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Hilary Kelly Date

Measles, Mumps, and Rubella Seroprevalence Among HIV Negative Women of Childbearing Age at High Risk for HIV in Zambia

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By

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The Pennsylvania State University – University Park
2013

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An abstract of
A thesis submitted to the Faculty of the
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Master of Public Health in Epidemiology
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Abstract

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By Hilary Kelly

Background: It is estimated that 2 to 3 million deaths every year are currently averted by immunization and an estimated 1.5 million additional deaths could be avoided if global vaccination coverage continues to increase (1). Measles, mumps, and rubella are vaccine-preventable diseases that can be effectively controlled by immunization. Unfortunately, Zambia's current immunization schedule only contains measles and the country has relatively low coverage of the vaccine.

Methods: This study aims to evaluate the immune response to measles, mumps, and rubella in a population of women who are HIV-negative but are at high risk for HIV-infection in Lusaka and Ndola, Zambia. Additionally, rubella antibody titers will be evaluated at time points pre- and post-vaccination. A predictive model was created to determine which covariates impacted the odds of being measles IgG negative. Paired t-tests were performed to evaluate the difference between pre- and post-vaccine mean titers for rubella.

Results: Of the three viruses, our population had the highest negativity for measles IgG. Our analysis showed no statistical significance of any covariates in the predictive model evaluating the odds of being measles IgG negative. The mean rubella titer pre- vs. post-vaccine were statistically significantly different.

Conclusions: Overall, these results identify gaps in MMR coverage in Zambia. Even though this study had no statistically significant predictors of sero-negativity for measles, multiple studies have shown that covariates such as age and BMI are typically predictors of sero-status. The mean increase in pre- vs post-vaccine rubella titers indicates that boosters are important for maintaining immunity.

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Table of Contents

Chapter I: Literature Review

Immunization provides protection from illness, disability, and death caused by vaccine-preventable diseases including measles, mumps, and rubella and is considered one of the most cost-effective and powerful means of preventing deaths and improving lives (2). It is estimated that 2 to 3 million deaths every year are currently averted by immunization and an estimated 1.5 million additional deaths could be avoided if global vaccination coverage continues to increase (1). To help increase global vaccination coverage for vaccine preventable diseases and possibly eradicate several infectious diseases in the world's most disease laden countries, the World Health Organization (WHO) implemented the Expanded Programme of Immunization (EPI) in 1974 (3). Unfortunately, the past year saw no significant changes to the 86% global coverage (1). Currently in Zambia, EPI is experiencing low uptake (<50%) of measles vaccine second dose (4).

After the eradication of Polio, one of the most desirable goals in public health is the elimination of measles with rubella and eventually mumps (5). In May 2012, all WHO Member States adopted the Global Vaccine Action Plan (GVAP). The mission of GVAP is to improve health by extending the full benefits of vaccinations to everyone no matter where they were born, who they are, or where they live. To achieve this mission, five goals and six strategic objectives were outlined. The second goal, to meet global and regional elimination targets, consists of three indicators: 1. Maternal and neonatal tetanus elimination, 2. Measles elimination, and 3. Rubella/Congenital Rubella Syndrome (CRS) elimination. The African Region set a goal to eliminate measles by 2020, but between 2010 and 2016 African Region and Eastern Mediterranean Region

failed to reach 80% coverage of vaccine. In 2016 the African Region had a high incidence of measles. These outbreaks are mainly the result of incomplete coverage of the first dose measles containing vaccine (MCV1). The African Region does not yet have a target for achieving Rubella and CRS elimination, but countries who have implemented measles elimination strategies are hoping to introduce rubella vaccine to their routine vaccination schedules (6).

