, which plays a role in defending against HBV infection[25-27]. Hence, while seroprevalence estimates offer valuable insights into direct immunity, careful consideration, especially regarding CoPs, is essential.

Similar to other countries where these estimates have been used to identify gaps in population immunity [6], inform targeted vaccination strategies [4] and inclusion of booster doses in vaccination schedules [7, 28] these estimates will be of immense value in monitoring the Kenyan immunisation program. For instance, the immunity gaps call for strengthened renewed efforts to increase the uptake of both MCV1 and 2 and pentavalent vaccines. The data will also be crucial in informing targeted interventions for SIAs in measles. Additionally, it will provide valuable guidance for the introduction of a pentavalent booster vaccine, particularly in light of the recent WHO recommendations advocating for three booster doses starting from the second year of life[29].

Existing evidence shows that serosurveillance has proven valuable in complementing case-based surveillance, particularly in situations where eradication of the disease necessitates reaching a herd immunity threshold like in the case of measles[30] or where the asymptomatic nature of the disease poses substantial challenges for surveillance like in the case of rubella[31] leading to substantial underreporting. Measles serial age-specific population measles immunity profiles in Kenyan children, crucial for assessing progress towards elimination, were previously unknown. This lack of data is not unique to Kenya, as indicated by a systematic review of measles serosurveillance studies that found a limited presence of such studies in most LMICs. Specifically, only 19% of all seroprevalence studies in the review were from countries in the African region, with the majority exhibiting high overall bias in sampling design and assay testing methods [32]. The impact of the rubella vaccine introduction on rubella immunity in children and adults was also previously unknown as was the effectiveness of the current HBV vaccination program in children since its introduction. The age-specific immunity profiles for measles, rubella and HBV in children and adults generated in this thesis will substantially complement the existing case surveillance data and contribute to filling these critical knowledge gaps.

The utilisation of serological data for monitoring the effective coverage of immunisation programs is gaining increased attention, particularly as organizations like Gavi and other international

agencies scrutinize how countries assess the efficacy of their immunisation services and provide credible data to justify financial investments[1, 33]. In this thesis, we linked tetanus serosurveys and vaccination records to compare crude and effective vaccination coverage. Additionally, we integrated data from HBV immune markers with vaccine records to update vaccination information for participants with missing vaccine records. We find this method, only undertaken in a few other areas [34-36]to be a useful way of identifying vaccination gaps particularly in communities with poor record keeping that can be filled during immunisation campaigns. However, use of serologic data is by no means a gold-standard as it may also result in inaccurate classification of vaccination history due to a range of factors.

One is the serologic profile generated by the vaccine. Infection by most natural pathogens results in the induction of a positive serologic response[2, 37]. Although, in tetanus natural infection is not thought to provide any immunity[38, 39],limited studies have suggested the possibility of natural immunity developing after asymptomatic colonization of the intestinal tract[40, 41].Consequently, the clinical implications of natural immunity to tetanus remains controversial which might affect the coverage estimates from these data. In our study, a substantial proportion with zero-recorded doses showed tetanus seropositivity, indicating potential inaccuracies with reported coverage or the presence of natural immunity. Another concern relates to the sensitivity and specificity of assays. In the case of HBV, we employed panel testing with various markers to differentiate between natural and vaccine-induced immunity. However, challenges with the sensitivity and specificity of assays can lead to false positive or false negative results, depending on the specific assays used for different markers[37]. Moreover, rare instances of chronic occult HBV, characterized by isolated anti-HBs, may occur, as evidenced by our study, where some children born before the vaccine introduction displayed serologic markers of immunity from Hepatitis B vaccination[42]. Integrating the collection and testing of biological samples into immunisation programs also adds a layer of complexity and requires significant financial resources. Therefore, it is crucial to carefully weigh the available resources against the survey goals and assess the potential added benefits of employing serologic methods to evaluate vaccination history[37].

8.3.2 Adapting methodology to local disease dynamics

Vaccine policy decisions for VPDs in LMICs frequently depend on modelled disease burden estimates due to a shortage of robust local data. However, this approach may not consistently capture the actual disease scenarios in these regions. As such, amother key strength of this thesis is the ability to utilize local serosurveillance data and tailor, customize and adapt existing methods to improve our understanding of disease susceptibility levels and control.

Measles epidemiology varies globally demanding customized interventions. A key concern in measles control is the interaction between different intervention programs in order to understand the added value of each program and optimize resource allocation. There are few studies that have explored interactions between different delivery strategies of MCV doses. For instance, a study in 9 high-burden countries indicated that SIAs effectively reach unvaccinated children, preventing more measles cases and deaths than MCV2 [43] while a study in Zambia found that MCV2 can sustain high levels of population immunity and that frequent, low-coverage SIAs might sustain higher levels of immunity than less frequent, high-coverage SIAs[44]. These studies did not explicitly assess changes in RI programs that would reduce reliance on SIAs, a critical consideration given WHO's concerns about SIA sustainability in LMICs[45].In Chapter 3, I formulated a static cohort model incorporating measles serological data, case-notification data, and records on timing, schedule, and coverage of RIs and SIAs to explore the interactions of natural infection and locally tailored interventions for measles control and elimination. In addition to contributing significantly to understanding the interplay between different MCV delivery strategies and natural infection in our context, this study provides a versatile modeling framework for measles control inquiries that can be extended in different settings.

Due to the highly infectious nature of measles, large outbreaks following disruptions to health care systems and reduced MCV1 coverage are typical [46, 47]. Following the COVID-19 pandemic, WHO recommended benefit–risk assessments on sustaining Routine Immunisation services for countries, considering local disease transmission dynamics and health system characteristics. Despite the proven benefits of sustaining RI services [48] and assessment of these disruptions to other VPDs elsewhere [49], a local assessment of the actual risk of measles outbreaks based on existing measles seroprevalence and the measles control program was missing. In this thesis, I extended an initial cohort model [3] incorporating local seroprevalence data, contact patterns, and

vaccination coverage estimates to enhance our understanding of local conditions related to projected measles immunity under various vaccination scenarios. The work not only informed expectations regarding the impact of COVID-19 on measles outbreaks but also provided insights for the utility of post-pandemic SIAs which were subsequently implemented in June 2021.

Subclinical cases of HBV infection are prevalent, but exposure can also lead to acute or chronic infections, significantly elevating the risk of liver disease and, ultimately, hepatocellular carcinoma (HCC)[68]. The subclinical nature of HBV coupled with limited data on chronic liver disease particularly in LMICs, often necessitates reliance on modelled estimates of HBV burden. In Chapter 7, I utilized local serological data on active HBV infections and vaccine records, integrating them into a catalytic model to estimate VE against HBV infections. The selection of the catalytic model's mechanistic approach aimed to glean deeper insights into VE, considering the inherent data limitations. This model also facilitated the estimation of the rate at which children acquire infection and how this rate has evolved over the years. Sensitivity analyses, incorporating variations of the catalytic model and a logistic regression model, were conducted to address uncertainties related to assumptions about immune markers. This study significantly contributes to understanding the current seroprevalence of HBV in Kenyan children and the effectiveness of the vaccination program against HBV infections. Furthermore, it provides valuable data applicable to modelling global disease estimates of chronic liver disease in LMICs settings. Lastly, it offers a versatile modelling framework for VE estimation, adaptable across diverse settings.

8.3.3 Data

Another key strength of this thesis is the comprehensive age-specific seroprevalence data obtained from a decade-long series of cross-sectional random samples in the Kilifi HDSS covering six VPDs.

The formidable logistic, financial, human, and laboratory resources required for conducting serosurveys make them particularly susceptible to various levels of bias. The mitigation of this bias is crucial for ensuring the validity and reliability of results. The random sampling strategy employed to collect the dataset ensured its representativeness for children in the KHDSS area, addressing a common challenge in many LMICs serosurveys that often rely on convenience samples, leading to limited representativeness[8]. This dataset also boasts a substantial number of

children (approximately 2,500) and adults (around 500) overcoming a common limitation of limited sample sizes due to the requirement for invasive samples [1, 8]. The data was also evaluated using serum specimens, which, although more invasive than oral fluid samples [50] and dried blood spots [51], have demonstrated higher sensitivity and greater suitability for estimating vaccine effectiveness [52]. Serum samples were simultaneously tested for the five pathogens using a highly sensitive and specific fluorescent bead-based multiplex immunoassay [13, 53] which has been shown to be the future of sero-diagnostics for surveillance and epidemiology [52, 54].

The dataset in our study was collected in a series of cross-sectional surveys conducted over different years offering a temporal perspective on the accumulation of seroprevalence for various diseases across age groups within the population. This approach facilitated statistical inferences about different cohorts, as demonstrated in chapter 3 and chapter 7. While conducting surveys at regular intervals provides valuable insights, the practicality of conducting serosurveys every two years, even for HICs, is constrained by logistical and cost implications. For instance, nationally representative serosurveys in Netherlands are conducted about every 10 years [3-5] and every 5 years in Australia [6].

A single cross-sectional serosurvey can provide a situational awareness of disease susceptibility levels. This is in fact one of the advantages of serosurveys over case-based surveillance, which require continuous maintenance over an extended period to accumulate sufficient information. Depending on the research question, analytical procedures allow for extrapolation of serosurvey results and the projection of immunity levels. For instance, in Chapter 4, I exclusively used results from a single serosurvey to assess the risk of disease outbreaks. Existing evidence also shows the utility of single cross-sectional surveys to predict the risk of measles outbreak risk across 17 European countries[55] while another study utilised results from a single serosurvey to assess the risk of measles resurgence in Belgium and France [56]. Nevertheless, the assumptions in the models and the data used for extrapolation may introduce bias, emphasizing the importance of maintaining a delicate balance and avoiding extrapolation well beyond the original purpose of the serosurvey.

The inclusion of seroprevalence data in adults is crucial for diseases like tetanus and rubella, providing insights into the risk of neonatal tetanus and CRS based on immunity gaps in women of

reproductive age. Overall, the availability of this comprehensive seroprevalence data enhances the research's depth and applicability, especially in the context of LMICs.

Another strength of the study in relation to data lies in the availability of an age-mixing matrix which consisted the number of contacts between six distinct age groups. This matrix which was derived from diary studies conducted in KHDSS enabled us to estimate predicted immunity in chapter 4 by considering the contribution of each age group to transmission[57].

We also had access to MCV1 and MCV2 vaccination coverage estimates derived from a birth-cohort analysis conducted in the KHDSS[58]. This invaluable data enabled us to estimate the increasing rate of seroconversion resulting from vaccination across various birth cohorts. Additionally, availability of measles case notification data[59] facilitated the calculation of the increasing rate of seroconversion attributed to natural infection in the static cohort model in Chapter 3. Lastly, the KVMS vaccination registry played a pivotal role in providing information on the number of vaccine doses received for the different participants which we utilised in Chapter 6 and Chapter 7 of this thesis.

8.3.4 Reproducibility and replicability

Throughout this PhD thesis, I have strived to enhance the reproducibility and replicability of my research. Reproducibility refers to the ability for the results of a study to be duplicated using the same materials as the original investigator and to obtain the same results. In epidemiological studies, ensuring reproducibility involves making code and analytical data publicly accessible, and utilizing open-source software[60]. However, it is crucial to ensure that data sharing processes respect individual anonymity and ethical considerations. On the other hand, replicability involves a researcher's ability to duplicate prior study results while using new data and following the same procedures[61].

The codes for all the projects are publicly available on GitHub repositories to promote transparency, enable replication, and facilitate code extensions and adaptations. These include: the code utilise to adjust measles, rubella, tetanus, diphtheria, and pertussis seroprevalence estimates for test imperfections in Chapters 2 and 5, employing Bayesian multilevel regression with

poststratification through the rjags package; the two distinct static cohort models of measles immunity in Chapters 3 and 4; the various versions of logistic models estimating effective tetanus coverage in Chapter 6; and the joint catalytic model employed to estimate the effectiveness of the hepatitis B vaccine in Chapter 7. To maintain anonymity, I have shared aggregated data in the repositories as the raw full datasets are not publicly available but can be requested with appropriate ethical approval.

8.4 Limitations

8.4.1 Data

Whilst this thesis benefits from the data used, it is not without its limitations. The conclusions from this thesis rely heavily on the quality of the data used throughout this research, and the models are only as good at the available data used to inform them.

The age-specific seroprevalence data comprises a comprehensive dataset covering six VPDs and includes a considerable number of participants. However, the data originated from three distinct primary cross-sectional surveys; Malaria Cross-Sectional Survey(2009-2013) [62, 63], PCVIS (2015-2019) [64] and COVID-19 serosurveillance study(2021)[65].Although the sampling methods were consistent across surveys, involving random selection of participants from the KHDSS with about 500 children under 14 years of age sampled in different age strata, the malaria samples were not independent as some children were repeatedly sampled over the years. While this did not affect the annual disease-specific seroprevalence estimates, I incorporated paired statistical tests to appropriately address correlation when comparing seroprevalence estimates across different years. In chapter 6, a sensitivity analysis was conducted by fitting a random effects regression model to estimate the waning rate of tetanus antibodies. The main predictive model in the same chapter included a sensitivity analysis where the model was fitted to the later years of the study.

It's important to acknowledge that the extended freezing duration could potentially have led to antibody degradation in older samples, although this seems less likely given the comparable correlation between vaccination records and seroprotection observed in both the early and later years of the study, as presented in Chapter 6 and chapter 7. Nonetheless, all samples were tested using the same assays and at the same time to ensure uniformity.

In this thesis, the serological data was categorized as either being seropositive or seronegative based on established cut-off values measured in International standards units(IU/ml) for the different pathogens[13, 53]. However, as emphasized in section 8.2.1, it is essential to specify the targets of Correlates of Protection (CoPs). CoPs exhibit variability both quantitatively and qualitatively, depending on whether the objective is to prevent systemic infection, mucosal infection, disease, or severe disease[18].CoPs also present several challenges including the added role of cellular immunity in the case of measles, rubella and HepB which challenges the conventional dependence solely on antibody levels for ascertaining immunity[18].CoPs may also vary based on individual characteristics such as age, gender, and genetic heterogeneity making it challenging to assign a definitive antibody level for protection[21, 23].Finally, diseases like pertussis present complexity, as the vaccine, in addition to pertussis toxin, incorporates other attachment factors that may contribute to protection [18]. Consequently, protection correlates with both antibodies and these additional factors, leading to controversy regarding the exact levels of antibodies required for protection, and no absolute threshold has been established[18, 66]. Moreover, beyond the concerns associated with Cops, it is essential to recognize that the diagnostic accuracy of assays, including both specificity and sensitivity, significantly impacts the resulting seroprevalence estimates. Low sensitivity and specificity can result in overestimation or underestimation of seroprevalence, respectively, thereby potentially compromising the validity of public health decisions based on this data. In both Chapter 2 and Chapter 5, we mitigated these test imperfections by appropriately adjusting the seroprevalence estimates.

The vaccination coverage data also had its limitations. In chapter 4, we used administrative MCV1 and MCV2 coverage estimates. However, these estimates are susceptible to inaccuracies stemming from errors in the number of vaccine doses administered and potential invalid assumptions about the size of the target population of children[67]. To enhance the robustness of our analysis, chapter 3 utilised MCV1 and MCV2 vaccination coverage estimates from a birth-cohort analysis conducted in the KHDSS[58]. However, this dataset was limited to the years 2010 to 2017, necessitating the extrapolation of MCV1 coverage estimates using administrative MCV1 coverage data for the remaining years. In Chapter 6 and Chapter 7, we incorporated data from the KVMS, established in 2009 in the KHDSS area, to ensure prompt and accurate real-time recording of all vaccinations administered during clinic visits[58]. While more accurate compared to administratively reported vaccination coverage, there were still instances of incomplete

vaccination records for a substantial proportion of the children. To address this, we employed imputation methods to account for missing vaccination records.

There are also limitations with measles surveillance data. The national measles case notification data utilised in chapter 3 rely on hospital admissions. Measles cases are only ascertained if individuals feel unwell enough to report to healthcare, the clinician suspects measles, and there is diagnostic capacity for testing. The cases reported to surveillance are therefore likely to be a small fraction of the true cases.

8.4.2 Model limitations

The dynamics of infectious diseases are intricately complex and interconnected. Attempting to incorporate every aspect of transmission is impractical due to the vast complexity and detail involved. Instead, our models must rely on simplifying assumptions based on our understanding of the underlying mechanisms and the questions we seek to address allowing us to derive credible and practical conclusions.

This was highlighted in chapter 4 where I used a static cohort model of measles immunity to predict the risk of measles outbreaks in the context of disruptions in vaccination coverage during the pandemic. I only focused on added immunity due to vaccination and did not account for other drivers of measles immunity in the model including added immunity due to wild-type circulation, waning immunity and demographic changes. The absence of data and the complexity involved in incorporating these factors into the model were the main reasons for their exclusion. Importantly, I did not anticipate these omissions to substantially affect the results, given the relatively short timeframe of the study period.

In Chapter 3, I developed a static birth cohort model to decipher the relative contributions of MCV1, MCV2, SIAs and natural infection to measles seroconversions in Kenyan children. Several factors influenced the selection of a static cohort model. First, I was interested in examining the interplay of MCV1, MCV2, SIA, and natural infection within a well-defined population. The availability of a comprehensive dataset, consisting of serial cross-sectional samples, facilitated the construction of clearly delineated birth cohorts from the KHDSS area, characterized by relatively stable mid-year population estimates throughout the study period. Secondly, while measles immunity profiles varied across different age groups, there was limited fluctuation across the years which allowed for a reasonable assumption of a constant force of infection over the study duration.

Additionally, considering the complexity already inherent in the interaction of various vaccination programs and natural infection, introducing time-dependent changes or additional transmission parameters through a dynamic model would have unnecessarily complicated the analysis and I deemed the static cohort model to be sufficient. Nevertheless, future work is needed to investigate if a dynamic model would reach a different conclusion.

The use of a static model, as implemented here also has limitations, notably in underestimating the ability to control measles circulation at high vaccine coverage due to the exclusion of indirect herd effects. Furthermore, there is merit in incorporating the build-up of immunity from subsequent vaccine doses in individuals and accounting for correlations between different vaccine doses for a more comprehensive and realistic representation of the vaccination dynamics in future works.

In Chapter 6, I employed a logistic regression model to evaluate the relationship between tetanus antibodies and vaccination coverage estimates derived from vaccine records. However, it's crucial to acknowledge that the true relationship between these variables is more intricate than portrayed in the model. Firstly, although there is evidence of high seroconversion rate after tetanus vaccination this is likely to vary across different settings due to challenges in maintaining cold chain, leading to potential loss of vaccine potency. Secondly, I did not account for the specificity and sensitivity of the assay and the likely inaccuracies that are commonly associated with vaccine records. While incorporating these factors would undoubtedly introduce complexity to the analysis, extending this work to account for issues related to the cold chain, assay characteristics, and the accuracy of vaccine records could provide a more comprehensive understanding of the relationship between tetanus antibodies and vaccination coverage. Future research endeavors could explore these complexities to refine the assessment and provide a more nuanced interpretation of the observed relationships.

8.4.3 Generalisability

Finally, a limitation of this thesis lies in the extent to which the conclusions can be applied to other settings. In particular, the seroprevalence study was carried out in a rural area within the African region, where most VPDs are endemic. The susceptibility profiles for the majority of VPDs exhibit significant variation between and even within countries primarily due to differences in vaccination

coverage, levels of natural exposure, and distinct mixing patterns across various age groups. Although the methods employed in the study are broadly applicable, the diverse epidemiological characteristics of these diseases in different settings restrict the generalisability of the results. Consequently, these findings may be applicable only within specific contexts.

In Chapter 4, I utilised this seroprevalence data and an age mixing matrix from the same area which was not representative of the entire country. In order to counteract this, I utilised national estimates of vaccination coverage, which was the main driver of predicted immunity in the model to ensure, at the very least, a level of national representativeness. Nonetheless, it's crucial to note that these results may vary across geographic regions and surveillance systems. As an illustration, a different study focusing on the impact of disruptions to vaccination programs related to COVID-19 in six countries discovered variations in projections of future outbreaks in terms of both timing and magnitude. These discrepancies were attributed to differences in RI coverage before COVID-19 disruptions and the local immunity gaps[49].

In Chapter 3, I utilised the same seroprevalence estimates and vaccination coverage estimates from a birth-cohort analysis conducted in the same area. Additionally, measles case notification data, based on hospital admissions and admittedly not fully representative, was incorporated in the model due to the absence of viable alternatives. While the results represent rural measles-endemic areas well, variations may occur in urban settings due to disparities in vaccination coverage and measles susceptibility profiles between urban and rural environments.

In Chapter 6, I utilised the same seroprevalence estimates and linked them with vaccination records from the KHDSS population registry to estimate the effective coverage of tetanus immunity. While an internal validation of the predictive model was successfully conducted, external validation was hindered by the absence of data from a different location. The relationship between crude coverage and the effective coverage of tetanus immunity is likely to vary across settings, influenced by factors such as the quality, accuracy, and completeness of vaccination records. Additionally, considerations must be given to the quality of seroprevalence data, including the specificity and sensitivity of the assay used for testing. As a result, the generalisability of these results to other settings remains uncertain, as different dynamics may be in play. To enhance the assessment of generalisability, it would be crucial to fit the same model, parameterized to a different location.

8.5 Implications and future works

The research presented in this thesis has several implications for the added value of serosurveillance, offering additional insights beyond the capabilities of syndromic surveillance and vaccine record keeping in the effective management of VPDs.

1. I have shown that serosurveys can substantially help with the situational awareness on the proportion of susceptible population that results either from waning of induced immunity or failure to seroconvert that may otherwise be overlooked while relying solely on vaccination coverage estimates. I have also shown with examples how these estimates can inform revisions to existing programs like guiding targeted SIAs in the case of measles or assessing the need for booster doses in the cases of diphtheria and tetanus.
2. I have illustrated the synergistic utility of combining serosurveillance data, case surveillance data, and routine coverage data in evaluating the trade-offs among various intervention programs. This proves instrumental for optimising resource allocation, especially in the context of diseases with multiple intervention programs and resource constraints. In addition to emphasizing the importance of optimising routine coverage timing and uptake to reduce dependence on SIAs and measles susceptibility in our context, this analysis underscores the significant value derived from integrating these diverse datasets. Therefore, beyond the integration of serosurveys into disease surveillance, it is imperative to enhance routine vaccination coverage and case surveillance data for optimal disease control.
3. I have shown the enhanced utility of integrating seroprevalence data into a modelling framework for predicting the risk of outbreaks, especially in situations where the control program hinges on herd protection thresholds, as seen in the case of measles. This approach proves valuable particularly when a rapid assessment of the potential impact of disruptions to healthcare systems is needed. It can also be useful in assessing how close a country is to eliminating measles
4. I have demonstrated the value of serosurveillance data in monitoring the effective coverage of immunisation programs. This approach offers additional advantages compared to the crude vaccination coverage as it provides insights into the population protected against infection or disease. I consider this method as a valuable means to identify vaccination

gaps, especially in communities with inadequate record-keeping, which can be addressed during immunisation campaigns. However, it is essential to carefully consider the cost implications and logistical challenges associated with serologic testing in relation to the potential benefits of incorporating immune markers for vaccination monitoring.

5. I have illustrated how these estimates can be utilised to assess the effectiveness of a vaccination program, either independently as demonstrated with rubella or through integration into a modelling framework as exemplified with hepatitis B. This methodology proves invaluable, especially when evidence is required for potential revisions to existing vaccination programs

While this thesis addresses the value of information added by serological surveys in control of VPDs in an LMIC setting, there is limited precedence for drawing firm conclusions about their potential inclusion for monitoring immunisation programs in LMICs settings. Consequently, there are several suggestions for future research.

One, the long-term potential for implementation of serosurveys will hinge on the added value of the additional information provided in improving performance of EPI. Further structured analyses, building on this work and other case studies, are essential to assess the cost-effectiveness of conducting serosurveys for detecting age-related immunity gaps in large populations. These evaluations should consider the technical resource requirements and the national capacity to deliver results that can be accurately interpreted. The issues we have discussed in this thesis including those inherent to the nature of serologic assays, the serologic profile generated by specific vaccines, and the cost and logistic implications of incorporating representative and well-powered biologic assays should also be considered in incorporation of biomarkers for monitoring immunisation programs.

Two, for any consideration of widespread use of biomarkers to monitor immunisation programs among LMICs settings further efforts to standardize assays and interpretations among countries and across implementing laboratories should be addressed. Additionally, future serosurveys and cohort studies must incorporate thorough documentation of the study design, population characteristics, testing procedures, uncertainties inherent in the estimates, and sensitivity analyses conducted. This comprehensive approach strengthens the reliability of the findings and is essential for accurately interpreting the results. Such transparency also facilitates the generation of accurate

data that can be utilised in models.The frequency of serosurveys should also be carefully considered. The current practice of conducting surveys every two years may offer limited value, and adopting an approach similar to the Netherlands, with surveys conducted every 10 years, might prove sufficient.

An alternative approach to expanding the use of serosurveys in LMICs, enhancing practicality and cost-effectiveness, could involve leveraging other nationally-representative surveys where blood is collected. This includes surveys such as Demographic Health Surveys, malaria indicator surveys, or nationally-representative HIV prevalence surveys. Additionally, serosurveys can be targeted to specific subpopulations where concerns exist that vaccination coverage and case surveillance data may not accurately reflect population immunity. The utility of the bead-based multiplex assay, utilised in this thesis, should be considered, as it allows for testing small sample quantities and multiplexing from tens to hundreds of pathogens. Consequently, it could be expanded to simultaneously detect antibodies to multiple pathogens of interest in children of different ages.

8.6 Concluding remarks

There is a role for serosurveys to support VPD control by providing a richer understanding of population immunity gaps. However, it is essential to explicitly consider the quality and selection of sampling and laboratory methods, along with the considerable resource implications, before undertaking such endeavours. As demonstrated in the majority of chapters, the integration of serosurveillance with vaccination coverage data and even syndromic surveillance data holds greater significance compared to assessing either result in isolation. Consequently, enhancing both routine vaccination coverage record keeping, and case surveillance remain crucial to enhance the utility of serosurveys and resulting inference for the control of VPDs.

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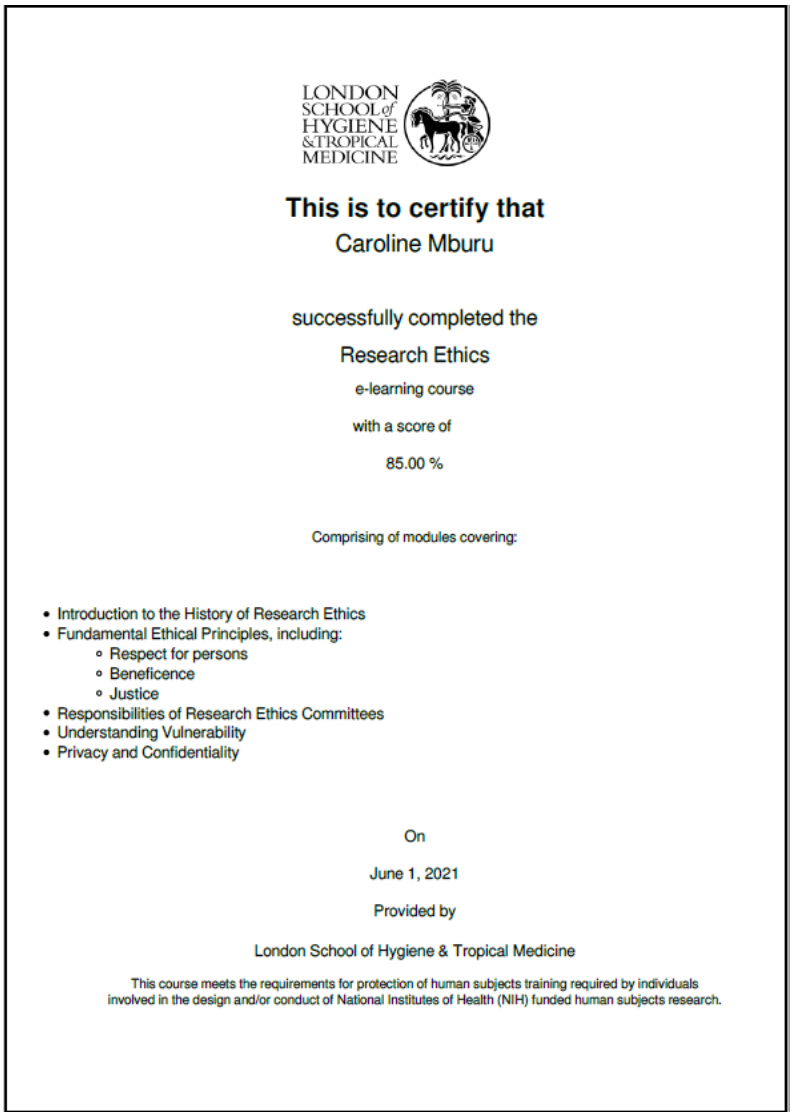
9. Chapter 9: Appendices

Appendix A: Supplementary material for ethical approval



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KEMRI/RES/7/3/1 **July 22, 2020**

TO: **DR. IFEDAYO ADETIFA,**
PRINCIPAL INVESTIGATOR.

THROUGH: **THE DEPUTY DIRECTOR, CGMR-C,**
KILIFI.

Dear Sir,

RE: **PROTOCOL NO. KEMRI/SERU/CGMR-C/194/4054 *(RESUBMISSION OF INITIAL SUBMISSION)*: A NATIONWIDE SEROLOGICAL SURVEY TO GENERATE EVIDENCE FOR VACCINE POLICY IN KENYA (SEROVACK STUDY 002) *(VERSION 2.0 DATED JULY 10, 2020)***

Reference is made to your letter dated July 10, 2020. The KEMRI Scientific and Ethics Review Unit (SERU) acknowledges receipt of the following revised study documents on July 17, 2020;

1. SERU response letter dated 06th July 2020
2. A copy of the protocol SERU 4054 with tracked and highlighted changes_v2.0 dated 10th July 2020
3. A clean copy of protocol SERU 4054_V2.0 dated 10th July 2020
4. Study tool Swahili version

This is to inform you that the issues raised by the Expedited Review Team of the KEMRI Scientific and Ethics Review Unit (SERU) on the letter dated **July 06, 2020** have been adequately addressed.

Consequently, the study is granted approval for implementation effective this day, **July 22, 2020** for a period of **one (1) year.** Please note that authorization to conduct this study will automatically expire on **July 21, 2021.** If you plan to continue with data collection or analysis beyond this date, please submit an application for continuation approval to SERU by **June 09, 2021.**

Please note that only approved documents including (informed consents, study instruments, Material Transfer Agreement) will be used. You are required to submit any proposed changes to this study to SERU for review and the changes should not be initiated until written approval from SERU is received. Any unanticipated problems resulting from the implementation of this study should be brought to the attention of SERU and you should advise SERU when the study is completed or discontinued.

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) https://oris.nacosti.go.ke and also obtain other clearances needed

[redacted]

ENOCK KEBENEI,
THE ACTING HEAD,
KEMRI SCIENTIFIC AND ETHICS REVIEW UNIT.

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KEMRI/RES/7/3/1 **April 27, 2020**

TO: **DR. IFEDAYO ADETIFA**
PRINCIPAL INVESTIGATOR

THROUGH: **THE DIRECTOR, CGMR-C**
KILIFI

Dear Sir,

RE: **KEMRI/SERU/CGMR-C/159/3847 (*REQUEST FOR EXPEDITED ANNUAL RENEWAL*): SEROLOGIC SURVEYS FOR ASCERTAINING VACCINATION COVERAGE AND POPULATION IMMUNITY IN KENYA (THE SEROVACK 001 STUDY).**

Thank you for the continuing review report for the period **May 10, 2019 to March 24, 2020**
This is to inform you that the Expedited Review Team of the KEMRI Scientific and Ethics Review Unit (SERU) was of the informed opinion that the progress made during the reported period is satisfactory. The study has therefore been granted **approval** for continuation.

This approval is valid from **May 10, 2020** through to **May 9, 2021.** Please note that authorization to conduct this study will automatically expire on **May 9, 2021.** If you plan to continue with data collection or analysis beyond this date please submit an application for continuing approval to the SERU by **March 28, 2021.**

You are required to submit any amendments to this protocol and any other information pertinent to human participation in this study to the SERU for review prior to initiation. You may continue with your study.

[redacted]

ENOCK KEBENEI,
THE ACTING HEAD,
KEMRI SCIENTIFIC AND ETHICS REVIEW UNIT.

Appendix B: Supplementary material for Chapter 2

Table s2.1: Changes in GMCs for Measles and Rubella adjusted for underlying population.

Survey year	**2009**			**2011**			**2013**			**2015**			**2017**			**2019**			**2021**		
Measles	n	GMC [95% CI]		n	GMC [95% CI]		n	GMC [95% CI]		n	GMC [95% CI]		n	GMC [95% CI]		n	GMC [95% CI]		n	GMC [95% CI]	
Age in years																					
<9m	4	0.0	[0.0-0.1]	8	0.0	[0.0-0.0]	3	0.0	[0.0-0.8]	14	0.0	[0.0-0.1]	15	0.0	[0.0-0.0]	20	0.0	[0.0-0.0]	5	0.1	[0.0-1.9]
9m-<1yr	9	0.1	[0.0-2.0]	6	0.5	[0.0-13.6]	4	0.1	[0.0-8.4]	21	0.1	[0.0-0.4]	10	0.0	[0.0-0.2]	19	0.1	[0.0-0.4]	4	0.2	[0.0-1.4]
1-<2yrs	94	1.6	[0.7-3.6]	59	1.0	[0.3-2.8]	95	0.7	[0.5-1.1]	104	0.6	[0.2-1.5]	124	0.5	[0.2-1.2]	150	0.9	[0.6-1.5]	69	0.9	[0.5-1.9]
2-4yrs	37	0.8	[0.5-1.5]	22	1.8	[1.2-2.8]	25	2.1	[1.5-3.0]	38	1.2	[0.7-2.1]	36	1.5	[0.9-2.4]	54	0.8	[0.5-1.2]	15	1.0	[0.4-2.6]
5-9yrs	197	0.5	[0.4-0.7]	137	0.6	[0.4-0.8]	143	0.9	[0.6-1.6]	152	0.6	[0.4-1.1]	182	0.7	[0.4-1.1]	221	0.6	[0.4-1.0]	102	0.7	[0.3-1.3]
10-14yrs	24	0.1	[0.0-0.1]	76	0.2	[0.1-0.3]	136	0.3	[0.2-0.5]	47	0.6	[0.3-3.9]	54	0.7	[0.4-1.2]	56	0.6	[0.3-1.0]	95	0.6	[0.4-1.0]
Total	365	0.5	[0.3-0.9]	308	0.7	[0.4-1.4]	406	0.9	[0.6-1.6]	376	0.7	[0.4-2.1]	421	0.8	[0.5-1.3]	520	0.6	[0.4-1.0]	290	0.7	[0.4-1.5]
P_value		<0.001			<0.001			<0.001			<0.001			<0.001			<0.001			0.03	
Rubella																					
<9m	4	1.1	[0.2-7.1]	8	0.3	[0.1-0.6]	3	2.3	[0.1-38.5]	14	0.9	[0.1-5.6]	15	1.4	[0.5-4.3]	20	1.1	[0.4-2.9]	5	32.3	[4.1-254.0]
9m-<1yr	9	0.1	[0.0-0.5]	6	0.1	[0.0-0.7]	4	0.2	[0.1-0.8]	21	0.2	[0.0-0.7]	10	0.8	[0.1-10.5]	19	7.1	[1.6-31.8]	4	3.9	[0.1-4.9]
1-<2yrs	94	0.1	[0.1-0.3]	59	0.8	[0.1-4.1]	95	0.1	[0.1-0.2]	104	0.5	[0.1-2.1]	124	8.3	[2.2-31.2]	150	87.6	[53.3-144.0]	69	133.0	[69.1-254.0]
2-4yrs	37	0.5	[0.2-1.6]	22	0.1	[0.0-0.8]	25	0.2	[0.1-0.5]	38	0.9	[0.2-4.0]	36	37.8	[12.6-114.3]	54	56.8	[30.0-107.7]	15	70.2	[25.4-208.9]
5-9yrs	197	6.6	[1.8-24.0]	137	14.7	[3.7-60.4]	143	2.0	[0.4-10.4]	152	3.8	[0.6-26.2]	182	119.0	[61.7-248.6]	221	69.0	[31.5-153.5]	102	77.6	[38.0-189.1]
10-14yrs	24	3.9	[0.7-21.5]	76	24.5	[6.5-95.9]	136	46.9	[11.6-197.2]	47	70.0	[26.1-255.7]	54	196.2	[99.2-502.6]	56	144.6	[64.8-369.4]	95	156.3	[90.1-308.9]
Total	365	11.5	[0.9-15.5]	308	12.6	[3.3-50.4]	406	15.2	[3.7-66.0]	376	23.0	[8.2-88.4]	421	109.1	[54.2-265.1]	520	234.8	[40.7-198.9]	290	131.3	[51.0-234.8]
P_value		<0.001			<0.001			<0.001			<0.001			<0.001			<0.001			0.01	

Table s2.2; Population immunity and GMCs in adults. Seroprevalence estimates were adjusted for test performance and underlying population structure using Bayesian modelling

Survey year	**2021**						
Measles	n	% [95% CI]		P_value	GMC [95% CI]		P_value
Age in years							
15_19	56	97	[87-100]	0.41	0.70	[0.36-1.69]	<0.001
20_24	51	96	[85-100]		0.92	[0.33-2.66]	
25_29	47	97	[88-100]		0.85	[0.30-2.79]	
30_34	46	99	[94-100]		1.99	[0.68-6.49]	
35_39	54	99	[91-100]		1.56	[0.54-4.91]	
40_44	44	97	[86-100]		1.76	[0.76-6.55]	
45-49	56	99	[95-100]		2.00	[0.86-5.49]	
50_54	48	99	[95-100]		0.31	[0.15-0.66]	
55_59	50	98	[88-100]		1.79	[0.68-6.32]	
60_64	48	99	[96-100]		1.83	[0.79-4.70]	
65+	55	99	[95-100]		0.47	[0.13-2.34]	
Total	555	99	[95-99]		0.95	[0.38-2.94]	
Rubella							
15_19	56	99	[94-100]	0.91	229.07	[147.37-358.65]	0.76
20_24	51	90	[80-98]		97.32	[28.18-510.94]	
25_29	47	88	[75-97]		65.84	[31.38-364.78]	
30_34	46	86	[74-96]		56.08	[24.72-181.51]	
35_39	54	95	[84-99]		118.30	[50.68-306.75]	
40_44	44	93	[81-99]		118.18	[55.63-468.27]	
45-49	56	93	[84-99]		76.16	[29.11-259.71]	
50_54	48	95	[87-100]		16.61	[7.06-40.42]	
55_59	50	92	[82-99]		66.39	[15.39-295.18]	
60_64	48	92	[80-99]		101.56	[37.78-350.59]	
65+	55	97	[90-100]		14.52	[6.17-47.46]	
Total	555	92	[89-96]		82.22	[40.36-249.27]	

Table s2.3: Impact of 2016 campaign on Measles and Rubella prop and GMCs

SIA period	Pre_SIA period (10-15months pre-SIA)					Post_SIA period (7-12months post-SIA)					P-value GMCs	P-value %
Measles	n	% [95% CI]		GMC [95% CI]		n	% [95% CI]		GMC [95% CI]			
Age in years												
<9m	14	47	[21-74]	0.0	[0.0-0.1]							
9m-<1yr	21	59	[34-81]	0.1	[0.0-0.4]							
1-<2yrs	33	93	[78-99]	0.6	[0.2-1.5]	15	97	[87-100]	2.0	[1.2-3.1]	0.02	0.42
2-4yrs	109	95	[88-100]	1.2	[0.7-2.1]	119	98	[94-100]	1.5	[0.9-2.4]	0.03	0.31
5-9yrs	152	97	[92-100]	0.6	[0.4-1.1]	182	98	[95-100]	0.7	[0.4-1.1]	0.81	0.67
10-14yrs	36	94	[79-99]	0.6	[0.3-1.0]	54	97	[88-100]	0.7	[0.4-1.2]	0.07	0.81
Total	365	90	[85-92]	0.7	[0.4-1.3]	370	91	[87-95]	0.9	[0.5-1.5]	<0.001	0.69
Rubella												
<9m	14	22	[04-46]	0.9	[0.1-5.6]							
9m-<1yr	21	12	[02-32]	0.2	[0.0-0.7]							
1-<2yrs	33	22	[06-41]	0.5	[0.1-2.1]	15	45	[17-69]	2.0	[0.1-29.4]	0.36	0.31
2-4yrs	109	27	[11-37]	0.9	[0.2-4.0]	119	79	[70-87]	37.8	[12.6-114.3]	<0.001	<0.001
5-9yrs	152	49	[38-59]	3.8	[0.6-26.2]	182	90	[84-96]	119.0	[61.7-248.6]	<0.001	<0.001
10-14yrs	36	62	[45-79]	10.6	[0.8-15.9]	54	94	[86-99]	196.2	[99.2-502.5]	<0.001	0.002
Total	365	45	[35-52]	4.8	[0.5-58.8]	370	79	[75-84]	108.6	[53.9-264.5]	<0.001	<0.001

Appendix C: Supplementary material for Chapter 3

Note s3:1. Model equations

The static birth cohort model developed to track proportion of children who are either susceptible to measles seroconversion (S) or seroconverted due to natural infection (NI) or due to vaccination with MCV1 (MCV1), MCV2 (MCV2) or SIA (SIA) is described in detail in the main text. Model parameters which are summarized in table 3:1 of the main text include; Ɛ1=1-vaccine failure of one dose given at less than 1 year, Ɛ2=1-vaccine failure of one dose given between 12-18months and Ɛ3=1-vaccine failure of one dose given at 18months or older. V1, V2 and V3 are the birth-cohort coverages of MCV1, MCV2 and SIA, b is the birth rate and FOI is the force of infection.FOI is the annual and $\overline{FOI}$ the average FOI during the study period, and C and $\overline{C}$ the annual and average reported number of measles cases respectively.In the model, children in each age group are divided into 5 compartments. The total population in age group a at time t is denoted as $N(a,t)$. There are total of 36 age groups with the first 24 representing the monthly age groups from 0 to 2 years and the rest yearly age groups between 3 and 14 years.

Transitions for each birth cohort is described by a system of ordinary differential equations which incorporates both aging and vaccination coverage implemented in monthly timesteps. Routine vaccination is delivered at fixed ages (11 months for MCV1 and 18 months for MCV2) and the seroconverted children age into the subsequent compartment while those who do not seroconvert age in the same compartment. SIAs are implemented at the beginning of the month in the respective calendar year by moving a proportion of children in the target age group who successfully seroconvert from S into the SIA compartment. Probability of movement of children from S to the NI compartment is governed by an annual force of infection FOI

$$N(a,t) = S(a,t) + NI(a,t) + MCV1(a,t) + MCV2(a,t) + SIA(a,t)$$

$$FOI(t) = \overline{FOI}(t) * C(t)/\overline{C}$$

$$S(a,t)=(1-(FOI(t)+(\varepsilon_1*V_1)+(\varepsilon_2*V_2)+(\varepsilon_1*V_3)+(\varepsilon_2*V_3)+(\varepsilon_3*V_3)))*age.in*S(a,t)-age.out*S(a,t)$$

$$NI(a,t)=FOI(t)*age.in*S(a,t)+age.in*NI(a,t)-age.out*NI(a,t)$$

$$MCV1(a,t)=(\varepsilon_1*V_1)*age.in*S(a,t)+age.in*MCV1(a,t)-age.out*MCV1(a,t)$$

$$MCV2(a,t)=(\varepsilon_2*V_2)*age.in*S(a,t)+age.in*MCV2(a,t)-age.out*MCV2(a,t)$$

$$SIA(a,t)=((\varepsilon_1*V_3)+(\varepsilon_2*V_3)+(\varepsilon_3*V_3))*age.in**S(a,t)+age.in*SIA(a,t)-age.out*SIA(a,t)$$

Note s3:2. Extrapolation of vaccination coverage estimates

Estimates for MCV1 and MCV2 vaccination coverage estimates for birth-cohort analysis in KHDSS were only available between 2010 and 2017[1]. To extrapolate birth-cohort vaccination coverage in KHDSS from administrative national MCV1 coverage [2] beyond this time frame, we first compared the trends and estimates for the time period in which both datasets were present and observed matching trends. On average, administrative coverage estimates were 11% higher and 21% higher than MCV1 and MCV2 KHDSS coverage estimates. We then adjusted the coverage in the rest of the modelled time frame by subtracting this average difference.

Note s3:3. Calculation of annual FOI

To calculate the proportion annually exposed to Measles between 2009 and 2021,we derived the annual force of infection (FOI) from the average monthly FOI $i.e.$ $\overline{FOI}$ which was defined as the probability to be exposed for the same period. The monthly probability not be exposed is therefore $1-\overline{FOI}$. Probability not be exposed for a year is $(1-\overline{FOI})$^12. It follows from this that annual probability exposed is for 1-$(1-\overline{FOI})$^12

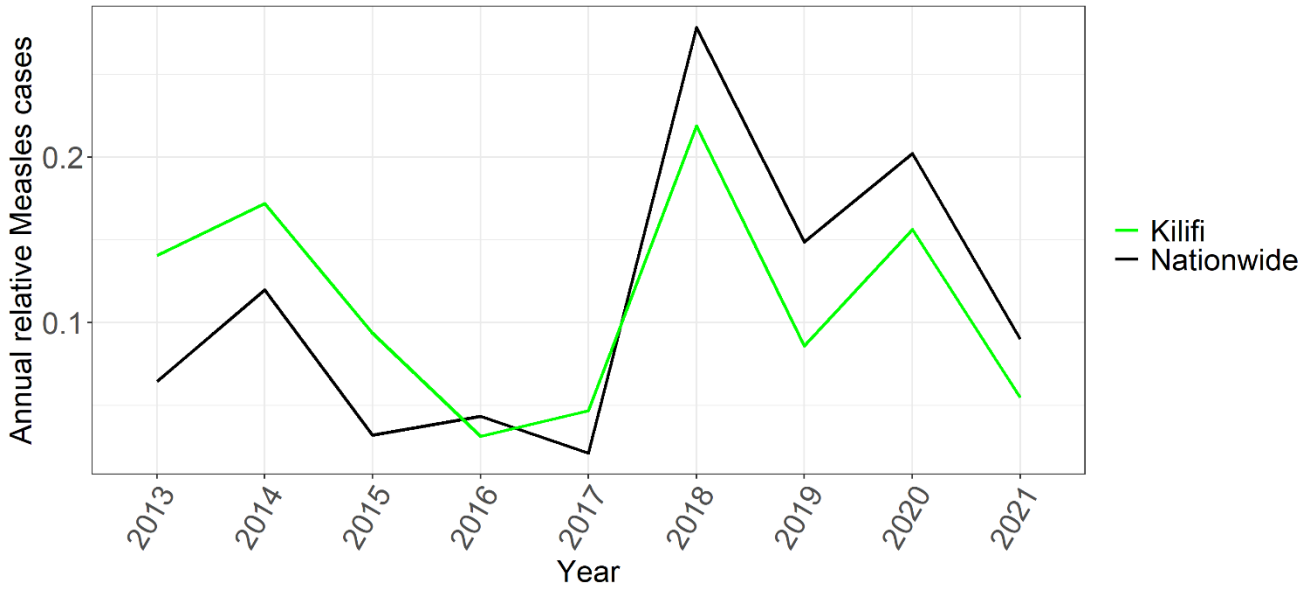

Figure s3:1: Comparison of the relative reported nationwide wide cases per year from WHO and the relative reported Measles cases from KHDSS surveillance for the years in which both datasets were present. Annual relative cases were calculated by dividing notified measles cases per year by the total measles cases in the entire period.