The World Health Assembly (WHA) endorsed the GVAP with the objective of eliminating measles in 4 out of 6 WHO regions by 2015 and in 5 regions by 2020. Measles elimination is defined as the absence of endemic measles transmission in a region or other defined geographic area for ≥12 months in the presence of a well performing surveillance system (7). Unfortunately, the 2015 elimination goals were not met, and a great effort is needed if the 2020 goal of elimination in 5 out of 6 regions is to be accomplished (6). This goal will require high and homogenous population immunity due to the high infectivity of the virus making the herd protection threshold (89% - 94% immunity) the highest of all vaccine preventable diseases (8). Since no non-human measles reservoir exists, the 2020 elimination goal is possible, but accurate diagnosis and effective vaccines are vital.

By the end of 2016, the first dose of measles vaccine had 85% worldwide coverage and 164 countries began including a second dose as part of their routine immunization plan. Currently, there is only 64% coverage of the second dose of the measles vaccine. By the end of 2016, 121 countries introduced nationwide mumps vaccinations and 152 countries introduced nationwide rubella vaccinations. The global coverage of rubella vaccination was estimated at a mere 25% (1).

Measles infection, caused by measles virus, is one of the most contagious diseases of humans. Outbreaks can occur in populations in which less than 10% of persons are susceptible. Prior to the creation of a vaccine in 1963, a major epidemic occurred approximately every 2 to 3 years with an estimated 30 million cases and more than 2 million deaths occurring globally each year. More than 95% of individuals have been infected with measles virus by age 15, but this varies depending on biological and epidemiological factors, mainly population immunity and birth rate. In low income countries an increase in transmission due to low population immunity, high birth rates, and high population density can be seen. Additionally, severity of measles can vary depending on several host factors such as malnutrition and being immunocompromised (8). In 2010, an estimated 8% of vaccine preventable deaths worldwide was attributed to measles (9).

As coverage of measles vaccination increases, the average age of infection begins to increase. These older groups remain susceptible to infection because they have not been vaccinated or exposed to wild-type measles virus. Without vaccine efforts, measles virus introduction can result in an outbreak in these older susceptible populations highlighting the immunity gaps. The estimated coverage of the first dose of measles vaccination in the African region was 74% with the estimated coverage of the second dose being much lower. Evidence indicates that a single dose of measles vaccine which results in seroconversion will afford lifelong protection in most, but levels of anti-measles-virus antibodies may diminish over time making it important to receive both doses of vaccination (8).

Mumps is a viral infection of humans that primarily affects the salivary glands. While this virus is mostly a mild childhood disease, infection in adults commonly leads

to complications such as meningitis and orchitis and rarely leads to encephalitis and permanent neurological sequelae. Without immunization the annual incidence of infection ranges from 100 – 1,000 cases per 100,000 population with epidemic peaks occurring every 2-5 years (10). Natural infection is thought to confer lifelong immunity, but outbreaks have been reported even with appropriate immunizations. This may suggest waning immunity over time (9). Analogous to measles, as vaccination coverage increases the average age of mumps infection also increases. Mumps is generally a mild self-limiting disease with a very low case-fatality rate (1/10,000 cases). Getting mumps during the first 12 weeks of pregnancy is associated with 25% incidence of spontaneous abortions and when the pregnancy goes to term, fetal malformations have not been found following infection. Even though the WHO recommends routine vaccination for mumps, based on mortality and disease burden, measles control and prevention of CRS are considered higher priorities in countries with well established, effective, vaccination programs (10).

Rubella is an acute viral disease that is generally mild. It is of public health importance due to the teratogenic potential of the virus. Rubella infection occurring just before conception or early in pregnancy may result in miscarriage, fetal death, or CRS. Rates of susceptibility to rubella vary dramatically among and within countries depending on many epidemiological and socioeconomic differences. Prior to vaccine introduction, incidence of CRS during epidemics ranged from 0.8-4/1,000 live births. The incidence of rubella and CRS has been drastically reduced in countries that have successfully implemented vaccination strategies. As described previously for measles and mumps, as vaccination coverage increases, the average age of rubella infection can

shift up due to these groups remaining susceptible to infection due to lack of exposure to wild-type virus or vaccination (11).

Barring CRS, rubella is a mild and self-limiting illness. Multiple fetal defects may result from rubella infection just before conception and during the first 8-10 weeks of gestation in up to 90% of cases. Infection may also result in fetal wastage. The risk of complication declines after 16th week of pregnancy and fetal defects associated with maternal rarely occur. When a baby develops CRS, serious ophthalmic, auditory, cardiac, or craniofacial defects may occur (11). CRS can be prevented by immunizing females prior to reaching child-bearing age, testing pregnant women for immunity and providing counseling regarding avoidance of exposure to rubella for those without immunity (9).

Zambia is a landlocked country in the center of southern Africa that is considered to be fairly stable due to the lack of war and upheaval. The country has one of the world's fastest growing populations and is currently projected to triple its population by 2050. This growth does not correlate to an overall lifestyle improvement. Two-thirds of Zambians still live in poverty (12). Currently, in Zambia, out of measles, mumps, and rubella, only measles is a part of the vaccination schedule and it is given at 9 and 18 months (See Appendix A) (13). Introducing MMR is valuable to our study population because mumps and rubella are typically acquired by adults, especially during pregnancy. Contracting either of these viruses during the early stages of pregnancy can be catastrophic to a fetus. Additionally, rubella is often under diagnosed because it is difficult to see the rash on black on skin (14). Furthermore, 6-16% of women nationwide of reproductive age in Zambia are susceptible to rubella virus. For a virus as infectious as rubella, this rate is high and provides an increased risk of CRS (15). In 2010

the country suffered a major measles outbreak with 15,754 cases and mortality of 160. This outbreak corresponded with a decrease in measles vaccination coverage (90% in 2009 and 80% in 2010). From 2003 to 2008, Zambia experience an increase in cases of measles and rubella and a decrease in cases of mumps (16).

Between 2010 and 2015 the country experienced measles outbreaks even though measles first dose vaccination coverage was 84%. These outbreaks could be contributed to the suboptimal second dose vaccination coverage at a mere 35%. The current dropout rate between measles dose 1 and measles dose 2 is 58%. A significant decline in measles sero-positivity was recorded between 2011 and 2015. From the measles negative samples obtained, 28.3% had rubella sero-positivity. This data supports validating the introduction of measles-rubella (MR) vaccine into the national immunization strategy (4).

The present study evaluates the immune response to measles, mumps, and rubella as well as sero-positivity rates and antibody titers to rubella. Rubella antibody titers will be evaluated at time points before and after vaccination in a population of women who are HIV-negative but are at high risk for HIV-infection in Lusaka and Ndola, Zambia. Female participants who met the eligibility criteria and were randomized to one of two study groups received vaccinations at two time points. MMR and Tdap-IPV vaccines were being used as a proxy for an HIV vaccine in a Simulated Vaccine Efficacy Trial (SiVET). These two licensed vaccines were used because they would provide direct benefit to the study participants due to the limited to no vaccination coverage for measles, mumps, and rubella. The participants were then followed for a 12-month period. As stated previously, vaccines are the most cost-effective intervention known to prevent death and disease worldwide. Recent measles

and mumps outbreaks make the importance of utilizing the MMR vaccine even more apparent. Additionally, with the surprising resurgence of measles, it is critical that a better understanding of the immune responses to MMR be obtained. Finally, this study obtains data that fills a gap in understanding of MMR immune response in African populations and adults (14).

Chapter II: Manuscript

Abstract

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Background: It is estimated that 2 to 3 million deaths every year are currently averted by immunization and an estimated 1.5 million additional deaths could be avoided if global vaccination coverage continues to increase (1). Measles, mumps, and rubella are vaccine-preventable diseases that can be effectively controlled by immunization. Unfortunately, Zambia's current immunization schedule only contains measles and the country has relatively low coverage of the vaccine.