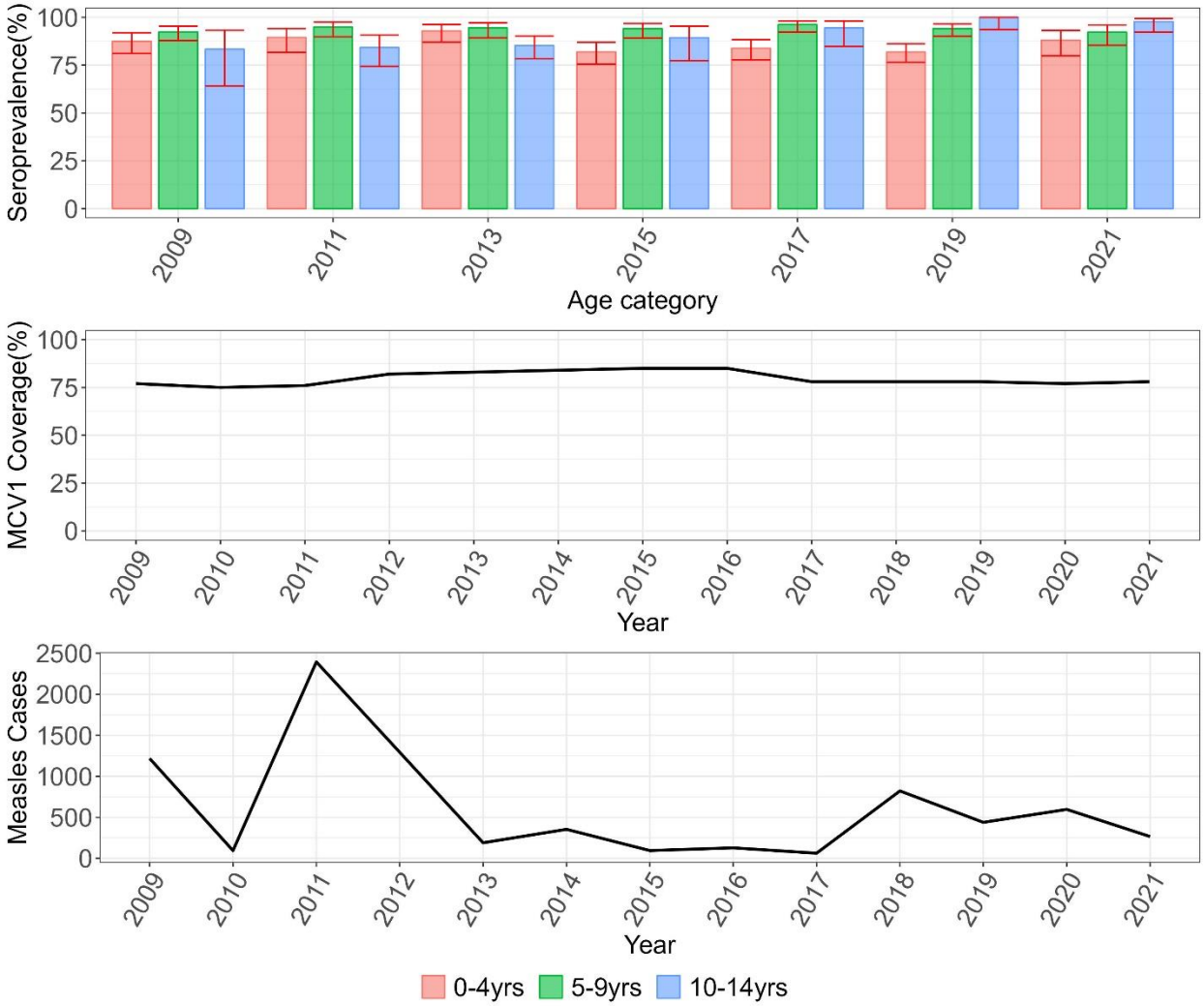

Figure s3:2: MCV1 coverage, Measles cases and seroprevalence estimates between 2009 and 2021

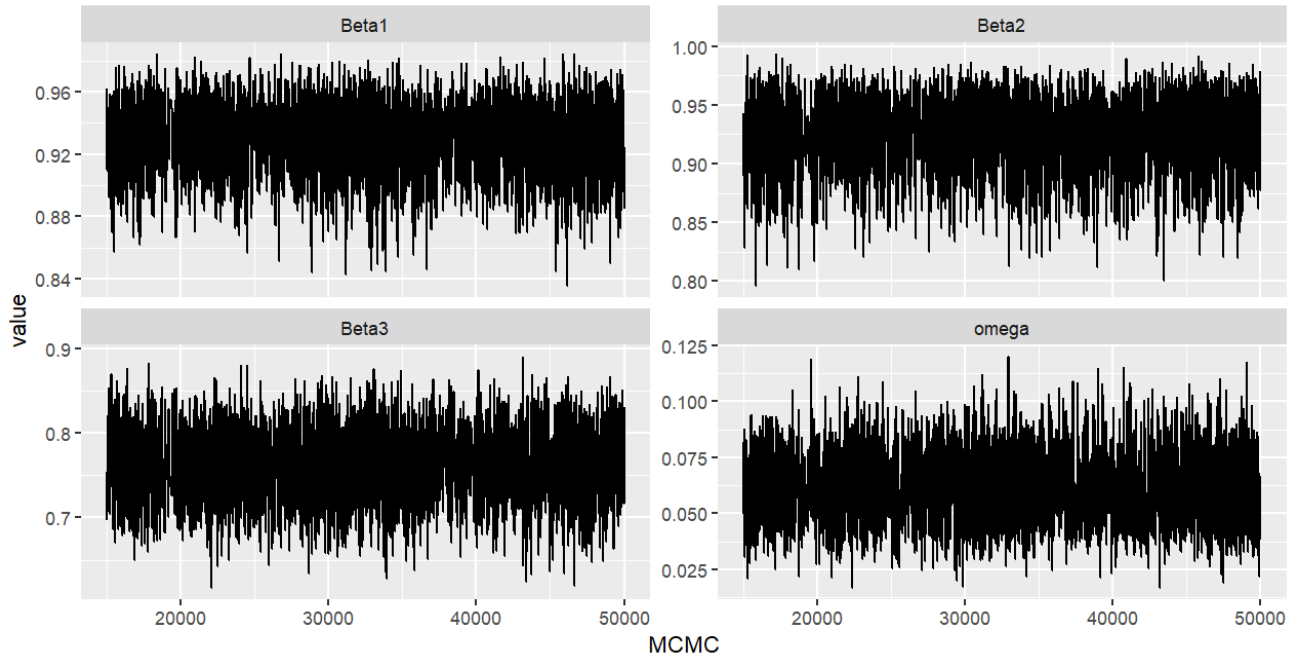

Figure s3:3. Convergence chains

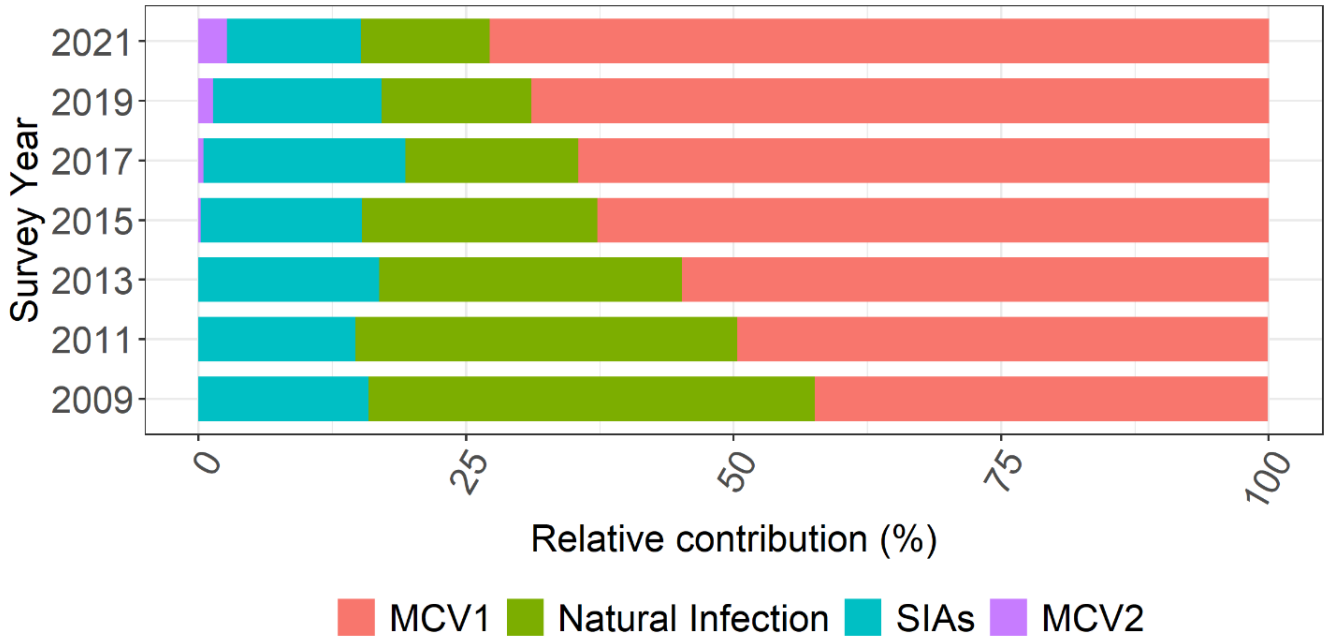

Figure s3:4. Estimated age-specific measles immunity profiles. The figure shows percentage of children that seroconverted either through MCV1, MCV2, SIA or natural infection in each year.

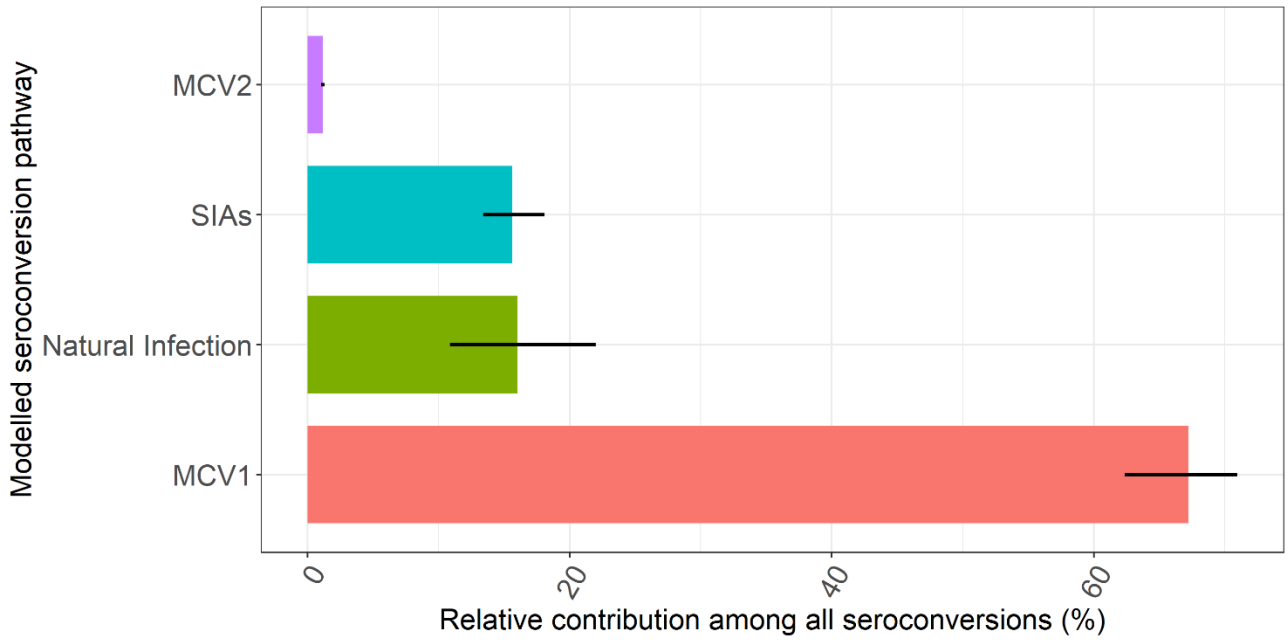

Figure s3:5. Percentage of children that seroconverted either through MCV1, MCV2, SIA or natural infection after MCV2 introduction (between 2015 and 2021). Error bars indicate the credible interval of the predictive posterior distribution.

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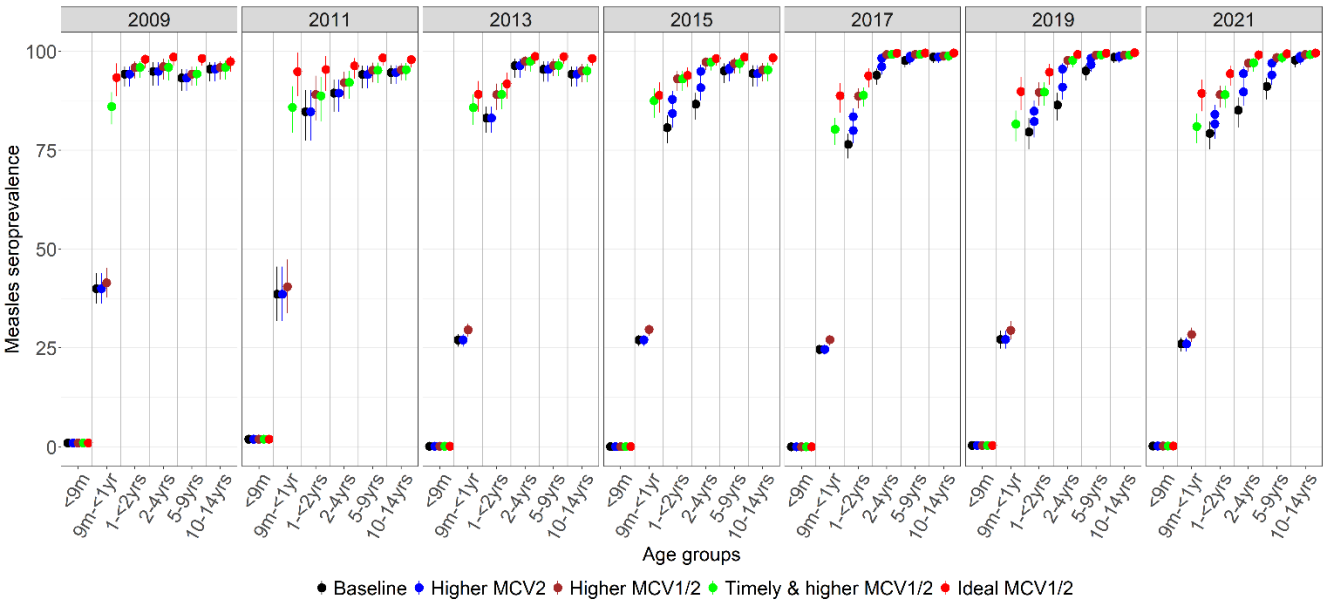


Figure s3:6. Predicted Measles seroprevalence from the sampled from the fitted model with 95% credible interval of the predictive posterior distribution

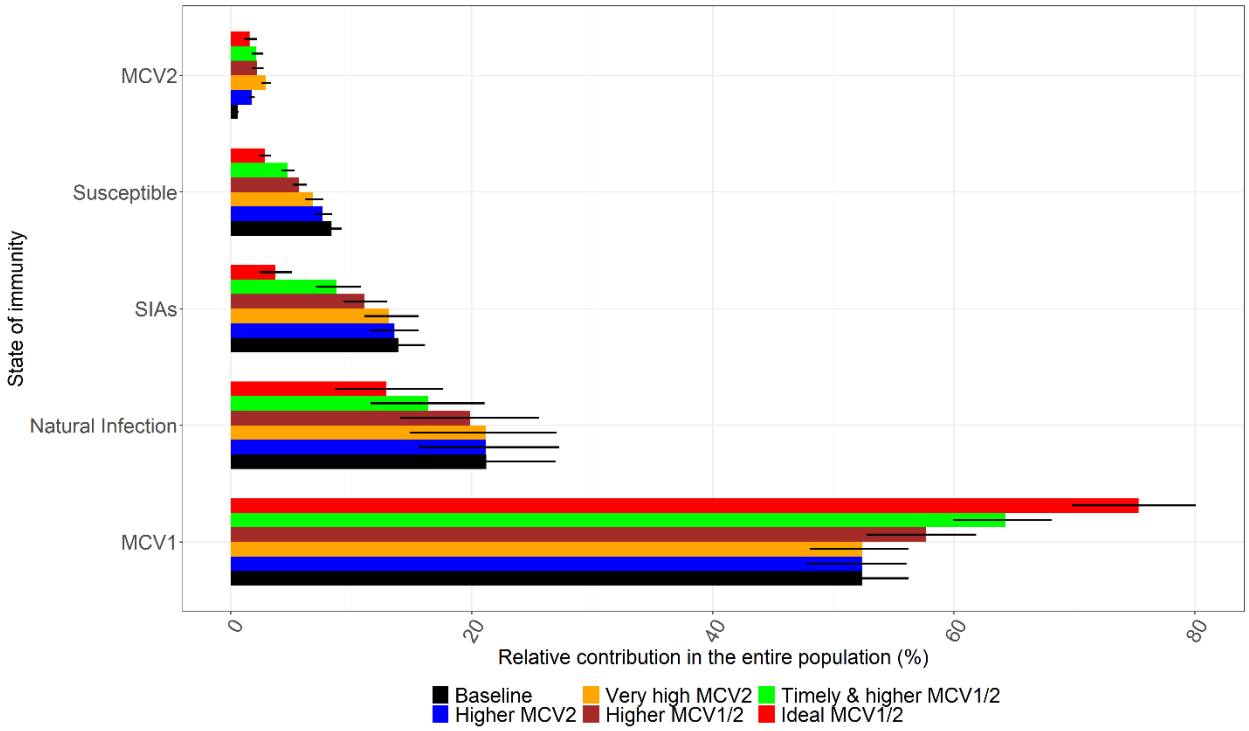


Figure s3:7: Estimated relative contribution of the different programs from the projection scenarios on increased MCV1 and MCV2 coverage over the entire period (2009-2021).

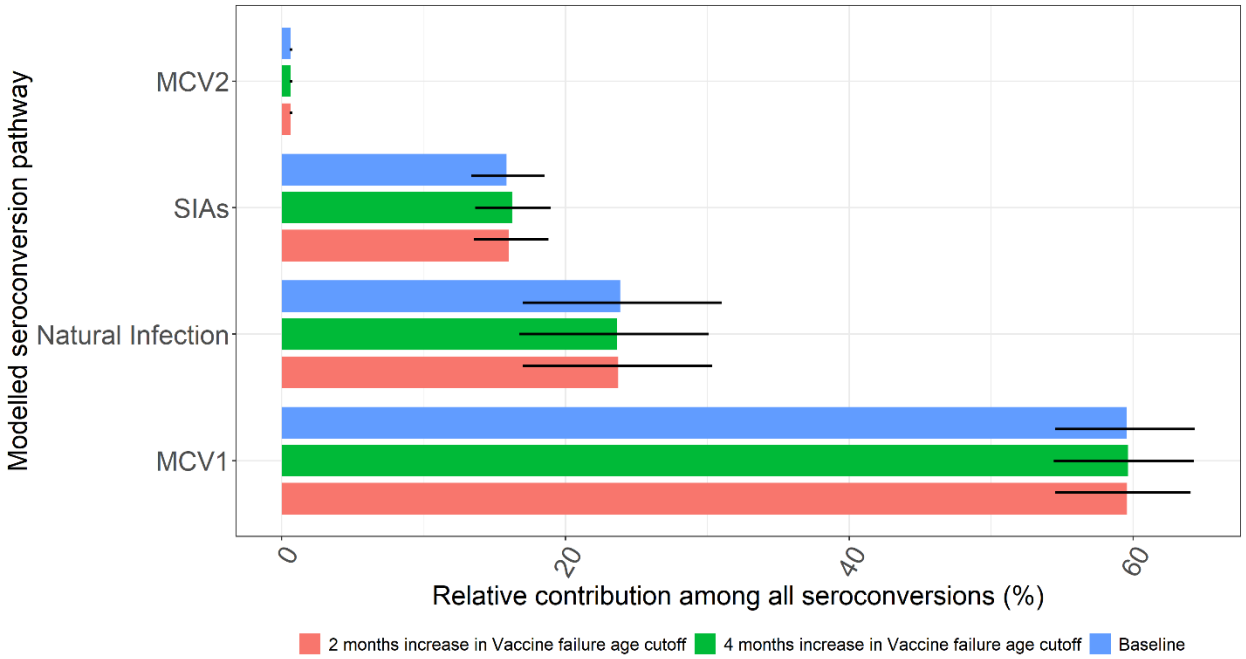


Figure s3:8. Impact of age cut off of priors of the vaccine failure on the relative contribution of the different programs to seroconversion

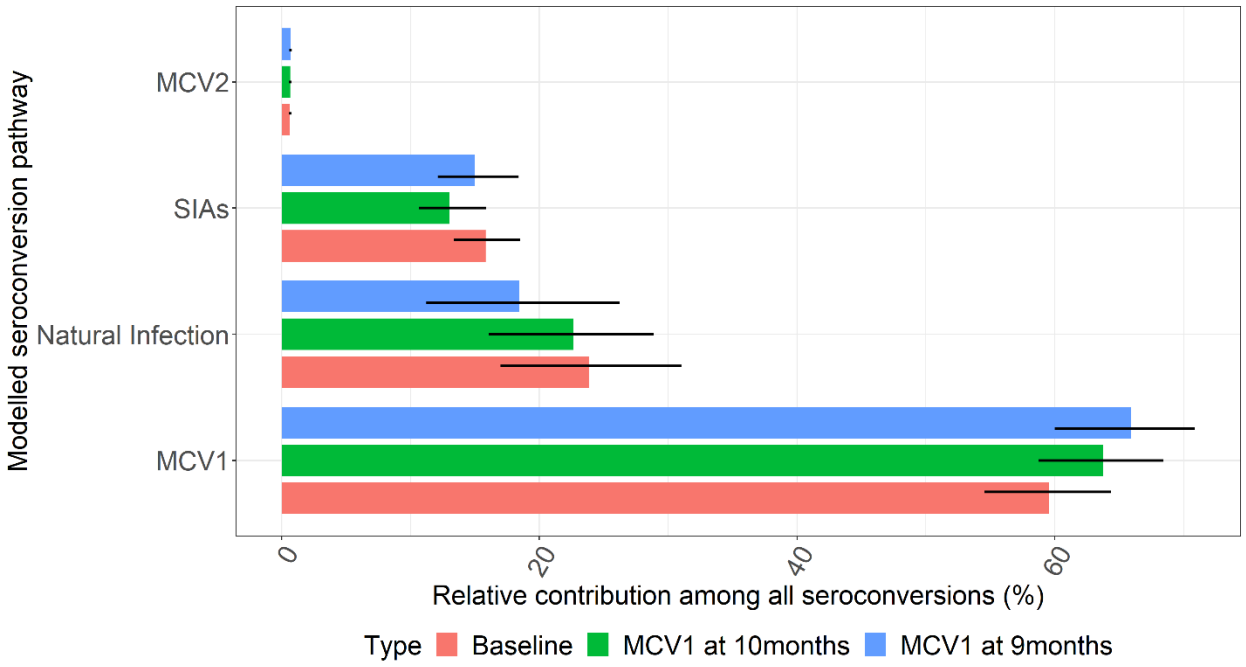


Figure s3:9. Impact of timeliness of MCV1 on the relative contribution of the different programs to seroconversion

Appendix D: Supplementary material for Chapter 4

Table s4:1: Serological data used in the analysis. The table shows counts of all tested individuals and positive individuals in the different age-categories

Age-categories	All samples	Positive samples
<9m	20	1
9m-<1yr	18	9
1yr	48	45
2yrs	47	42
3yrs	53	49
4-8yrs	237	228
9-14yrs	74	71
Total	497	445

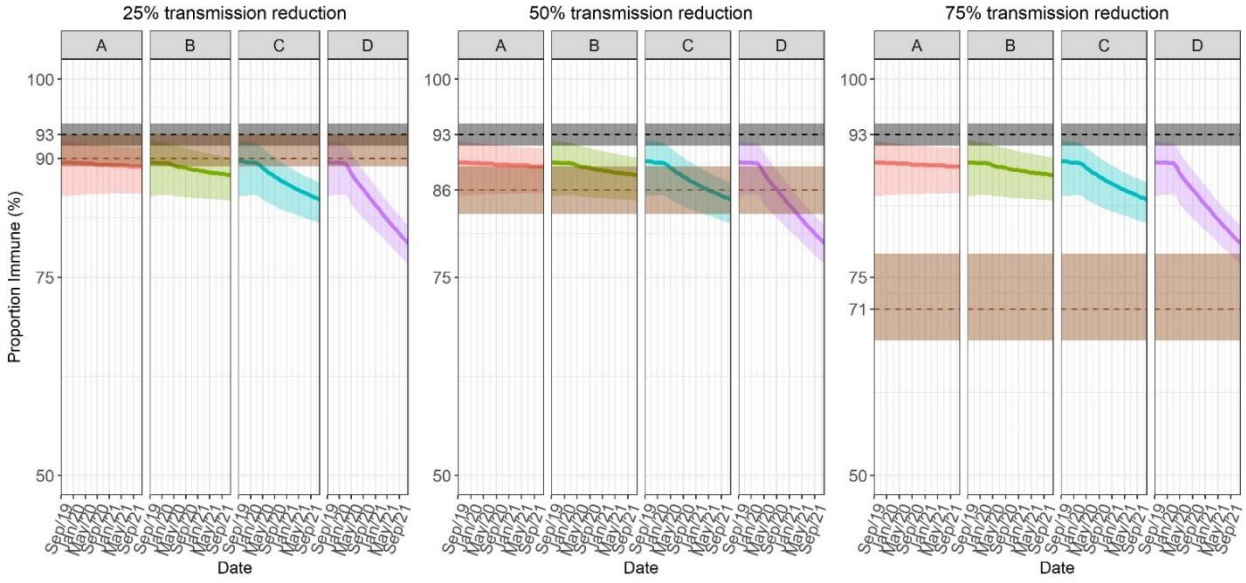


Figure s4:1. Age stratified population immunity profiles. The three HITs are based on the three assumptions of reduction in transmission of measles during the pandemic.