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Introduction

Without global immunizations, an estimated 2 to 3 million deaths per year would occur due to vaccine-preventable diseases (1). In an attempt to increase global vaccination coverage, the World Health Organization (WHO) implemented the Expanded Programme of Immunization (EPI) in 1974 (3). Even with this increased effort, the global coverage has stalled at 86% (1). Measles, mumps, and rubella are vaccine-preventable diseases that should no longer be contributing to deaths worldwide due to effective vaccinations, but countries like Zambia experience low-uptake of these vaccines. Currently, Zambia has less than 50% uptake of the second dose of measles vaccine and they do not provide mumps and rubella vaccination as part of the routine immunization schedule (4, 13).

In May 2012, all WHO Member States adopted the Global Vaccine Action Plan (GVAP) whose mission is to improve health by extending the full benefits of vaccinations to everyone no matter where they were born, who they are, or where they live (6). One of the goals set to assist in meeting global and regional elimination targets consists of indicators for measles elimination and rubella/congenital rubella syndrome (CRS) elimination. The African region set a goal to eliminate measles by 2020, but the region is currently failing to reach 80% coverage. In addition, the region had a high incidence of measles in 2016. At this time, the region does not have a target for achieving Rubella and CRS elimination (6).

Measles elimination is defined as the absence of endemic measles transmission in a region or other defined geographic area for ≥12 months in the presence of a well performing surveillance system (7). Reaching elimination will require very high and homogenous vaccination coverage (89% - 94%) amongst populations due to the

extremely high infectivity of the virus (8). Measles infection is caused by the measles virus. By age 15, more than 95% of individuals have typically been infected with measles virus, but this varies depending on biological and epidemiologic factors (8). In 2010, an estimated 8% of vaccine preventable deaths worldwide was attributed to measles (9).

Mumps is a viral infection that mostly causes a mild childhood disease. Without immunization the annual incidence of infection ranges form 100 – 1,000 cases per year (10). Infection with the virus is thought to confer lifelong immunity, but there have been reported outbreaks in areas with appropriate immunizations suggesting waning immunity over time (9). Getting mumps during the first 12 weeks of pregnancy is associated with 25% incidence of spontaneous abortions. Even though the WHO recommends routine vaccination for mumps, based on mortality and disease burden, measles control and prevention of CRS are considered higher priorities in countries with well established, effective, vaccination programs (10).

Rubella is a generally mild acute viral disease that is of public health important due to the teratogenic potential of the virus. If a rubella infection occurs just before conception or early in pregnancy, the pregnancy may result in miscarriage, fetal death, or CRS. The incidence of rubella and CRS has been drastically reduced in countries that have successfully implemented vaccination strategies. Barring CRS, rubella is a mild and self-limiting illness. When a baby develops CRS, serious ophthalmic, auditory, cardiac, or craniofacial defects may occur (11). CRS can be prevented by immunizing females prior to reaching child-bearing age, testing pregnant women for immunity and providing counseling regarding avoidance of exposure to rubella for those without immunity (9).

As coverage of measles, mumps, or rubella vaccination increases, the average age of infection increases due to the susceptibility of older groups to infection because of a lack of either vaccination or exposure to wild-type virus. Without an increase in vaccination efforts, outbreaks for these viruses will occur in older populations highlighting these gaps in immunity (8, 10, 11, 17).

Zambia is in the center of southern Africa. The country has one of the world's fastest growing populations and is projected to triple in population by 2050. Unfortunately, this growth does not equate to an improvement in lifestyle. Two-thirds of Zambians still live in poverty (12). As stated previously, the current vaccination schedule includes measles vaccines given at 9 and 18 months, but it excludes mumps and rubella (13). Introducing MMR is valuable to our study population because mumps and rubella are typically acquired by adults, especially during pregnancy. Contracting either of these viruses during the early staged of pregnancy cane be catastrophic to a fetus. Additionally, rubella is often under diagnosed because it is difficult to see the rash on black on skin (14). Furthermore, 6-16% of women of reproductive age in Zambia are susceptible to rubella virus. For a virus as infectious as rubella, this rate is high and provides an increased risk of CRS (15).

Even with measles first dose vaccination coverage at 84%, the country still experienced a major outbreak in 2010 with 15,754 cases and mortality of 160 (4). This outbreak corresponded with a decrease in measles vaccination coverage (90% in 2009 and 80% in 2010). From 2003 to 2008, Zambia experience an increase in cases of measles and rubella and a decrease in cases of mumps (16). These outbreaks could be contributed to the suboptimal second dose vaccination coverage at a mere 35%. The current dropout rate between measles dose 1 and measles dose 2 is 58%. A significant

decline in measles sero-positivity was recorded between 2011 and 2015. From the measles negative samples obtained, 28.3% had rubella sero-positivity. This data supports validating the introduction of measles-rubella (MR) vaccine into the national immunization strategy (4).

The present study evaluates the immune response to measles, mumps, and rubella as well as sero-positivity rates and antibody titers to rubella in a population of women who are HIV-negative but are at high risk for HIV-infection in Lusaka and Ndola, Zambia. Rubella antibody titers will also be evaluated at time points before and after vaccination. Female participants who met the eligibility criteria and were randomized to one of two study groups received vaccinations at two time points. MMR and Tdap-IPV vaccines were being used as a proxy for an HIV vaccine in a Simulated Vaccine Efficacy Trial (SiVET). These two licensed vaccines were used because they would provide direct benefit to the study participants due to the limited to no vaccination coverage for measles, mumps, and rubella. The participants were then followed for a 12-month period. Recent measles and mumps outbreaks make the importance of utilizing the MMR vaccine even more apparent. Additionally, with the surprising resurgence of measles, it is critical that a better understanding of the immune responses to MMR be obtained. Finally, this study obtains data that fills a gap in understanding of MMR immune response in African populations and adults (14).

Methods

From July 2015 to June 2016, Rwanda Zambia HIV Research Group (RZHRG), Zambia HIV Research Group (ZEHRP), and Emory University, with sponsorship from International AIDS Vaccine Initiative (IAVI), began a Simulated Vaccine Efficacy Trial (SiVET) using MMR and Tdap-IPV vaccines in healthy HIV negative women at high risk of HIV infection in Lusaka and Ndola, Zambia. The study was a double-blinded, randomized trial with the primary objective of determining the feasibility of conducting an HIV vaccine efficacy trial in HIV negative women at risk of HIV. A secondary objective of the trial was to evaluate immune response to measles, mumps and rubella before and after vaccination.

The participating women were randomly assigned to two arms, Group A (received MMR and then Tdap-IPV three months later) and Group B (received Tdap-IPV and then MMR three months later). Participants were screened and enrolled prior to vaccination and were then followed for 12 months after enrollment. Study participants provided informed consent and the study was approved by University of Zambia Biomedical Research Ethics Committee and the Emory University IRB. These analyses were done to evaluate the SiVET study's secondary objective. More specifically, these analyses will assess baseline sero-prevalence to measles, mumps, and rubella, predictors for measles sero-negativity at baseline, and change in rubella titer at time points before and after vaccination.

Study Population

The study population was drawn from a cohort of healthy female adults aged 18 to 40 years currently enrolled in the ZEHRP study, "A prospective cohort study to

determine the incidence and risk factors of HIV amongst female sex workers (FSW) and single, sexually active [mothers] (SM) … in Rwanda and Zambia". The FSW-SM cohort study is a separate IRB-approved research study. Women enrolled in the FSW cohort were invited from known hot spots in the local community. Women enrolled in the SM cohort were referred to the research clinic from Government of the Republic of Zambia infant vaccination clinics. Data on HIV status, sexually transmitted infections (STI), demographic factors, sexual behavior, and sexual reproductive history were collected quarterly on members of the cohort.