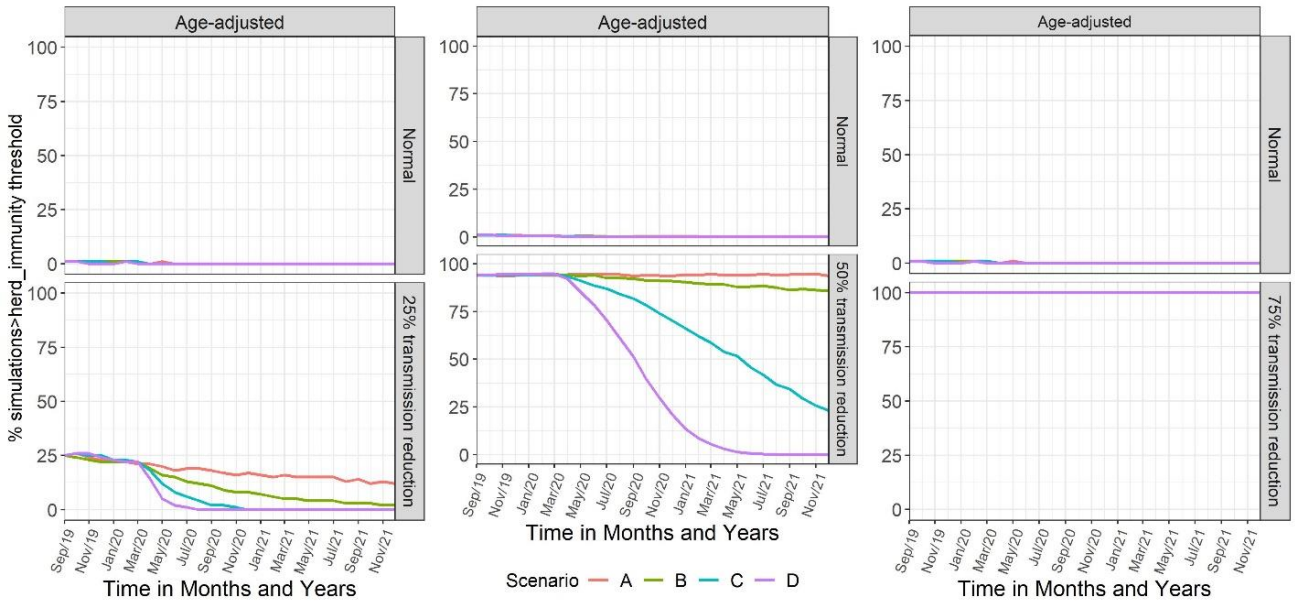


Figure s4:2. Percentage of simulations with proportion immune > herd immunity threshold

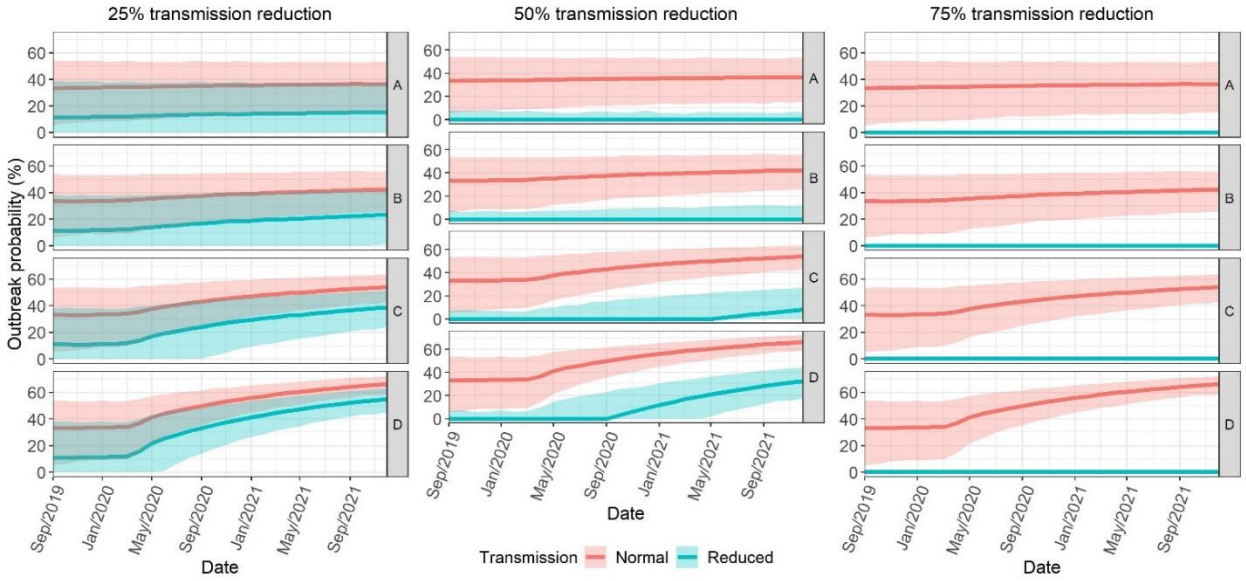


Figure s4:3. Probability of a large outbreak sparked by a single infectious individual assuming different levels of reduction in measles transmission during the pandemic.

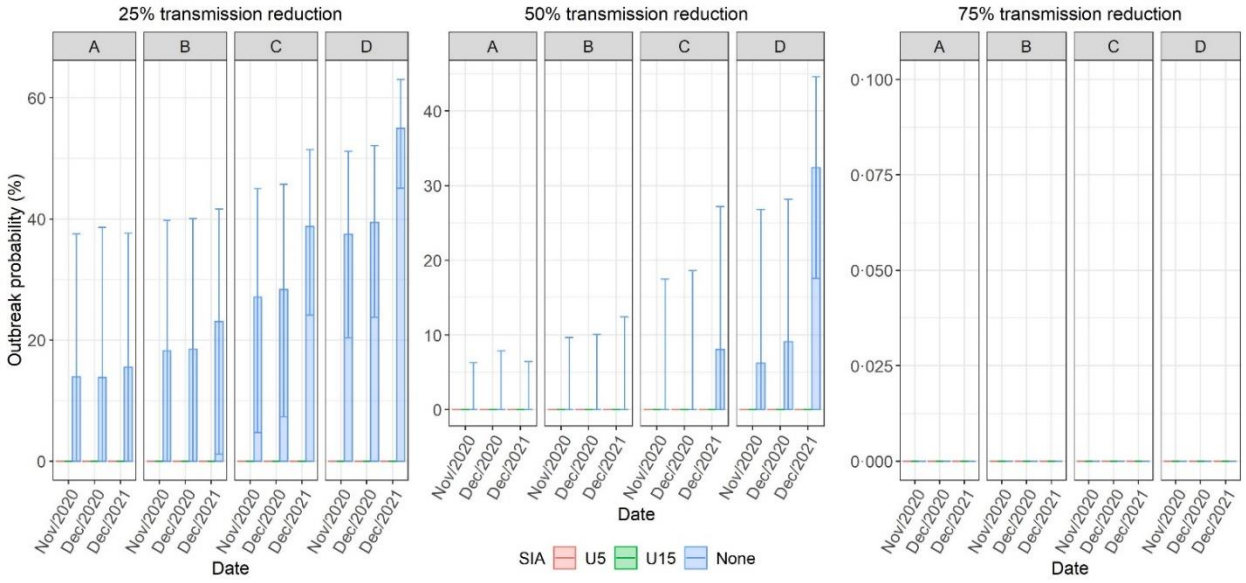


Figure s4:4. Probability of a single infectious person seeding a large outbreak before (none) and after implementing a SIA in children 9 months to 5 years old (U5) and in 9 months to 15 years old (U15) at different time points during lockdown (25%, 50% and 75% reduction in measles transmission).

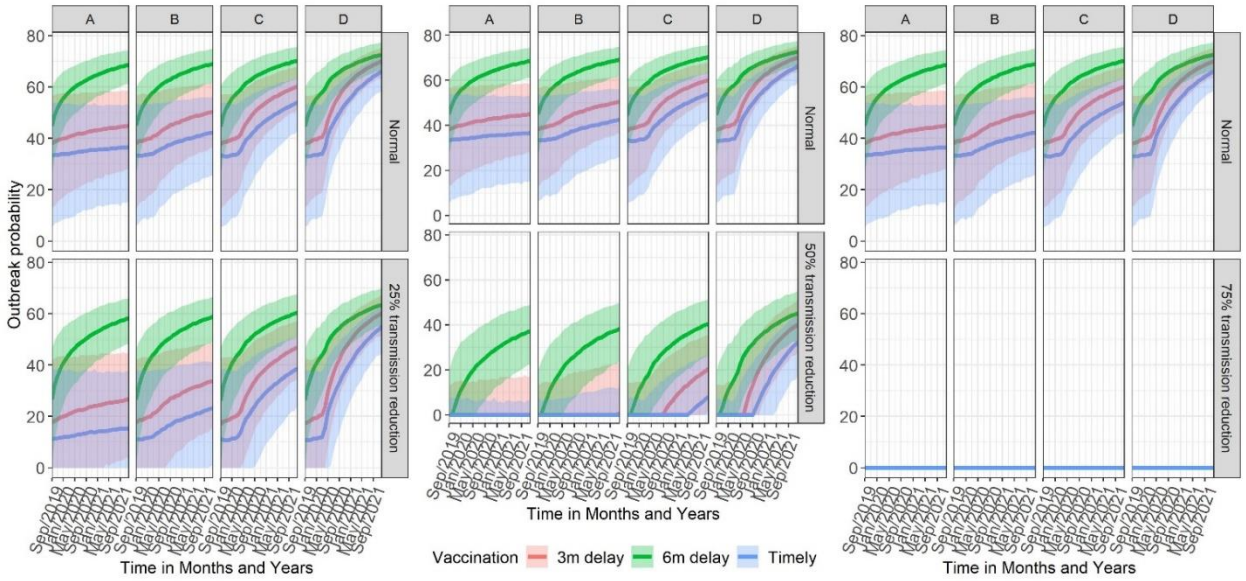


Figure s4:5. Impact of delayed vaccination on outbreak probability after lifting of contact reducing measures (Normal transmission) and assuming different levels of reduction in measles transmission during the pandemic.

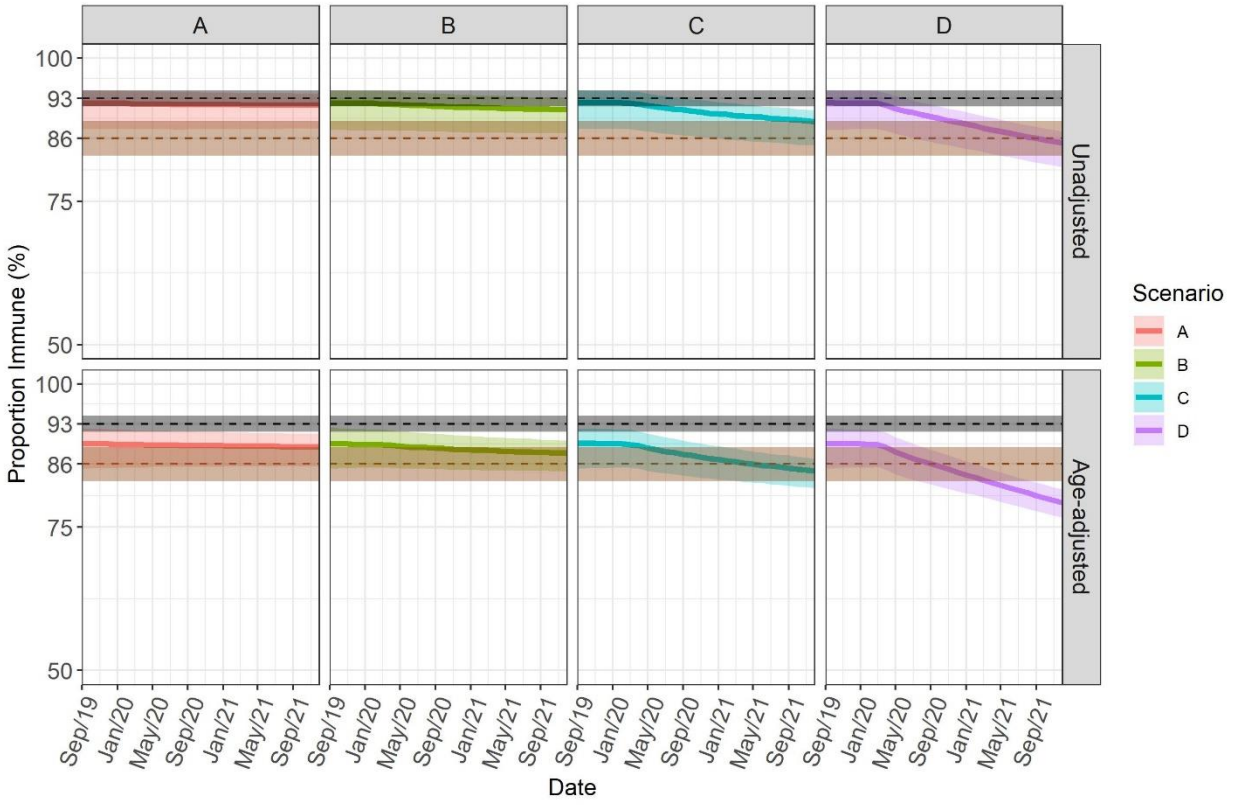


Figure s4:6. Monthly projected unadjusted and contact adjusted immunity profiles from September 2019 to December 2021**.** The changes in coverage took effect in April 2020· The black line shows the herd immunity threshold for measles before the COVID-19 pandemic 0·93 (0·92 to 0·94) and the brown line shows the herd immunity threshold during COVID-19 pandemic of 50%, 0·86 [·83-0·89], assuming the lockdown measures are still in effect.

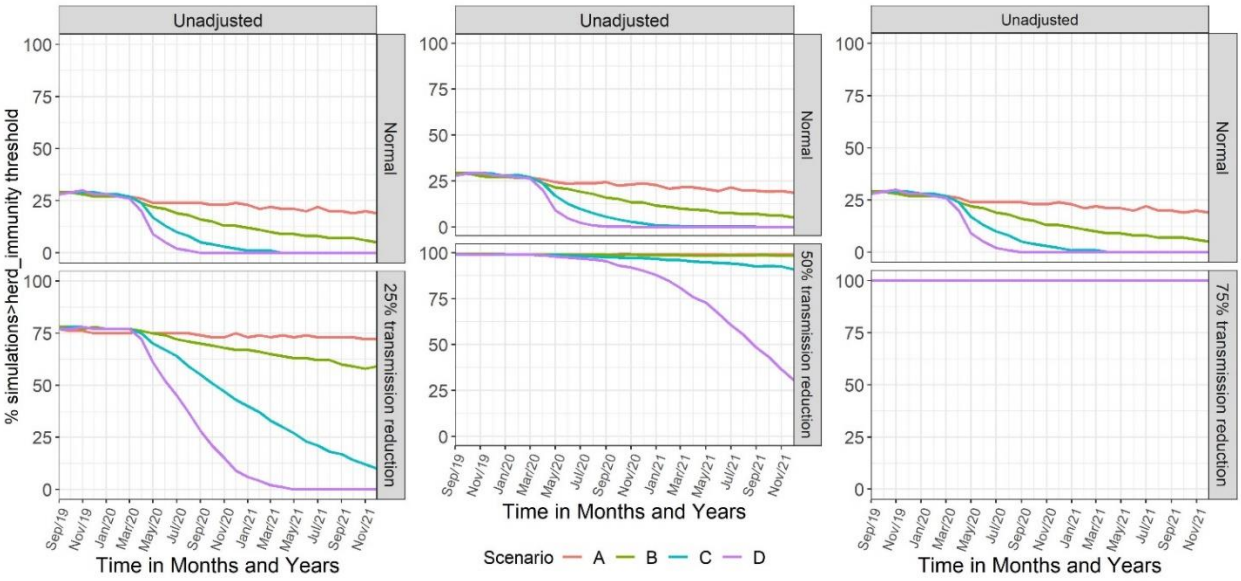


Figure s4:7. Percentage of simulations with proportion immune > herd immunity threshold for crude population immunity

Appendix E: Supplementary material for Chapter 5

Table s51: Age-specific changes in tetanus and diphtheria population immunity categorised into minimal seroprotection (0.011≤IgG<0.1 IU/ml), full seroprotection (0.1≤IgG<1 IU/ml) and long-term protection (IgG≥1 IU/ml). Seroprevalence estimates were adjusted for test performance and underlying population structure using Bayesian modelling.

Survey year	**Age in years**		**Diphtheria**			**Tetanus**		
	Level of seroprotection	n	Minimal%	Full%	Long-term%	Minimal%	Full%	Long-term%
2009	<1yr	14	40[20-63]	46[18-72]	02[00-07]	10[02-27]	49[23-73]	25[08-50]
	1-4yrs	133	56[47-65]	08[01-19]	01[00-02]	21[14-29]	60[46-70]	05[01-11]
	5-9yrs	202	50[41-59]	08[01-17]	01[00-03]	32[25-39]	30[11-43]	21[14-28]
	10-14yrs	16	45[26-68]	10[01-30]	01[00-08]	16[04-36]	23[05-46]	49[26-72]
	Total	365	50[41-58]	11[05-22]	01[00-04]	23[17-30]	37[24-49]	25[17-33]
	P value		0.054	0.001	0.483	0.038	<0.001	<0.001
2011	<1yr	14	21[06-39]	72[44-91]	03[00-14]	10[02-27]	19[04-43]	67[43-88]
	1-4yrs	82	13[05-24]	59[46-69]	10[04-17]	05[01-12]	26[07-42]	57[46-68]
	5-9yrs	142	51[42-60]	09[01-20]	01[00-04]	38[31-47]	23[07-37]	18[10-25]
	10-14yrs	70	57[44-69]	10[01-23]	01[00-05]	21[12-31]	24[06-42]	40[28-54]
	Total	308	40[34-46]	28[21-36]	04[02-07]	22[17-27]	24[09-37]	39[33-45]
	P value		<0.001	<0.001	0.001	<0.001	0.444	<0.001
2013	<1yr	7	34[13-62]	52[17-83]	02[00-09]	09[01-29]	28[06-59]	55[24-82]
	1-4yrs	123	50[40-59]	37[24-47]	01[00-04]	09[03-16]	57[43-69]	23[14-31]
	5-9yrs	148	51[42-60]	15[05-26]	02[00-04]	30[22-39]	40[23-53]	12[06-19]
	10-14yrs	128	58[48-67]	11[02-22]	01[00-02]	28[20-37]	20[04-36]	31[22-39]
	Total	406	52[45-58]	22[15-31]	01[00-03]	22[17-27]	38[25-49]	24[18-29]
	P value		0.256	<0.001	0.384	<0.001	<0.001	<0.001
2015	<1yr	35	19[08-34]	68[50-82]	07[01-16]	04[01-13]	38[15-57]	48[32-64]
	1-4yrs	144	53[43-62]	28[17-38]	01[00-03]	11[04-17]	64[51-73]	15[09-23]
	5-9yrs	153	49[40-58]	15[04-26]	01[00-02]	36[29-45]	39[23-52]	08[02-14]
	10-14yrs	44	59[43-73]	07[01-20]	01[00-04]	33[20-48]	21[04-40]	22[11-37]
	Total	376	51[44-58]	20[13-28]	01[01-03]	26[20-32]	41[28-51]	17[11-23]
	P value		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
2017	<1yr	27	18[06-35]	60[38-78]	11[03-25]	08[02-22]	45[20-65]	32[18-52]
	1-4yrs	160	48[38-57]	34[24-44]	01[00-04]	08[03-13]	69[59-78]	15[08-21]
	5-9yrs	185	51[42-59]	17[07-27]	02[0-05]	40[33-47]	40[25-52]	04[01-09]
	10-14yrs	49	45[31-59]	08[01-22]	02[00-06]	53[39-66]	18[04-35]	08[01-18]
	Total	421	46[39-53]	22[15-30]	03[01-04]	33[27-38]	42[31-52]	10[06-15]
	P value		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
2019	<1yr	39	25[12-40]	57[39-74]	08[02-20]	05[01-14]	29[08-48]	55[40-71]
	1-4yrs	205	52[43-60]	33[23-42]	02[00-04]	10[05-15]	71[62-80]	08[03-14]
	5-9yrs	220	51[44-58]	10[02-20]	02[00-04]	33[27-40]	45[30-56]	04[01-09]
	10-14yrs	56	55[41-68]	08[01-21]	04[00-09]	37[24-50]	30[10-47]	09[02-19]
	Total	520	51[44-57]	19[13-27]	03[01-05]	26[20-31]	46[34-56]	11[06-15]
	P value		0.003	<0.001	0.002	<0.001	<0.001	<0.001

2021	<1yr	8	27[10-50]	35[08-68]	07[01-29]	09[01-30]	27[06-56]	59[28-85]
	1-4yrs	85	30[18-41]	51[39-63]	04[01-09]	07[02-14]	55[37-68]	25[15-35]
	5-9yrs	102	52[41-62]	29[17-42]	03[00-07]	24[15-33]	49[34-63]	11[04-20]
	10-14yrs	95	44[32-55]	24[11-37]	05[01-10]	38[28-49]	21[05-37]	19[11-28]
	Total	290	41[34-48]	34[26-43]	04[02-07]	23[17-28]	41[29-52]	21[15-26]
	P value		0.004	0.004	0.109	<0.001	<0.001	<0.001

Table s5:2. Age-specific changes in Diphtheria and Tetanus geometric mean concentrations (GMCs) adjusted for underlying population.

Survey year	**2009**			**2011**			**2013**			**2015**			**2017**			**2019**			**2021**		
Diphtheria	n	GMC [95% CI]		n	GMC [95% CI]		n	GMC [95% CI]		n	GMC [95% CI]		n	GMC [95% CI]		n	GMC [95% CI]		n	GMC [95% CI]	
Age categories																					
<1yr	14	0.15	[0.08-0.27]	14	0.31	[0.16-0.59]	7	0.22	[0.08-0.63]	35	0.29	[0.20-0.43]	27	0.29	[0.15-0.56]	39	0.27	[0.19-0.38]	8	0.26	[0.05-1.29]
1-4yrs	133	0.03	[0.02-0.05]	82	0.18	[0.09-0.39]	123	0.11	[0.07-0.17]	144	0.06	[0.04-0.10]	160	0.09	[0.06-0.12]	205	0.08	[0.06-0.11]	85	0.13	[0.07-0.26]
5-9yrs	202	0.02	[0.01-0.04]	142	0.03	[0.01-0.06]	148	0.04	[0.02-0.08]	153	0.03	[0.02-0.05]	185	0.04	[0.02-0.07]	220	0.04	[0.02-0.07]	102	0.09	[0.05-0.16]
10-14yrs	16	0.01	[0.00-0.01]	70	0.02	[0.01-0.03]	128	0.03	[0.02-0.05]	44	0.03	[0.01-0.09]	49	0.02	[0.01-0.08]	56	0.04	[0.01-0.13]	95	0.06	[0.03-0.13]
Total	365	0.03	[0.02-0.05]	308	0.09	[0.04-0.18]	406	0.07	[0.04-0.13]	376	0.06	[0.04-0.10]	421	0.07	[0.04-0.12]	520	0.07	[0.04-0.12]	290	0.10	[0.05-0.26]
P_value		<0.001			<0.001			<0.001			<0.001			<0.001			<0.001			<0.001	
Tetanus																					
<1yr	14	0.54	[0.30-0.98]	14	1.25	[0.65-2.42]	7	1.67	[0.53-5.27]	35	1.03	[0.76-1.39]	27	0.82	[0.54-1.25]	39	1.03	[0.75-1.41]	8	1.20	[0.44-3.23]
1-4yrs	133	0.22	[0.15-0.33]	82	1.06	[0.58-1.96]	123	0.58	[0.40-0.85]	144	0.42	[0.30-0.60]	160	0.37	[0.27-0.52]	205	0.30	[0.22-0.41]	85	0.49	[0.29-0.86]
5-9yrs	202	0.27	[0.15-0.50]	142	0.18	[0.09-0.37]	148	0.17	[0.10-0.30]	153	0.15	[0.09-0.25]	185	0.11	[0.07-0.18]	220	0.13	[0.08-0.21]	102	0.25	[0.15-0.44]
10-14yrs	16	0.09	[0.04-0.22]	70	0.31	[0.16-0.62]	128	0.37	[0.15-0.93]	44	0.41	[0.14-1.63]	49	0.09	[0.02-1.81]	56	0.10	[0.04-0.31]	95	0.16	[0.06-0.42]
Total	365	0.22	[0.13-0.40]	308	0.55	[0.29-1.04]	406	0.46	[0.23-1.00]	376	0.37	[0.21-0.85]	421	0.23	[0.15-0.85]	520	0.23	[0.16-0.38]	290	0.36	[0.18-0.75]
P_value		0.012			<0.001			<0.001			<0.001			<0.001			<0.001			<0.001	

Table s5:3. Age-specific population immunity and GMCs in Diphtheria and Tetanus in adults. Seroprevalence estimates were adjusted for test performance and underlying population structure using Bayesian modelling

		Level of seroprotection							
Diphtheria	n	Minimal% [95% CI]		Full% [95% CI]		Long-term% [95% CI]		GMC [95% CI]	
15_19	53	52	[39-65]	13	[02-29]	01	[00-04]	0.03	[0.01-0.09]
20_24	51	41	[27-55]	21	[06-38]	01	[00-04]	0.04	[0.01-0.19]
25_29	49	44	[31-57]	15	[02-31]	01	[00-04]	0.03	[0.01-0.11]
30_34	51	54	[40-67]	07	[01-22]	01	[00-04]	0.03	[0.01-0.08]
35_39	49	51	[37-65]	11	[01-26]	01	[00-04]	0.04	[0.01-0.14]
40_44	50	59	[45-72]	08	[01-21]	01	[00-04]	0.03	[0.01-0.10]
45-49	50	57	[43-70]	08	[01-22]	01	[00-06]	0.03	[0.01-0.13]
50_54	48	41	[27-55]	22	[05-39]	04	[01-10]	0.06	[0.02-0.75]
55_59	50	43	[30-57]	12	[02-28]	01	[00-06]	0.03	[0.01-0.16]
60_64	49	58	[44-70]	09	[01-25]	02	[00-06]	0.04	[0.01-0.20]
65+	50	62	[48-74]	10	[01-25]	01	[00-04]	0.02	[0.00-0.90]
Total	550	50	[45-54]	17	[14-21]	01	[00-02]	0.04	[0.01-0.24]
P value		0.10		0.53		0.11		0.76	
Tetanus									
15_19	53	42	[30-56]	19	[03-37]	10	[02-21]	0.12	[0.00-1.76]
20_24	51	15	[06-26]	13	[02-31]	56	[42-70]	0.88	[0.01-4.19]
25_29	49	03	[01-09]	19	[04-39]	60	[46-74]	1.70	[0.02-4.02]
30_34	51	05	[01-13]	10	[02-25]	70	[56-82]	1.47	[0.02-3.92]
35_39	49	08	[02-18]	23	[05-42]	48	[35-63]	1.42	[0.02-4.29]
40_44	50	08	[02-17]	30	[09-49]	37	[23-52]	0.67	[0.01-2.65]
45-49	50	20	[10-33]	26	[06-45]	28	[15-42]	0.32	[0.00-1.98]
50_54	48	10	[03-21]	28	[07-47]	41	[27-54]	1.01	[0.03-3.89]
55_59	50	18	[09-30]	26	[05-44]	31	[19-47]	0.45	[0.01-1.23]
60_64	49	17	[08-29]	27	[08-46]	24	[14-38]	0.29	[0.01-1.41]
65+	50	26	[15-39]	22	[05-42]	20	[09-33]	0.06	[0.00-1.12]
Total	550	09	[06-13]	29	[25-34]	36	[32-41]	0.76	[0.01-2.87]
P value		<0.001		0.19		<0.001		<0.001	

Table s5:4. Age-specific changes in Pertussis population immunity categorised into; IgG ≥125 IU/mL (infection in the past 6 months), 62.5≤IgG <125 IU/mL (infection in the past 12 months), 20≤IgG <62.5 IU/mL (infection in the past ≥12 months or vaccination response) and 0≤IgG <20 IU/mL (no recent infection). Seroprevalence estimates were adjusted for test performance and underlying population structure using Bayesian modelling while the GMCs were adjusted for underlying population.

			Level of seroprotection				**GMCs [95% CI]**	
Survey year	Age category	n	0≤IgG <20 IU/mL	20≤IgG <62.5 IU/mL	62.5≤IgG <125 IU/mL	IgG≥125 IU/mL		
2009	<1yr	14	63[26-81]	10[02-24]	05[00-39]	00[00-18]	4.04	[1.48-11.00]
	1-4yrs	133	66[33-77]	11[02-22]	00[00-02]	00[00-04]	5.02	[2.40-10.50]
	5-9yrs	202	61[24-72]	08[01-18]	04[00-09]	02[02-07]	7.49	[3.83-14.83]
	10-14yrs	16	55[17-72]	16[03-32]	00[00-15]	03[03-21]	1.82	[0.62-5.41]
	Total	365	61[28-71]	11[03-21]	01[00-05]	01[00-05]	4.81	[2.28-10.45]
	P value		0.25	0.11	0.07	0.52	0.12	
2011	<1yr	14	58[21-75]	15[3-31]	01[00-27]	00[00-17]	7.23	[2.87-18.20]
	1-4yrs	82	74[51-84]	08[01-19]	00[00-03]	00[00-01]	3.24	[1.56-6.84]
	5-9yrs	142	59[21-71]	16[04-28]	00[00-04]	00[00-02]	9.37	[5.08-17.56]
	10-14yrs	70	46[09-61]	22[07-34]	09[02-19]	00[00-03]	9.23	[4.33-19.95]
	Total	308	59[29-69]	15[06-25]	01[00-05]	00[00-03]	7.44	[3.70-15.30]
	P value		<0.001	0.02	0.01	0.33	<0.001	
2013	<1yr	7	52[17-71]	17[03-36]	14[02-62]	00[00-32]	34.80	[14.00-86.30]
	1-4yrs	123	58[23-71]	16[04-27]	03[00-09]	00[00-01]	7.88	[4.27-14.83]
	5-9yrs	148	67[36-78]	08[01-19]	00[00-03]	01[01-07]	5.45	[2.44-12.40]
	10-14yrs	128	55[17-67]	18[06-29]	00[00-04]	01[01-07]	11.61	[4.82-28.79]
	Total	406	60[27-69]	14[05-23]	00[00-03]	00[00-04]	10.11	[4.51-23.36]
	P value		0.02	0.02	0.03	0.08	0.01	
2015	<1yr	35	58[19-73]	13[02-27]	06[00-21]	00[00-04]	5.22	[2.68-10.10]
	1-4yrs	144	56[18-68]	19[07-31]	00[00-05]	00[00-02]	9.75	[5.58-17.09]
	5-9yrs	153	68[40-79]	09[01-20]	00[00-04]	00[00-01]	4.73	[2.35-9.61]
	10-14yrs	44	59[21-73]	11[02-23]	03[00-15]	03[02-15]	7.63	[2.98-24.81]
	Total	376	61[30-71]	13[05-23]	00[00-04]	00[00-04]	7.07	[3.48-16.41]

	P value		0.08	0.07	0.51	0.16	<0.001	
2017	<1yr	27	60[23-75]	11[02-24]	06[00-26]	02[02-20]	8.65	[3.81-19.70]
	1-4yrs	160	58[22-70]	18[07-30]	00[00-02]	00[00-01]	7.83	[4.39-14.07]
	5-9yrs	185	71[46-80]	09[02-20]	00[00-01]	00[00-00]	4.23	[2.09-8.80]
	10-14yrs	49	59[23-73]	12[02-24]	05[00-17]	00[00-07]	5.49	[1.77-22.01]
	Total	421	63[34-73]	12[05-23]	00[00-01]	00[00-05]	5.95	[2.77-15.10]
	P value		0.04	0.06	0.02	0.18	0.004	
2019	<1yr	39	64[28-78]	10[02-22]	00[00-12]	02[02-15]	4.14	[2.03-8.47]
	1-4yrs	205	66[36-76]	13[03-24]	00[00-00]	00[00-00]	5.56	[3.16-9.78]
	5-9yrs	220	59[26-70]	14[04-24]	00[00-02]	00[00-05]	7.58	[3.96-14.92]
	10-14yrs	56	56[16-71]	14[03-27]	01[00-12]	03[03-14]	11.18	[5.53-26.00]
	Total	520	61[28-70]	13[04-24]	00[00-01]	00[00-05]	7.86	[4.08-16.39]
	P value		0.23	0.53	0.54	0.07	0.003	
2021	<1yr	8	58[19-76]	12[02-29]	07[00-44]	07[07-44]	14.90	[3.11-71.20]
	1-4yrs	85	45[09-60]	25[11-37]	08[02-17]	00[00-02]	13.02	[5.82-29.19]
	5-9yrs	102	58[21-70]	15[04-27]	04[00-12]	00[00-00]	8.27	[3.55-19.30]
	10-14yrs	95	50[13-66]	17[05-30]	07[01-16]	00[00-05]	12.82	[5.02-34.04]
	Total	290	49[42-55]	21[16-27]	06[03-11]	00[00-05]	11.48	[4.61-30.30]
	P value		0.11	0.22	0.91	0.06	0.23	

Table s5:5. Population immunity and GMCs in Pertussis in adults. Seroprevalence estimates were adjusted for test performance and underlying population structure using Bayesian modelling.

			Level of seroprotection				GMCs	
Survey year	Age category	n	0≤IgG <20 IU/mL	20≤IgG <62.5 IU/mL	62.5≤IgG <125 IU/mL	IgG≥125 IU/mL		
2021	15-19yrs	53	58[20-73]	15[03-30]	00[00-07]	00[00-07]	15.51	[6.90-35.41]
	20-24yrs	51	55[17-71]	16[03-29]	02[00-13]	02[00-13]	15.84	[4.97-40.90]
	25-29yrs	49	59[23-74]	14[03-28]	02[00-14]	02[00-14]	08.46	[2.90-26.54]
	30-34yrs	51	63[29-76]	11[02-23]	04[00-16]	04[00-16]	11.75	[5.06-29.02]
	35-39yrs	49	58[18-71]	18[04-32]	00[00-11]	00[00-11]	12.87	[4.34-42.40]
	40-44yrs	50	63[27-76]	14[03-28]	00[00-05]	00[00-5]	11.05	[4.47-28.07]
	45-49yrs	50	58[21-73]	15[03-28]	00[00-11]	00[00-11]	11.82	[4.64-42.87]
	50-54yrs	48	65[28-78]	11[02-24]	00[00-12]	00[00-12]	15.26	[5.31-52.50]
	55-59yrs	50	62[25-76]	15[03-28]	00[00-05]	00[00-05]	09.32	[3.28-33.62]
	60-64yrs	49	53[15-69]	19[05-33]	05[00-17]	05[00-17]	16.15	[8.04-35.59]
	>=65yrs	50	46[11-62]	32[17-48]	00[00-08]	00[00-08]	03.97	[1.40-37.12]
	Total	550	63[58-67]	23[19-27]	00[00-02]	00[00-01]	11.88	[4.71-35.81]
	P value		0.17	0.05	0.75	0.37	0.22	

Appendix F: Supplementary material for Chapter 6

Table s6:1: Table showing including those with and without repeated measurements, their ages at the time of data collection and corresponding tetanus IgG levels. ** Study Id N1021 exhibited an unusual spike in IgG levels between surveys outside the typical vaccination window.

Study ID	Age(yrs)	Tetanus IgG	Test
N1122	1	0.0165	Pos
	3	0.0143	Pos
	7	0.0138	Pos
J648/7	4	0.0531	Pos
	6	0.0001	Neg
J649/5	4	1.067	Pos
	6	0.562	Pos
N1070	3	0.0014	Neg
	5	0.0012	Neg
	9	0.0014	Neg
J223/1	6	0.0685	Pos
	8	0.0451	Pos
	10	0.0366	Pos
N1021**	4	0.0027	Neg
	6	0.0567	Pos
	10	0.0409	Pos
4951	6	0.0168	Pos
5187	9	1.175	Pos

Note s6:1: Model validation

We conducted a comprehensive assessment of the internal validity of our model. Internal validity refers to the model's ability to consistently predict outcomes within the same population from which the data was drawn.

To gauge internal validity, we employed a bootstrap resampling technique, generating 1,000 samples from the model development dataset. This allowed us to internally validate the model by estimating optimism-adjusted metrics for discrimination and goodness-of-fit in each of the bootstrap samples. We then compared the performance of the refitted model in each bootstrap sample with that of the refitted model in the original development sample. To obtain optimism-adjusted measures, we calculated the average of these differences and subtracted them from the original metrics.

Appendix G: Supplementary material for Chapter 7

Table s7:9:1:The table shows Immunological status and vaccination status from vaccine records before the vaccine information was updated using serological markers

Vaccination status	Immune(Vaccination)	Susceptible	Immune(Resolved Infection)	Active infections	Isolated anti-HBc	Unknown	Total
Vaccinated	1102	879	49	78	7	2	2117
Unvaccinated	4	0	0	40			44
Unknown	124	166	5	6	2	2	305
Total	1230	1045	54	124	9	4	2466

Table s7:2: displays the updated vaccination status (as depicted in the main Fig. 7:1) and the corresponding coverage rates according to the year. A chi-square trend test indicated a statistically significant upward trend in the vaccination coverage rates

Year	Unvaccinated	Vaccinated	Total	mid	lo	hi
2007	49	167	216	77.31	71.28	82.39
2009	77	278	355	78.31	73.73	82.28
2011	64	208	272	76.47	71.08	81.12
2013	51	214	265	80.75	75.58	85.05
2015	2	221	223	99.1	96.79	99.75
2017	0	305	305	100	98.76	100
2019	0	422	422	100	99.1	100
2021	0	240	240	100	98.42	100

Note s7:1: Immunological markers

Defining Acute and Chronic HBV infections using combination markers

HBV data comprised results of tests for hepatitis B surface antigen (HBsAg), antibody to hepatitis B surface (Anti-HBs), antibody to hepatitis B core antigen (Anti-HBc) and IgM antibody to hepatitis B core antigen (Anti-HBc IgM). We initially used the commonly test result combinations used to define the HBV status of each child from CDC[23] but ran into several challenges in further classifying the HBsAg positive samples. First, 97/136(71%) of HBsAg positive samples were not tested for anti-HBs due to insufficient volume which required several assumptions to be made in cases where these results were missing or incomplete (Fig.s7:1).

To avoid introducing bias in our results due to these assumptions, we decided to leave out the anti-HBs test results in the HBsAg positive samples and only utilize IgM to anti-HBc to distinguish acute from chronic cases [23](Fig.s7:2).

Unusual transitions

The consistency checks for the above algorithm validated some of the interpretation of the HBV markers as shown in Fig.s7:3 including persistence of immunity from a resolved infection over-time, progression of acute infection to either chronic or resolved infection and persistence of chronic states of infection over time.

The algorithm also revealed some unusual transitions including transition from chronic infection to susceptible (1/368), transition from infection (acute, early acute, undefined) to susceptible (27/368) and to immune(vaccination), 22/368. A closer look at the children transitioning from any infection to immune (vaccination) revealed that 6/22 (27%) of these children were positive for both HBsAg and anti-HBs and negative for anti-HBc with no evidence of vaccination. Although simultaneous positive HBsAg and anti-HBs without evidence of vaccination has been described before[47] it's a rare occurrence and is likely to be a case of false positives. Consequently, we decided to remove this from further analysis.5/22 (23%) were born before the vaccine was introduced but had a serologic combination of immune from vaccine (anti-HBs positive only). We reclassified this as immune(unclassified) and any other child with this representation in the rest of the dataset. The rest 11/22(50%) had evidence of vaccination from the vaccine records, coexistence of anti-HBs and HBsAg which waned in subsequent surveys without development of anti-HBc resulting to an isolated anti-HBs. These could be cases of breakthrough infections which have been shown to sometime present with milder symptoms, shorter duration of HBsAg, lack of anti-HBc and isolated anti-HBs[48, 49].This rationale could also be applied to further classify children in the "undefined infection" category with a positive HBsAg, negative anti-HBc and IgM to anti-HBc and evidence of vaccination as breakthrough infections.

We also reviewed the transitions from infection to susceptible (28/368). The Acute (1/368) and Chronic infections (1/368) both had a positive anti-HBc and transition to susceptible would imply clearance of anti-HBc which is rare and so these were termed as misclassifications. Transition from early acute and undefined infections to susceptible would imply a loss of HBsAg without development of either anti-HBc or anti-HBs. This could be a case of mild HBV infection termed

as HBV2 characterized by positive HBsAg results only and clearance of the marker after a certain duration without the development of anti-HBc and anti-HBs antibodies[44, 45, 50]. In some cases, this could be due to sensitivity of the anti-HBc assay.

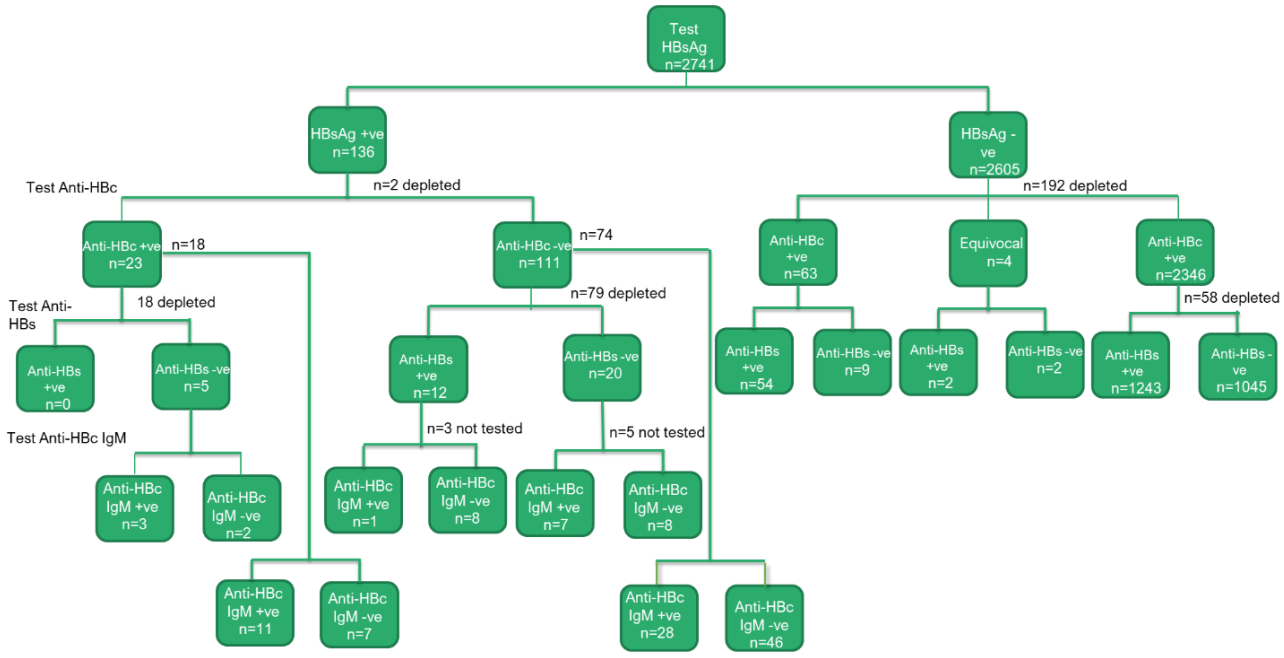


Figure s7:1: Initial HBV testing algorithm utilizing all the markers. 97/136(71%) of HBsAg positive samples were not tested for anti-HBs due to insufficient volume.

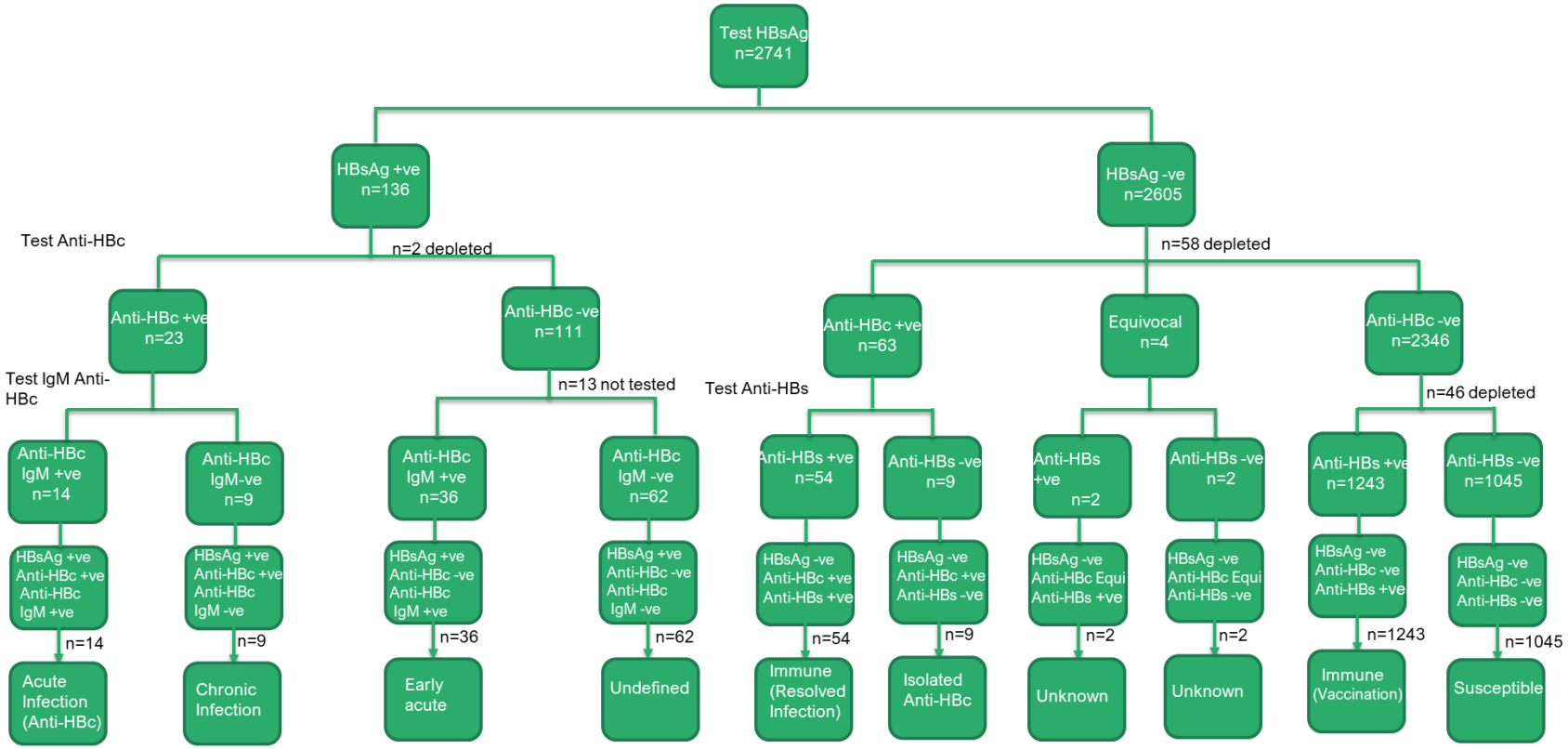


Figure s7:2: HBV testing algorithm without data on anti-HBs test results for HBsAg positive samples.

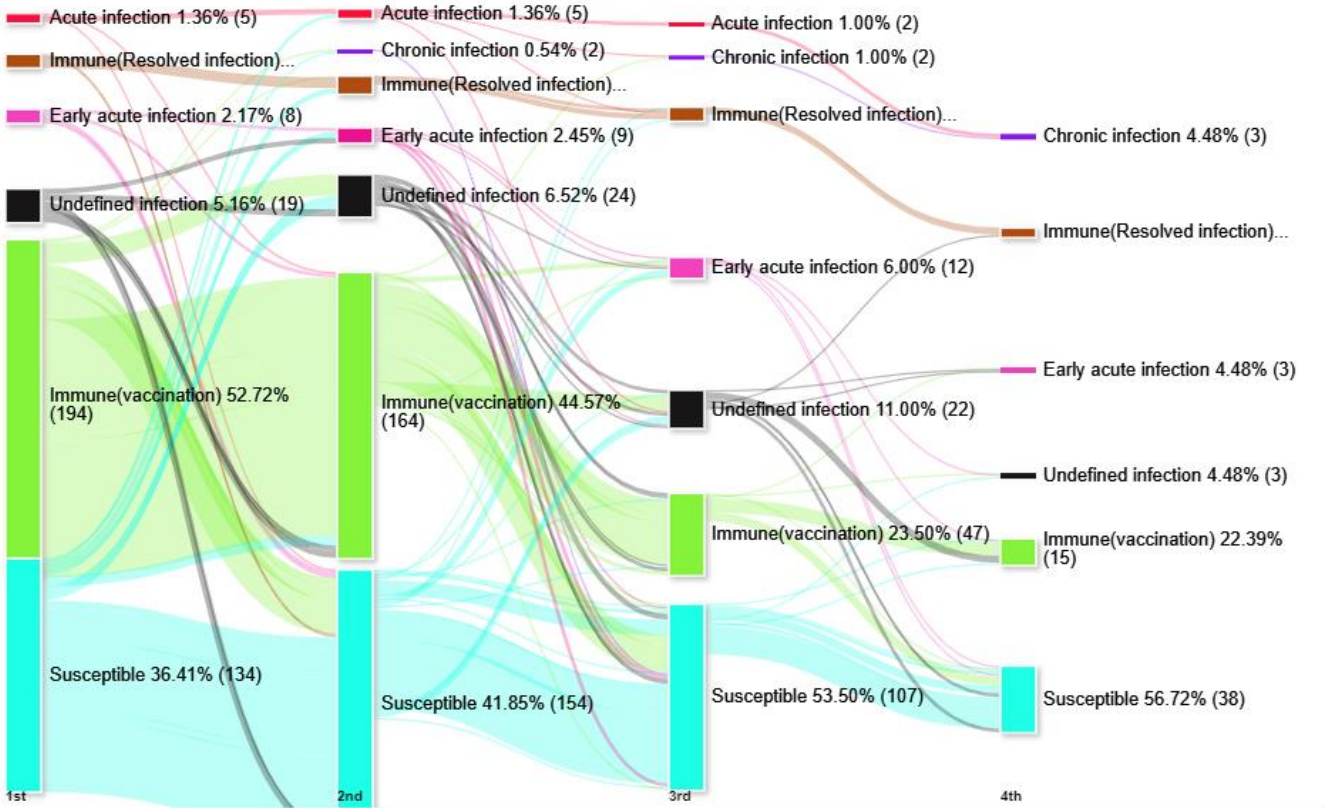


Figure s7:3: Consistency checks for algorithm utilizing IgM anti-HBc using longitudinal data for samples that are repeated over the 4-year survey (2007-2013). Most of the infections classified as undefined are likely breakthrough infections based on the combination of different marker

Table s7:3: Number and proportion of children with active HBV infections and evidence of ever having had a HBV infection stratified by survey year and age category. Estimates were adjusted for underlying population structure

Age category	**<1yr**			**1-4yrs**			**5-9yrs**			**10-14yrs**			**P value**	**Total**		
Active HBV infections	n	%[95% CI]		n	%[95% CI]		n	%[95% CI]		n	%[95% CI]			n	%[95% CI]	
2007	0	0.00	[0.00-32.44]	4	2.20	[0.87-15.97]	8	25.90	[5.81-40.57]	0	0.00	[0.00-33.31]	0.372	12	9.43	[2.22-30.81]
2009	0	0.00	[0.00-0.00]	7	4.55	[1.47-17.77]	24	12.53	[5.65-26.27]	9	12.57	[4.29-41.79]	0.001	40	9.39	[3.65-26.74]
2011	0	0.00	[0.00-0.00]	6	6.71	[1.74-24.56]	16	13.70	[4.83-35.02]	18	34.36	[11.05-46.51]	0.010	40	17.06	[5.52-33.08]
2013	0	0.00	[0.00-0.00]	3	2.82	[0.50-17.54]	8	5.51	[2.13-19.69]	10	7.17	[2.31-22.96]	0.367	21	4.86	[1.57-18.68]
2015	0	0.25	[0.00-48.99]	0	0.00	[0.00-11.35]	0	0.00	[0.00-14.83]	0	0.00	[0.00-38.33]	NA	0	0.00	[0.00-23.46]
2017	0	0.38	[0.00-32.44]	0	0.00	[0.00-9.82]	0	0.00	[0.00-10.51]	0	0.00	[0.00-26.70]	NA	0	0.00	[0.00-16.83]
2019	0	0.14	[0.00-35.43]	1	0.50	[0.09-7.74]	2	0.84	[0.15-10.02]	0	0.00	[0.00-25.44]	0.838	3	0.43	[0.08-15.90]
2021	0	0.00	[0.00-39.03]	0	0.00	[0.00-17.59]	3	3.25	[0.78-21.11]	5	4.82	[1.25-24.58]	0.211	8	2.58	[0.65-22.45]
Current and past HBV	n	%[95% CI]		n	%[95% CI]		n	%[95% CI]		n	%[95% CI]			n	%[95% CI]	
2007	0	0.00	[0.00-32.44]	5	2.75	[1.20-16.65]	9	26.80	[6.25-41.66]	0	0.00	[0.00-33.31]	0.395	14	9.89	[2.47-31.37]
2009	0	0.00	[0.00-0.00]	9	6.12	[2.15-19.91]	31	16.91	[8.60-31.07]	14	22.31	[9.53-47.63]	<0.001	54	14.31	[6.45-30.76]
2011	0	0.00	[0.00-0.00]	6	6.71	[1.74-24.56]	19	17.41	[6.51-39.01]	26	40.46	[15.31-52.78]	<0.001	51	20.19	[7.39-36.36]
2013	0	0.00	[0.00-0.00]	5	4.68	[0.97-20.31]	9	6.42	[2.48-20.90]	17	13.39	[5.10-30.63]	0.163	31	7.60	[2.68-22.22]
2015	1	25.00	[4.56-69.94]	2	1.84	[0.33-14.24]	0	0.00	[0.00-14.83]	1	1.30	[0.23-40.17]	<0.001	4	2.70	[0.49-26.33]
2017	3	37.50	[13.68-69.43]	0	0.00	[0.00-9.82]	1	0.48	[0.09-11.31]	2	4.56	[0.82-31.93]	<0.001	6	4.23	[1.25-21.33]
2019	1	14.29	[2.57-51.31]	4	1.83	[0.41-9.90]	2	0.84	[0.15-10.02]	1	1.94	[0.35-27.96]	0.06	8	2.41	[0.45-18.41]
2021	0	0.00	[0.00-39.03]	4	4.30	[0.98-23.98]	6	6.52	[1.99-25.32]	8	7.84	[2.81-28.06]	0.729	18	5.83	[1.81-26.76]

Table s7:4: Number and proportion of children who are susceptible or immune from vaccination stratified by survey year and age category. Estimates were adjusted for underlying population structure

Age category	**<1yr**			**1-4yrs**			**5-9yrs**			**10-14yrs**			**P value**	**Total**		
Susceptible	n	%[95% CI]		n	%[95% CI]		n	%[95% CI]		n	%[95% CI]		P value	n	%[95% CI]	
2007	2	25	[7-63]	24	19	[9-39]	63	55	[39-92]	2	42	[9-50]	<0.001	91	39	[19-62]
2009	0	0	[0-0]	23	19	[9-38]	94	48	[33-71]	16	37	[12-60]	<0.001	133	33	[18-53]
2011	0	0	[0-0]	19	19	[11-40]	51	45	[26-73]	47	38	[25-78]	<0.001	117	32	[20-60]
2013	0	0	[0-0]	16	12	[6-31]	38	27	[14-50]	74	54	[36-87]	<0.001	128	29	[18-53]
2015	0	0	[0-52]	21	17	[8-36]	46	41	[23-68]	24	78	[40-110]	<0.001	91	43	[22-71]
2017	1	13	[2-50]	33	23	[13-42]	70	40	[25-63]	34	56	[29-98]	<0.001	138	38	[21-67]
2019	1	14	[3-55]	66	32	[22-49]	108	51	[37-73]	39	61	[35-102]	<0.001	214	46	[30-74]
2021	0	0	[0-42]	28	36	[18-62]	34	34	[18-63]	60	64	[41-100]	<0.001	122	41	[24-73]
Immune (Vaccination)	n	%[95% CI]		n	%[95% CI]		n	%[95% CI]		n	%[95% CI]			n	%[95% CI]	
2007	5	63	[31-86]	87	77	[59-87]	19	13	[7-43]	0	0	[0-33]	<0.001	111	30	[21-56]
2009	0	0	[0-0]	95	71	[54-84]	70	31	[21-46]	0	0	[0-30]	<0.001	165	31	[22-49]
2011	0	0	[0-0]	61	71	[53-83]	40	32	[17-54]	2	1	[0-26]	<0.001	103	31	[21-50]
2013	0	0	[0-0]	57	55	[37-72]	31	22	[11-40]	17	10	[4-26]	<0.001	105	26	[15-42]
2015	2	50	[15-85]	91	74	[57-86]	62	54	[35-73]	7	11	[4-51]	<0.001	162	47	[30-71]
2017	2	25	[7-59]	85	59	[43-73]	91	44	[30-60]	23	31	[14-60]	<0.001	201	43	[27-64]
2019	5	71	[36-92]	126	60	[46-72]	96	44	[31-59]	19	32	[14-60]	<0.001	246	47	[30-65]
2021	6	100	[61-100]	46	56	[35-76]	57	58	[37-77]	26	28	[12-51]	<0.001	135	51	[31-70]

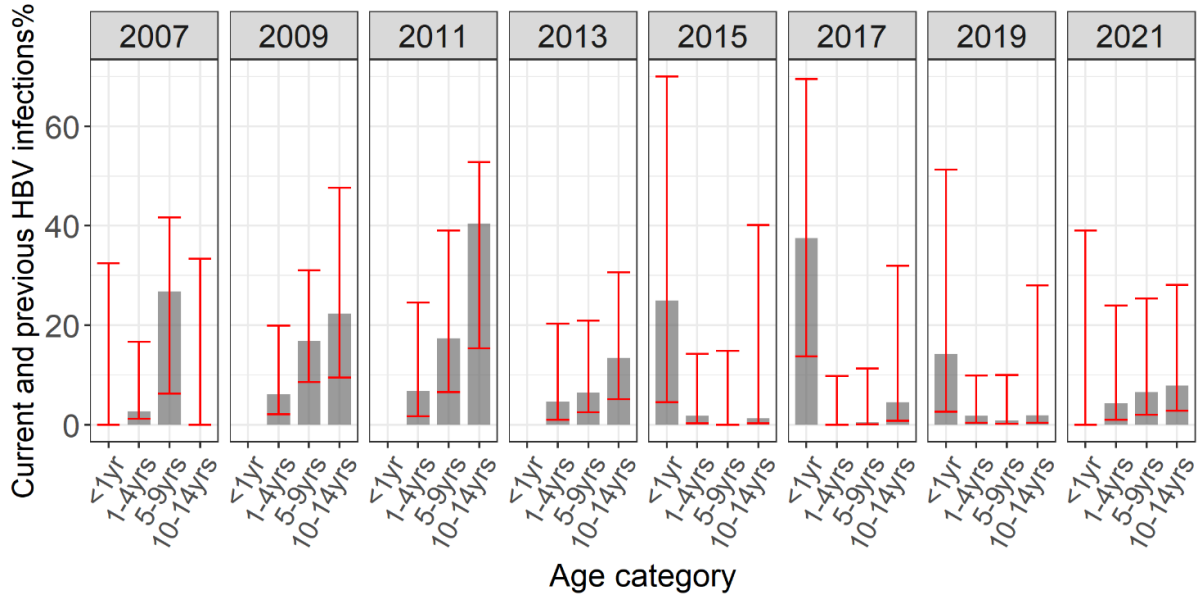
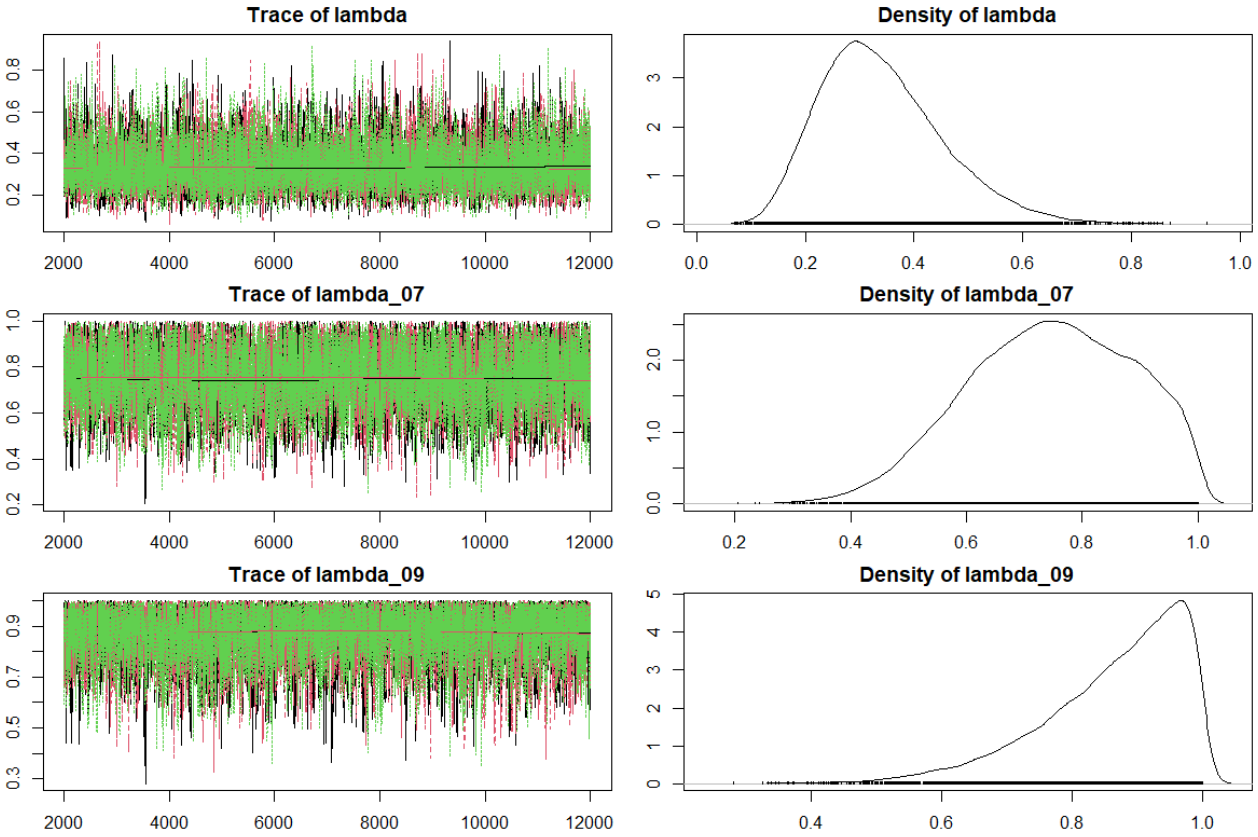
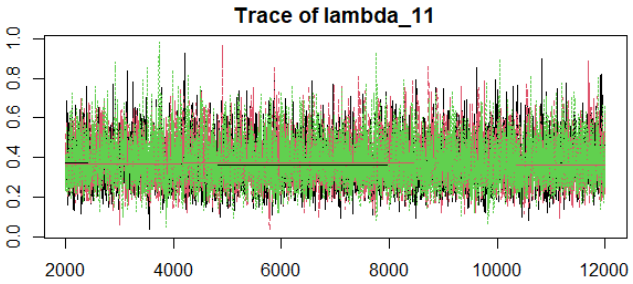


Figure s7:4: Proportion of children in Kilifi with evidence of ever having been infected with HBV adjusted for underlying pop




Trace of lambda_11
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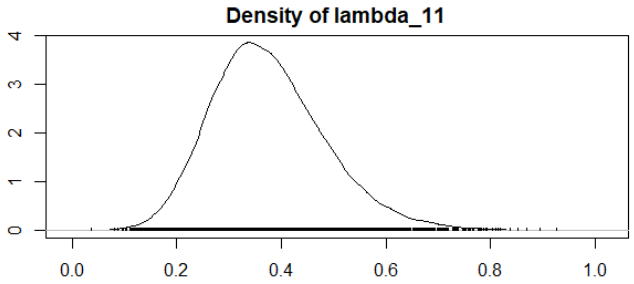
Density of lambda_11
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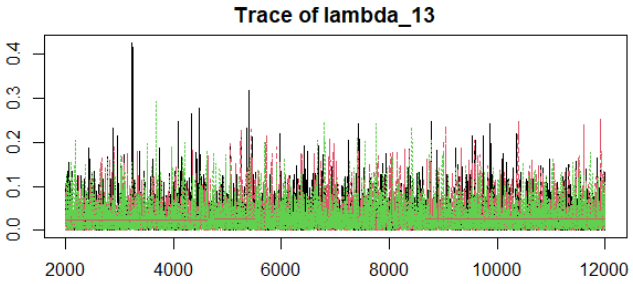
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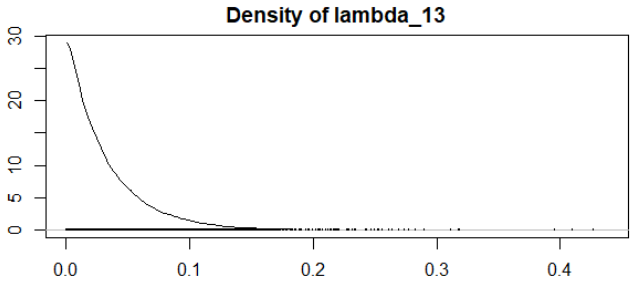
Density of lambda_13
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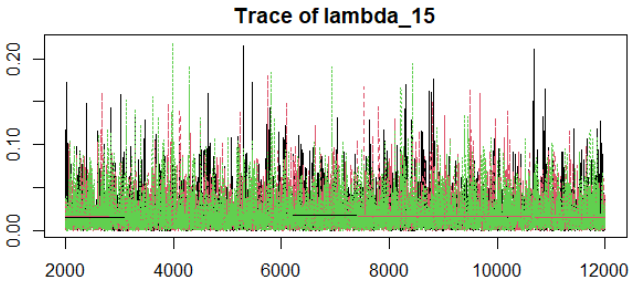
Trace of lambda_15
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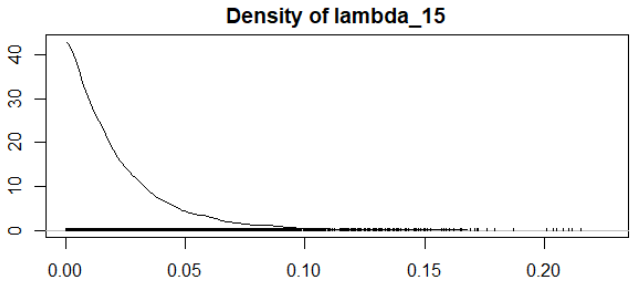
2000 4000 6000 8000 10000 12000


Density of lambda_15
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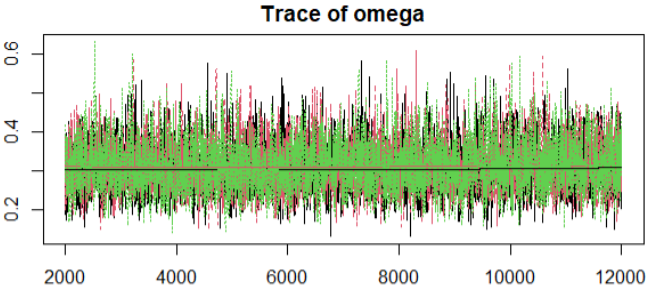
0.00 0.05 0.10 0.15 0.20


Trace of omega
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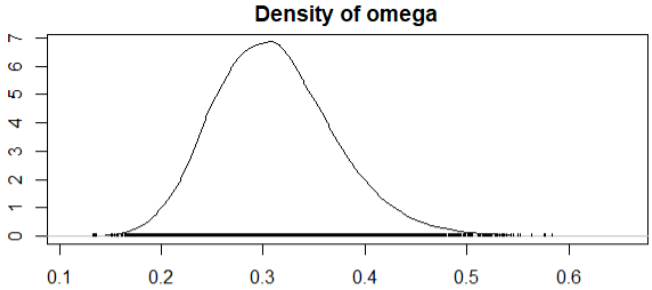
Density of omega
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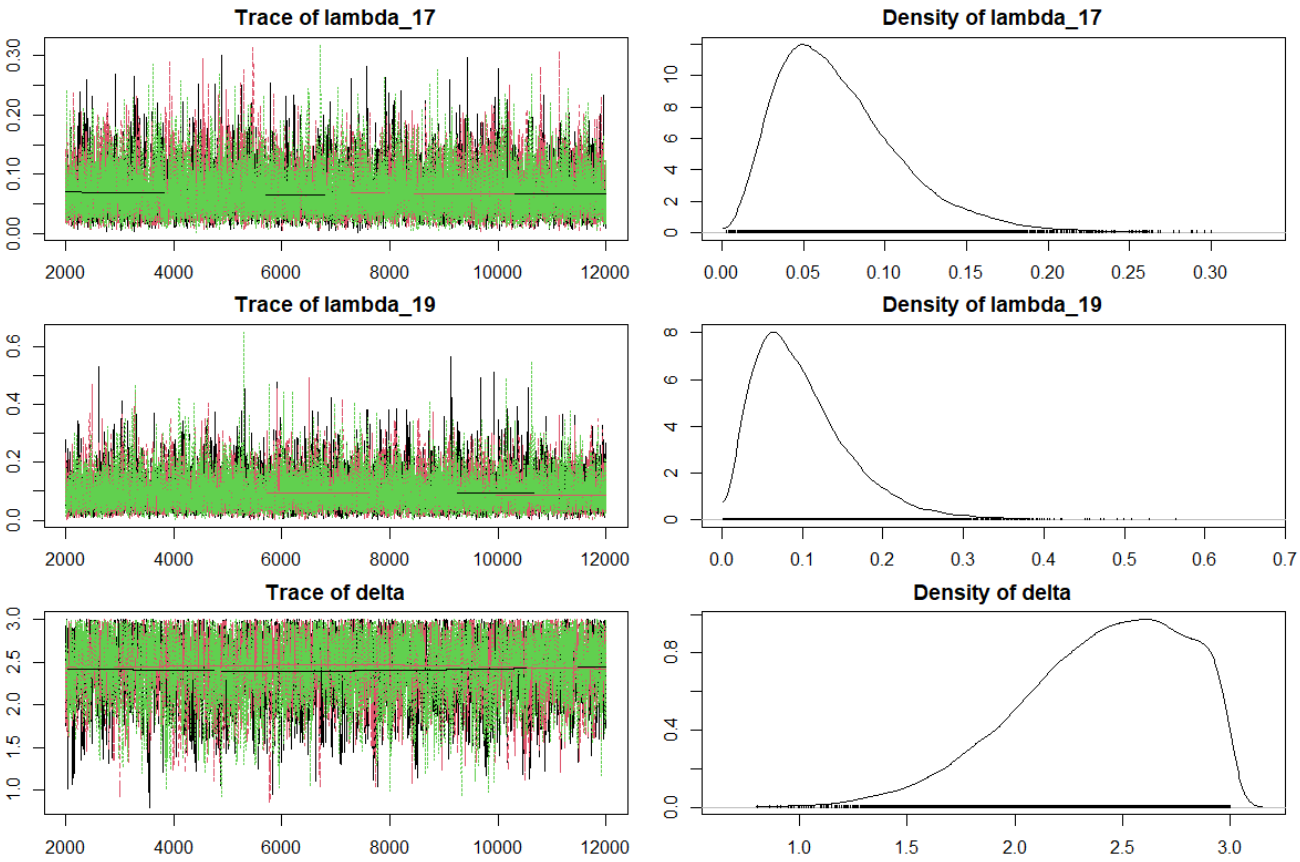

Figure s7:5: Convergence chains for the joint reverse catalytic model (main model). lambda is the force of infection for the different time perods,Omega is 1-vaccine effectiveness while delta is the waning rate.

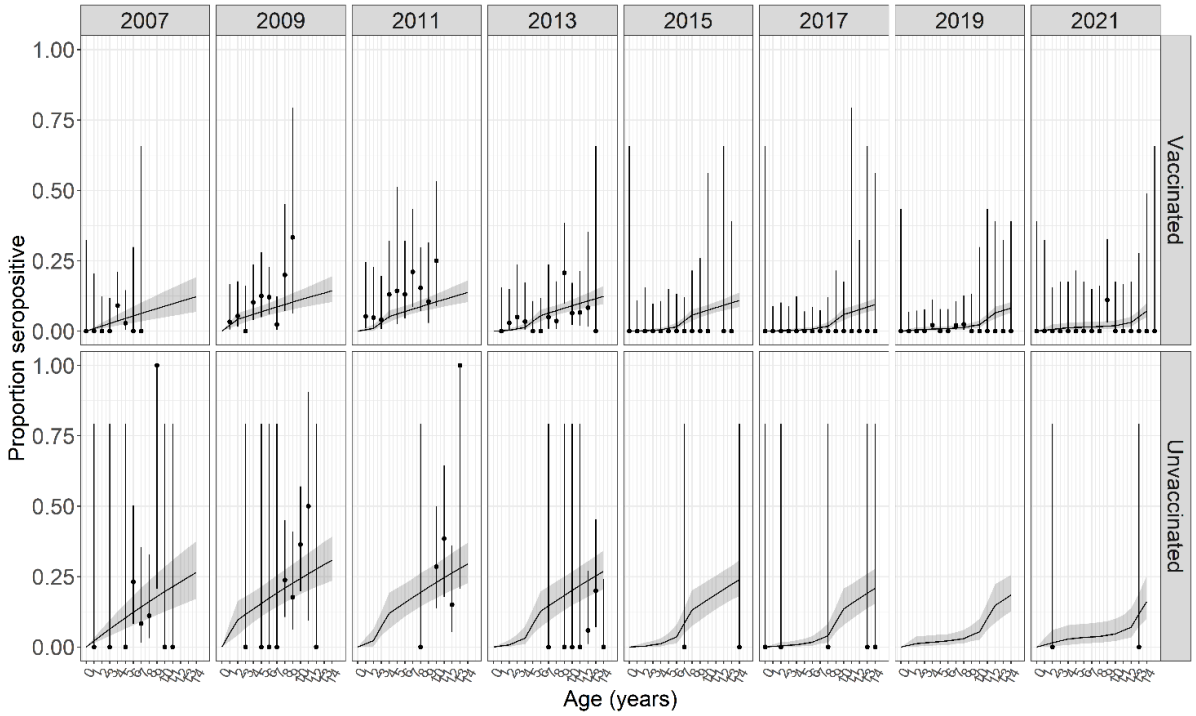

Figure s7:6: Simple catalytic model fit (Sensitivity analysis). The points are the observed proportion of HBsAg seropositive individuals. Lines are the seroprevalence curves, sampled from the fitted model where shaded region represents 95% credible interval of the predictive posterior distribution

Table s7:5: Sensitivity analysis using different variations of the catalytic model

Model	**Parameters**	**Period**	**Estimates(95% cry)**	**Waning**	**1-VE**	**VE(%)**
Joint reverse catalytic model(2015-2021)	lambda	Pre_2015	0.012 (0 - 0.066)	4.339 (2.329 - 4.974)	0.498 (0.025 - 0.975)	50(25-98)%
	lambda_15	2015-2017	0.008 (0 - 0.044)			
	lambda_17	2017-2019	0.035 (0.009 - 0.087)			
	lambda_19	2019-2021	0.048 (0.01 - 0.136)			
Joint simple catalytic model(2015-2021)	lambda	Pre_2015	0 (0 - 0.001)		0.488 (0.024 - 0.975)	51(24-98)%
	lambda_15	2015-2017	0.001 (0 - 0.004)			
	lambda_17	2017-2019	0.003 (0 - 0.008)			
	lambda_19	2019-2021	0.003 (0 - 0.013)			
Joint simple catalytic model all surveys	lambda	Pre_2007	0.022 (0.014 - 0.032)		0.418 (0.277 - 0.632)	58(37-72)
	lambda_07	2007-2009	0.05 (0.022 - 0.09)			
	lambda_09	2009-2011	0.012 (0.001 - 0.033)			
	lambda_11	2011-2013	0.004 (0 - 0.013)			

	lambda_13	2013-2017	0.001 (0 - 0.007)			
	lambda_15	2017-2019	0.002 (0 - 0.01)			
	lambda_17	2017-2019	0.006 (0.001 - 0.02)			
	lambda_19	2019-2021	0.007 (0 - 0.032)			

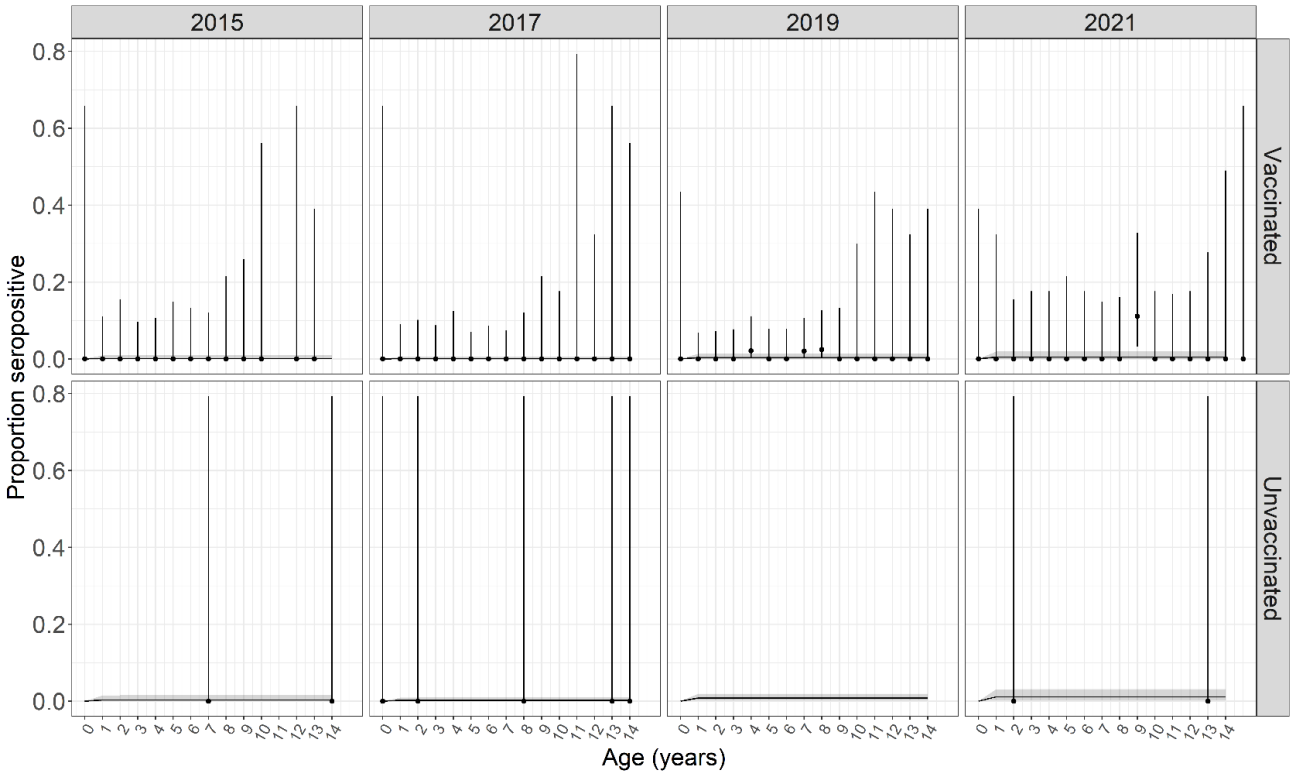

Figure s7:7: Joint reverse catalytic model 2015-2021.The points are the observed proportion of HBsAg seropositive individuals. Lines are the seroprevalence curves, sampled from the fitted model where shaded region represents 95% credible interval of the predictive posterior distribution

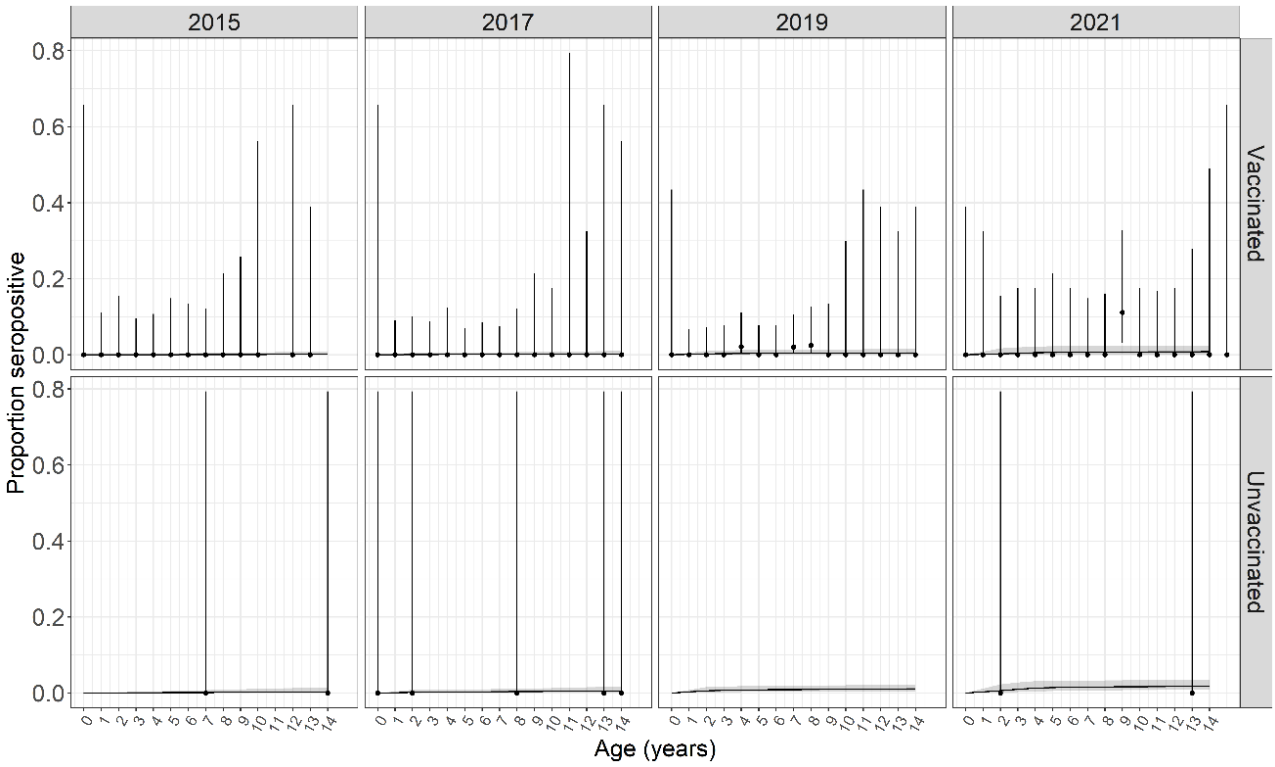

Figure s7:8: Simple catalytic model fit 2015-2021(Sensitivity analysis). The points are the observed proportion of HBsAg seropositive individuals. Lines are the seroprevalence curves, sampled from the fitted model where shaded region represents 95% credible interval of the predictive posterior distribution

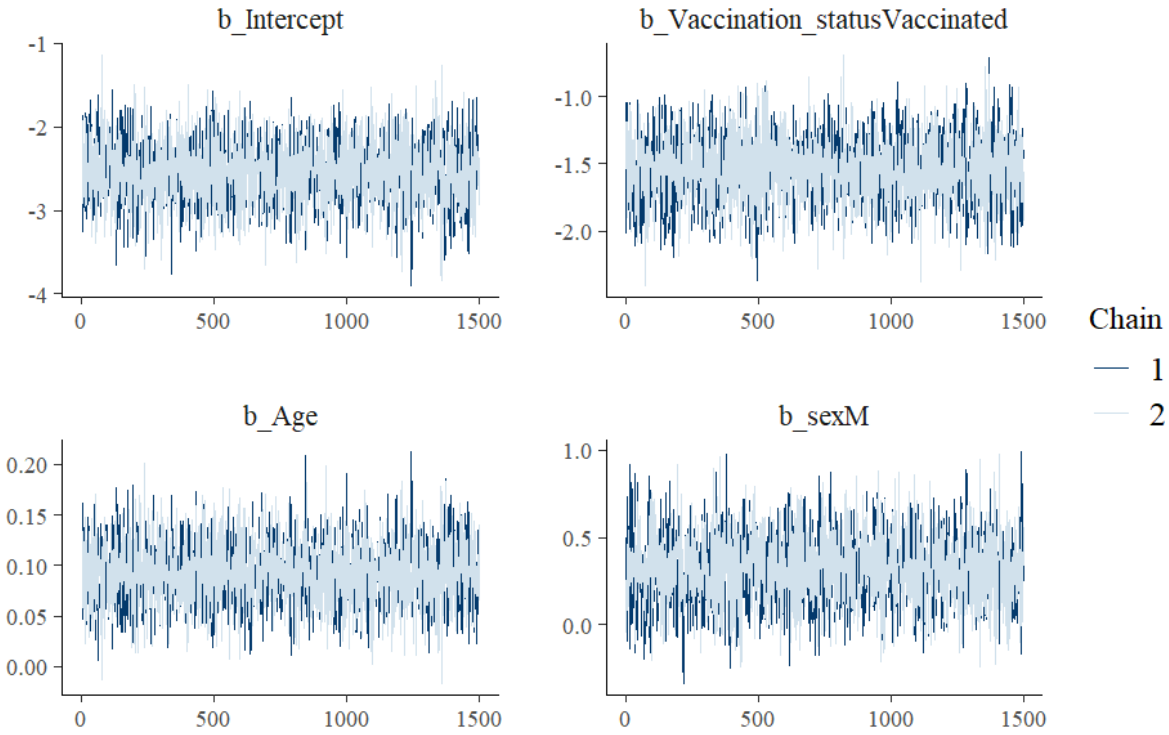

Figure s7:9: Convergence chains of the binary logistic model. There was no evidence of non-convergence

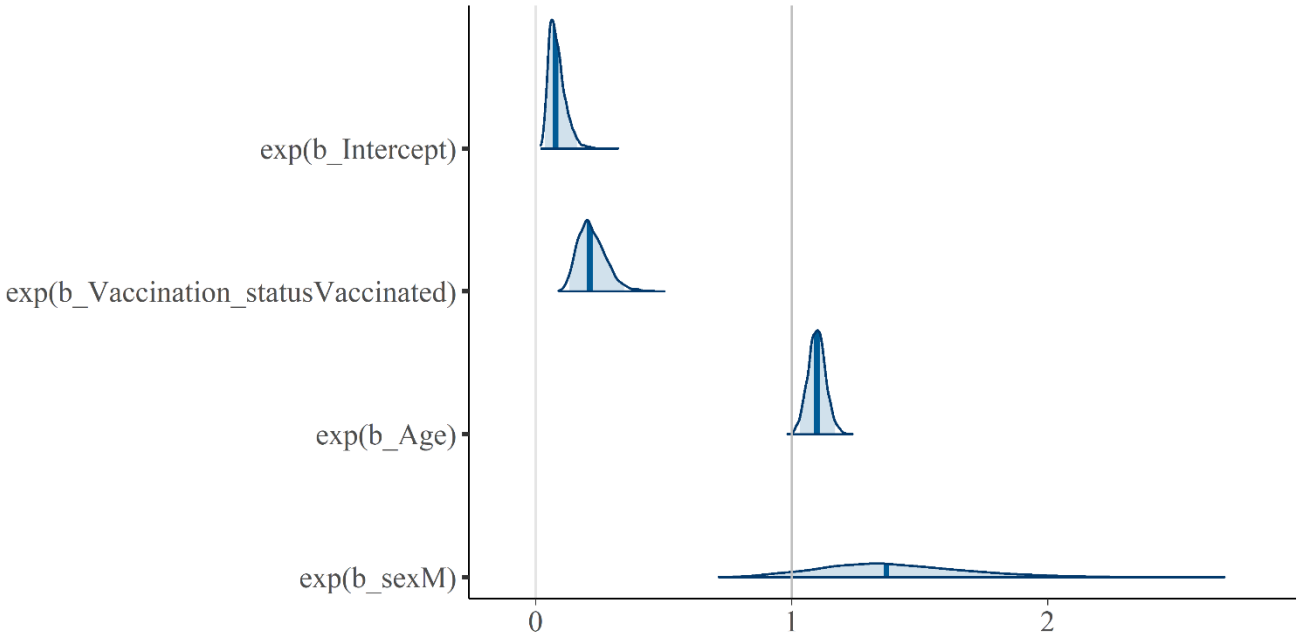

Figure s7:10: Densities of the binary logistic regression model exponentiated parameter estimates showing the odds of having an active HBV infection. The dark blue line in each density represents the point estimate, while the light-blue area indicates the 95% credibility intervals

Note s7:1 Model equations in the unvaccinated cohort. Similar equations were used for the vaccinated cohort but multiplied by μ(1-VE)

$$Z_{2007}(a) = \frac{\lambda_{pre2007}}{\lambda_{pre2007\ +}\,\omega}(1 - exp(-a * (\lambda_{pre2007\ +}\,\omega))$$

$$Z_{2009}(a) = \frac{\lambda_{2007}}{\lambda_{2007}+\omega}(1 - exp(-a * (\lambda_{2007} + \omega)) \qquad a < 2$$

$$\left(\frac{\lambda_{pre2007}}{\lambda_{pre2007\ +}\,\omega}(1\text{-}exp(-(a-2) * (\lambda_{pre2007\ +}\,\omega))) - \frac{\lambda_{2007}}{\lambda_{2007}+\omega}\right) * exp(-a*(\lambda_{2007} + \omega)) + \frac{\lambda_{2007}}{\lambda_{2007}+\omega} \qquad a \geq 2$$

$$Z_{2011}(a) = \frac{\lambda_{2009}}{\lambda_{2009}+\omega}(1 - exp(-a * (\lambda_{2009} + \omega))) \qquad a < 2$$

$$\left(\left(\frac{\lambda_{2007}}{\lambda_{2007}+\omega}(1\text{-}exp(-(a-2) * (\lambda_{2007} + \omega))) - \frac{\lambda_{2009}}{\lambda_{2009}+\omega}\right) * exp(-a*(\lambda_{2009} + \omega)) + \frac{\lambda_{2009}}{\lambda_{2009}+\omega}\right) \qquad 2 \leq a < 4$$

$$\left(\left(\frac{\lambda_{pre2007}}{\lambda_{pre2007\ +}\,\omega}(1\text{-}exp(-(\lambda_{pre2007\ +}\,\omega)*(a\text{-}4)) - \frac{\lambda_{2007}}{\lambda_{2007}+\omega}\right) * exp(-a*(\lambda_{2007} + \omega)) + \frac{\lambda_{2007}}{\lambda_{2007}+\omega}\right) - \frac{\lambda_{2009}}{\lambda_{2009}+\omega}\right) *$$

$$exp(-a*(\lambda_{2009} + \omega)) + \frac{\lambda_{2009}}{\lambda_{2009}+\omega} \qquad a \geq 4$$

$$Z_{2013}(a) = \frac{\lambda_{2011}}{\lambda_{2011}+\omega}(1 - exp(-a * (\lambda_{2011} + \omega))) \qquad a < 2$$

$$\left(\frac{\lambda_{2009}}{\lambda_{2009}+\omega}(1\text{-}exp(-(a-2) * (\lambda_{2009} + \omega))) - \frac{\lambda_{2011}}{\lambda_{2011}+\omega}\right) * exp(-a*(\lambda_{2011} + \omega)) + \frac{\lambda_{2011}}{\lambda_{2011}+\omega} \qquad 2 \leq a < 4$$

$$\left(\frac{\lambda_{2007}}{\lambda_{2007}+\omega}(1\text{-}exp(-(\lambda_{2007} + \omega)*(a\text{-}4)) - \frac{\lambda_{2009}}{\lambda_{2009}+\omega}\right) * exp(-a*(\lambda_{2009} + \omega)) + \frac{\lambda_{2009}}{\lambda_{2009}+\omega}\right) - \frac{\lambda_{2011}}{\lambda_{2011}+\omega}\right) * exp(-a*(\lambda_{2011} + \omega)) + \frac{\lambda_{2011}}{\lambda_{2011}+\omega} \qquad 4 \leq a < 6$$

$$\left(\frac{\lambda_{pre2007}}{\lambda_{pre2007\ +}\,\omega}(1\text{-}exp(-(\lambda_{pre2007\ +}\,\omega)*(a\text{-}6)) - \frac{\lambda_{2007}}{\lambda_{2007}+\omega}\right) * exp(-a*(\lambda_{2007} + \omega)) + \frac{\lambda_{2007}}{\lambda_{2007}+\omega}\right) - \frac{\lambda_{2009}}{\lambda_{2009}+\omega}\right) * exp(-a*(\lambda_{2009} + \omega)) + \frac{\lambda_{2009}}{\lambda_{2009}+\omega}\right) - \frac{\lambda_{2011}}{\lambda_{2011}+\omega}\right) *$$

$$exp(-a*(\lambda_{2011} + \omega)) + \frac{\lambda_{2011}}{\lambda_{2011}+\omega} \qquad a \geq 6$$

$$Z_{2015}(a) = \frac{\lambda_{2013}}{\lambda_{2013}+\omega}(1 - exp(-a * (\lambda_{2013} + \omega))) \qquad a < 2$$

$$(\frac{\lambda_{2011}}{\lambda_{2011}+\omega}(1\text{-}exp (- (a - 2) * (\lambda_{2011} + \omega))) - \frac{\lambda_{2013}}{\lambda_{2013}+\omega}) * exp (-a*(\lambda_{2013} + \omega)) + \frac{\lambda_{2013}}{\lambda_{2013}+\omega} \qquad 2 < a \leq 4$$

$$((\frac{\lambda_{2009}}{\lambda_{2009}+\omega}(1\text{-}exp (- (\lambda_{2009} + \omega) *(a\text{-}4)) - \frac{\lambda_{2011}}{\lambda_{2011}+\omega}) * exp (-a*(\lambda_{2011} + \omega)) + \frac{\lambda_{2011}}{\lambda_{2011}+\omega})-\frac{\lambda_{2013}}{\lambda_{2013}+\omega})* \quad exp (-a*(\lambda_{2013} + \omega)) + \frac{\lambda_{2013}}{\lambda_{2013}+\omega} \qquad 4 < a \leq 6$$

$$(((\frac{\lambda_{2007}}{\lambda_{2007}+\omega}(1\text{-}exp (- (\lambda_{2007} + \omega) *(a\text{-}6)) \frac{\lambda_{2009}}{\lambda_{2009}+\omega})*exp (-a*(\lambda_{2009} + \omega)) + \frac{\lambda_{2009}}{\lambda_{2009}+\omega})-\frac{\lambda_{2011}}{\lambda_{2011}+\omega})* \quad exp (-a*(\lambda_{2011} + \omega))+\frac{\lambda_{2011}}{\lambda_{2011}+\omega} - \frac{\lambda_{2013}}{\lambda_{2013}+\omega}$$

$$exp (-2*(\lambda_{2013} + \omega))+\frac{\lambda_{2013}}{\lambda_{2013} + \omega} \qquad 6 < a \leq 8$$

$$((((\frac{\lambda_{pre2007}}{\lambda_{pre2007_{+}}\omega}(1\text{-}exp (- (\lambda_{pre2007_{+}}\omega) *(a\text{-}8)) - \frac{\lambda_{2007}}{\lambda_{2007}+\omega}) * exp (-a*(\lambda_{2007} + \omega)) + \frac{\lambda_{2007}}{\lambda_{2007}+\omega})-\frac{\lambda_{2009}}{\lambda_{2009}+\omega})*$$

$$exp (-a*(\lambda_{2009} + \omega)) + \frac{\lambda_{2009}}{\lambda_{2009}+\omega})-\frac{\lambda_{2011}}{\lambda_{2011}+\omega})* \quad exp (-a*(\lambda_{2011} + \omega)) + \frac{\lambda_{2011}}{\lambda_{2011}+\omega} - \frac{\lambda_{2013}}{\lambda_{2013}+\omega})* \quad exp (-a*(\lambda_{2013} + \omega)) + \frac{\lambda_{2013}}{\lambda_{2013}+\omega} \qquad a > 8$$

$$Z_{2017}(a) = \frac{\lambda_{2015}}{\lambda_{2015}+\omega}(1 - exp(-a * (\lambda_{2015} + \omega))) \qquad a < 2$$

$$(\frac{\lambda_{2013}}{\lambda_{2013}+\omega}(1\text{-}exp (- (a - 2) * (\lambda_{2013} + \omega))) - \frac{\lambda_{2015}}{\lambda_{2015}+\omega}) * exp (-a*(\lambda_{2015} + \omega)) + \frac{\lambda_{2015}}{\lambda_{2015}+\omega} \qquad 2 < a \leq 4$$

$$((\frac{\lambda_{2011}}{\lambda_{2011}+\omega}(1\text{-}exp (- (\lambda_{2011} + \omega) *(a\text{-}4)) - \frac{\lambda_{2013}}{\lambda_{2013}+\omega}) * exp (-a*(\lambda_{2013} + \omega)) + \frac{\lambda_{2013}}{\lambda_{2013}+\omega})-\frac{\lambda_{2015}}{\lambda_{2015}+\omega})* \quad exp (-a*(\lambda_{2015} + \omega)) + \frac{\lambda_{2015}}{\lambda_{2015}+\omega} \qquad 4 < a \leq 6$$

$$(((\frac{\lambda_{2009}}{\lambda_{2009}+\omega}(1\text{-}exp (- (\lambda_{2009} + \omega) *(a\text{-}6)) - \frac{\lambda_{2011}}{\lambda_{2011}+\omega})*exp (-a*(\lambda_{2011} + \omega)) + \frac{\lambda_{2011}}{\lambda_{2011}+\omega})-\frac{\lambda_{2013}}{\lambda_{2013}+\omega})* \quad exp (-a*(\lambda_{2013} + \omega))+\frac{\lambda_{2013}}{\lambda_{2013}+\omega} - \frac{\lambda_{2015}}{\lambda_{2015}+\omega})* exp (-2*(\lambda_{2015} + \omega))+\frac{\lambda_{2015}}{\lambda_{2015}+\omega} \qquad 6 < a \leq 8$$

$$((((\frac{\lambda_{2007}}{\lambda_{2007_{+}}\omega}(1\text{-}exp (- (\lambda_{2007_{+}}\omega) *(a\text{-}8)) - \frac{\lambda_{2009}}{\lambda_{2009}+\omega})*exp (-a*(\lambda_{2009} + \omega)) + \frac{\lambda_{2009}}{\lambda_{2009}+\omega} exp (-a*(\lambda_{2011} + \omega)) + \frac{\lambda_{2011}}{\lambda_{2011}+\omega} - \frac{\lambda_{2013}}{\lambda_{2013}+\omega})* \quad exp (-a*(\lambda_{2013} + \omega)) + \frac{\lambda_{2013}}{\lambda_{2013}+\omega} - \frac{\lambda_{2015}}{\lambda_{2015}+\omega}) * exp (-a*(\lambda_{2015} + \omega)) + \frac{\lambda_{2015}}{\lambda_{2015}+\omega} \qquad 8 < a \leq 10$$

$$(((((\frac{\lambda_{pre2007}}{\lambda_{pre2007_{+}}\omega}(1\text{-}exp (- (\lambda_{pre2007_{+}}\omega) *(a\text{-}10)) - \frac{\lambda_{2007}}{\lambda_{2007}+\omega}) * exp (-a*(\lambda_{2007} + \omega)) + \frac{\lambda_{2007}}{\lambda_{2007}+\omega})-\frac{\lambda_{2009}}{\lambda_{2009}+\omega})*$$

$$exp (-a*(\lambda_{2009} + \omega)) + \frac{\lambda_{2009}}{\lambda_{2009}+\omega})-\frac{\lambda_{2011}}{\lambda_{2011}+\omega})* \quad exp (-a*(\lambda_{2011} + \omega)) + \frac{\lambda_{2011}}{\lambda_{2011}+\omega} - \frac{\lambda_{2013}}{\lambda_{2013}+\omega})* \quad exp (-a*(\lambda_{2013} + \omega)) +$$

$$\frac{\lambda_{2013}}{\lambda_{2013}+\omega} - \frac{\lambda_{2015}}{\lambda_{2015}+\omega})* exp (-2*(\lambda_{2015} + \omega))+\frac{\lambda_{2015}}{\lambda_{2015}+\omega} \qquad a>10$$

$$Z_{2019}(a) = \frac{\lambda_{2017}}{\lambda_{2017}+\omega}\left(1 - exp\left(-a * (\lambda_{2017} + \omega)\right)\right) \qquad a < 2$$

$$\left(\frac{\lambda_{2015}}{\lambda_{2015}+\omega}\left(1\text{-}exp\left(- (a-2) * (\lambda_{2015} + \omega)\right)\right) - \frac{\lambda_{2017}}{\lambda_{2017}+\omega}\right) * exp\left(-a*(\lambda_{2017} + \omega)\right) + \frac{\lambda_{2017}}{\lambda_{2017}+\omega} \qquad 2 < a \leq 4$$

$$\left(\left(\frac{\lambda_{2013}}{\lambda_{2013}+\omega}\left(1\text{-}exp\left(- (\lambda_{2013} + \omega) *(a-4)\right) - \frac{\lambda_{2015}}{\lambda_{2015}+\omega}\right) * exp\left(-a*(\lambda_{2015} + \omega)\right) + \frac{\lambda_{2015}}{\lambda_{2015}+\omega}\right) - \frac{\lambda_{2017}}{\lambda_{2017}+\omega}\right) *$$

$$exp\left(-a*(\lambda_{2017} + \omega)\right) + \frac{\lambda_{2017}}{\lambda_{2017} + \omega} \qquad 4 < a \leq 6$$

$$\left(\left(\left(\frac{\lambda_{2011}}{\lambda_{2011}+\omega}\left(1\text{-}exp\left(- (\lambda_{2011} + \omega) *(a-6)\right) \frac{\lambda_{2013}}{\lambda_{2013}+\omega}\right) * exp\left(-a*(\lambda_{2013} + \omega)\right) + \frac{\lambda_{2013}}{\lambda_{2013}+\omega}\right) - \frac{\lambda_{2015}}{\lambda_{2015}+\omega}\right) *$$

$$exp\left(-a*(\lambda_{2015} + \omega)\right) + \frac{\lambda_{2015}}{\lambda_{2015} + \omega} - \frac{\lambda_{2017}}{\lambda_{2017} + \omega}\right) * exp\left(-2*(\lambda_{2017} + \omega)\right) + \frac{\lambda_{2017}}{\lambda_{2017} + \omega} \qquad 6 < a \leq 8$$

$$\left(\left(\left(\left(\frac{\lambda_{2009}}{\lambda_{2009_+}\omega}\left(1\text{-}exp\left(- (\lambda_{2009_+} \omega) *(a-8)\right) - \frac{\lambda_{2011}}{\lambda_{2011}+\omega}\right) * exp\left(-a*(\lambda_{2011} + \omega)\right) + \frac{\lambda_{2011}}{\lambda_{2011}+\omega}\right) - \frac{\lambda_{2013}}{\lambda_{2013}+\omega}\right) *$$

$$exp\left(-a*(\lambda_{2013} + \omega)\right) + \frac{\lambda_{2013}}{\lambda_{2013}+\omega} - \frac{\lambda_{2015}}{\lambda_{2015}+\omega}\right) * exp\left(-a*(\lambda_{2015} + \omega)\right) + \frac{\lambda_{2015}}{\lambda_{2015}+\omega} - \frac{\lambda_{2017}}{\lambda_{2017}+\omega}\right) * exp\left(-a*(\lambda_{2017} + \omega)\right) + \frac{\lambda_{2017}}{\lambda_{2017}+\omega} \qquad 8 < a \leq 10$$

$$\left(\left(\left(\left(\left(\frac{\lambda_{2007}}{\lambda_{2007_+}\omega}\left(1\text{-}exp\left(- (\lambda_{2007_+} \omega) *(a-10)\right) - \frac{\lambda_{2009}}{\lambda_{2009}+\omega}\right) * exp\left(-a*(\lambda_{2009} + \omega)\right) + \frac{\lambda_{2009}}{\lambda_{2009}+\omega}\right) - \frac{\lambda_{2011}}{\lambda_{2011}+\omega}\right) * exp\left(-a*(\lambda_{2011} + \omega)\right) + \frac{\lambda_{2011}}{\lambda_{2011}+\omega} - \frac{\lambda_{2013}}{\lambda_{2013}+\omega}\right) *$$

$$exp\left(-a*(\lambda_{2013} + \omega)\right) + \frac{\lambda_{2013}}{\lambda_{2013}+\omega} - \frac{\lambda_{2015}}{\lambda_{2015}+\omega}\right) * exp\left(-a*(\lambda_{2015} + \omega)\right) + \frac{\lambda_{2015}}{\lambda_{2015}+\omega} - \frac{\lambda_{2017}}{\lambda_{2017}+\omega}\right) * exp\left(-a*(\lambda_{2017} + \omega)\right) + \frac{\lambda_{2017}}{\lambda_{2017}+\omega} \qquad 10 < a \leq 12$$

$$\left(\left(\left(\left(\left(\left(\frac{\lambda_{pre2007}}{\lambda_{pre2007_+}\omega}\left(1\text{-}exp\left(- (\lambda_{pre2007_+} \omega) *(a-12)\right) - - \frac{\lambda_{2007}}{\lambda_{2007}+\omega}\right) * exp\left(-a*(\lambda_{2007} + \omega)\right) + \frac{\lambda_{2007}}{\lambda_{2007}+\omega}\right) - \frac{\lambda_{2009}}{\lambda_{2009}+\omega}\right) * exp\left(-a*(\lambda_{2009} + \omega)\right) + \frac{\lambda_{2009}}{\lambda_{2009}+\omega}\right) - \frac{\lambda_{2011}}{\lambda_{2011}+\omega}\right) *$$

$$exp\left(-a*(\lambda_{2011} + \omega)\right) + \frac{\lambda_{2011}}{\lambda_{2011}+\omega} - \frac{\lambda_{2013}}{\lambda_{2013}+\omega}\right) * exp\left(-a*(\lambda_{2013} + \omega)\right) + \frac{\lambda_{2013}}{\lambda_{2013}+\omega} - \frac{\lambda_{2015}}{\lambda_{2015}+\omega}\right) * exp\left(-2*(\lambda_{2015} + \omega)\right) + \frac{\lambda_{2015}}{\lambda_{2015}+\omega} -$$

$$\frac{\lambda_{2017}}{\lambda_{2017} + \omega}\right) * exp\left(-a*(\lambda_{2017} + \omega) + \frac{\lambda_{2017}}{\lambda_{2017} + \omega}\right.$$

$$Z_{2021}(a) = \frac{\lambda_{2019}}{\lambda_{2019}+\omega}(1 - exp(-a * (\lambda_{2019} + \omega))) \qquad a < 2$$

$$(\frac{\lambda_{2017}}{\lambda_{2017}+\omega}(1\text{-}exp (- (a - 2) * (\lambda_{2017} + \omega))) - \frac{\lambda_{2019}}{\lambda_{2019}+\omega}) * exp (-a*(\lambda_{2019} + \omega)) + \frac{\lambda_{2019}}{\lambda_{2019}+\omega} \qquad 2 < a \leq 4$$

$$((\frac{\lambda_{2015}}{\lambda_{2015}+\omega}(1\text{-}exp (- (\lambda_{2015} + \omega) *(a-4)) - \frac{\lambda_{2017}}{\lambda_{2017}+\omega}) * exp (-a*(\lambda_{2017} + \omega)) + \frac{\lambda_{2017}}{\lambda_{2017}+\omega})-\frac{\lambda_{2019}}{\lambda_{2019}+\omega})* exp (-a*(\lambda_{2019} + \omega)) + \frac{\lambda_{2019}}{\lambda_{2019}+\omega} \qquad 4 < a \leq 6$$

$$(((\frac{\lambda_{2013}}{\lambda_{2013}+\omega}(1\text{-}exp (- (\lambda_{2013} + \omega) *(a-6)) \frac{\lambda_{2015}}{\lambda_{2015}+\omega})*exp (-a*(\lambda_{2015} + \omega)) + \frac{\lambda_{2015}}{\lambda_{2015}+\omega})-\frac{\lambda_{2017}}{\lambda_{2017}+\omega})* exp (-a*(\lambda_{2017} + \omega))+\frac{\lambda_{2019}}{\lambda_{2019}+\omega} -$$

$$exp (-2*(\lambda_{2019} + \omega))+\frac{\lambda_{2019}}{\lambda_{2019} + \omega} \qquad 6 < a \leq 8$$

$$((((\frac{\lambda_{2011}}{\lambda_{2011_+}\omega}(1\text{-}exp (- (\lambda_{2011\ +}\omega) *(a-8)) \frac{\lambda_{2013}}{\lambda_{2013}+\omega})*exp (-a*(\lambda_{2013} + \omega)) + \frac{\lambda_{2013}}{\lambda_{2013}+\omega})-\frac{\lambda_{2015}}{\lambda_{2015}+\omega})* exp (-a*(\lambda_{2015} + \omega)) + \frac{\lambda_{2015}}{\lambda_{2015}+\omega} -$$

$$\frac{\lambda_{2017}}{\lambda_{2017}+\omega})* exp (-a*(\lambda_{2017} + \omega)) + \frac{\lambda_{2017}}{\lambda_{2017}+\omega} - \frac{\lambda_{2019}}{\lambda_{2019}+\omega}) * exp (-a*(\lambda_{2019} + \omega)) + \frac{\lambda_{2019}}{\lambda_{2019}+\omega} \qquad 8 < a \leq 10$$

$$((((\frac{\lambda_{2009}}{\lambda_{2009_+}\omega}(1\text{-}exp (- (\lambda_{2009\ +}\omega) *(a-10)) \frac{\lambda_{2011}}{\lambda_{2011}+\omega})*exp (-a*(\lambda_{2011} + \omega)) + \frac{\lambda_{2011}}{\lambda_{2011}+\omega})-\frac{\lambda_{2013}}{\lambda_{2013}+\omega})* exp (-a*(\lambda_{2013} + \omega)) + \frac{\lambda_{2013}}{\lambda_{2013}+\omega} - \frac{\lambda_{2015}}{\lambda_{2015}+\omega})*$$

$$exp (-a*(\lambda_{2015} + \omega)) + \frac{\lambda_{2015}}{\lambda_{2015}+\omega} - \frac{\lambda_{2017}}{\lambda_{2017}+\omega})* exp (-a*(\lambda_{2017} + \omega))+\frac{\lambda_{2017}}{\lambda_{2017}+\omega} - - \frac{\lambda_{2019}}{\lambda_{2019}+\omega}) * exp (-a*(\lambda_{2019} + \omega)) + \frac{\lambda_{2019}}{\lambda_{2019}+\omega} \qquad 10 < a \leq 12$$

$$((((\frac{\lambda_{2007}}{\lambda_{2007_+}\omega}(1\text{-}exp (- (\lambda_{2007\ +}\omega) *(a-12))- \frac{\lambda_{2009}}{\lambda_{2009}+\omega})*exp (-a*(\lambda_{2009} + \omega)) + \frac{\lambda_{2009}}{\lambda_{2009}+\omega})-\frac{\lambda_{2011}}{\lambda_{2011}+\omega})* exp (-a*(\lambda_{2011} + \omega)) + \frac{\lambda_{2011}}{\lambda_{2011}+\omega} - \frac{\lambda_{2013}}{\lambda_{2013}+\omega})*$$

$$exp (-a*(\lambda_{2013} + \omega)) + \frac{\lambda_{2013}}{\lambda_{2013}+\omega} - \frac{\lambda_{2015}}{\lambda_{2015}+\omega})* exp (-a*(\lambda_{2015} + \omega))+\frac{\lambda_{2015}}{\lambda_{2015}+\omega} - \frac{\lambda_{2017}}{\lambda_{2017}+\omega}) * exp (-a*(\lambda_{2017} + \omega)) + \frac{\lambda_{2017}}{\lambda_{2017}+\omega} - \frac{\lambda_{2019}}{\lambda_{2019}+\omega}) * exp (-a*(\lambda_{2019} + \omega)) + \frac{\lambda_{2019}}{\lambda_{2019}+\omega}$$

$$12 < a \leq 14$$

$$(((((\frac{\lambda_{pre2007}}{\lambda_{pre2007_+}\omega}(1\text{-}exp (- (\lambda_{pre2007\ +}\omega) *(a-14))- - \frac{\lambda_{2007}}{\lambda_{2007}+\omega})*exp (-a*(\lambda_{2007} + \omega)) + \frac{\lambda_{2007}}{\lambda_{2007}+\omega})-\frac{\lambda_{2009}}{\lambda_{2009}+\omega})*exp (-a*(\lambda_{2009} + \omega)) + \frac{\lambda_{2009}}{\lambda_{2009}+\omega})-\frac{\lambda_{2011}}{\lambda_{2011}+\omega})*$$

$$exp (-a*(\lambda_{2011} + \omega)) + \frac{\lambda_{2011}}{\lambda_{2011}+\omega} - \frac{\lambda_{2013}}{\lambda_{2013}+\omega})* exp (-a*(\lambda_{2013} + \omega)) + \frac{\lambda_{2013}}{\lambda_{2013}+\omega} - \frac{\lambda_{2015}}{\lambda_{2015}+\omega})* exp (-2*(\lambda_{2015} + \omega))+\frac{\lambda_{2015}}{\lambda_{2015}+\omega} -$$

$$\frac{\lambda_{2017}}{\lambda_{2017}+\omega}) * exp (-a*(\lambda_{2017} + \omega) + \frac{\lambda_{2017}}{\lambda_{2017}+\omega} \frac{\lambda_{2019}}{\lambda_{2019}+\omega}) * exp (-a*(\lambda_{2019} + \omega)) + \frac{\lambda_{2019}}{\lambda_{2019}+\omega} \qquad a > 14$$