Inclusion criteria for the SiVET trial population simulated those that would be expected of a real HIV vaccine trial and were as follows: are at high-risk of HIV, not pregnant or intending to become pregnant for the duration of this trial, live in Lusaka or Ndola, are available for the duration of the trial, willing to undergo HIV testing, willing to use injectables, implant, or intrauterine device (IUD) contraceptive methods, and must understand the study and provide written informed consent. The women in the study are considered at high risk of HIV due to their occupation as female sex workers or due to recent pregnancy while not being married or cohabiting. Participants were excluded from the trial if they had a confirmed HIV-1/2 infection, were pregnant, had a chronic disease, recently received an investigational blood product or vaccine, or had a previous severe local or systemic reaction to vaccination or a history of severe allergic reactions. 159 participants were enrolled across two clinic sites in Lusaka and Ndola.

Even though Lusaka (population 1,747,152) and Ndola (451,246) are two of the largest cities in Zambia, they are very different. Lusaka, Zambia's capital, is a very densely populated city that spans 360 km^2 and is one of central Africa's fastest growing cities with an annual rate of population growth of 4.9% from 2000 to 2010. In 2010,

Lusaka had the highest population density in the country at 4,853.2 people per km^2. Ndola, the third largest city in Zambia is located in the Copperbelt Province. Ndola district contains 22.9% of the entire province's population with a population density of 409.1 per km^2 and total size of 1,103 km^2. Lusaka and Ndola are both urban areas, but Ndola is much closer to rural areas of the country (18).

Study Design

Participants involved in the FSW-SM study were pre-screened prior to or on the day of SiVET screening/enrollment to ensure that they met the inclusion criteria. If the inclusion criteria was satisfied an initial screening would be performed. Within three days of the screening visit, enrollment of eligible participants will be conducted. At the enrollment visit (V1), participants were randomized into either Group A (n = 79) or Group B (n=80) (See Figure 1).

Also, at enrollment and after randomization, participants in Group A received MMR and participants in Group B received Tdap-IPV via intramuscular injection. At month 3 (V7), Group A received Tdap-IPV and Group B received MMR vaccines. The MMR vaccine used was a trivalent live attenuated virus vaccine for measles, mumps, and rubella (TRIMOVAX). The Tdap-IPV vaccine used was a combined tetanus toxoid, reduced diphtheria toxoid and acellular pertussis vaccine adsorbed, combined with inactivated poliomyelitis vaccine (Adacel Quadra).

During each vaccination visit HIV-prevention counseling was provided. Following each vaccination certain procedures occurred at the time period indicated in the Schedule of Procedures (See Appendix B). Additional follow-up visits were also performed in compliance with the Schedule of Procedures.

Data collected during this trial was provided from the in-country lab at ZEHRP Lusaka that performed all of the antibody testing. VIDAS Measles IgG, VIDAS Mumps IgG, and VIDAS RUB IgG II assays by bioMérieux were used to test for immune response in participants. All three assays combine a two-step enzyme immunoassay sandwich method with a final enzyme-linked fluorescence assay (ELFA). Measles, mumps, and rubella had a relative fluorescence value (FRV) calculated by subtracting the background reading from the final result generated. Measles and mumps assays had test values generated by forming a ratio from the RFV of the sample to that of a standard. The rubella assay provided concentrations expressed in IU/ml, the WHO standard, calculated by using calibration curves. The measles and mumps assays provided qualitative results and the rubella assay provided quantitative results. All three assays listed interference with certain sera containing antibodies directed against reagent components as possible limitation. Hence, assay results should be interpreted while taking into account participant's history.

Variable Specification

All participants were asked a baseline questionnaire that pertains to socio demographic information, sexual history (both lifetime and recent), reproductive and contraceptive follow-up, and physical exam data. Clinical and demographic data was collected on hard-copy questionnaires and was then entered into either an offline Access database or REDCap, an online data entry system. All laboratory data was recorded in excel.

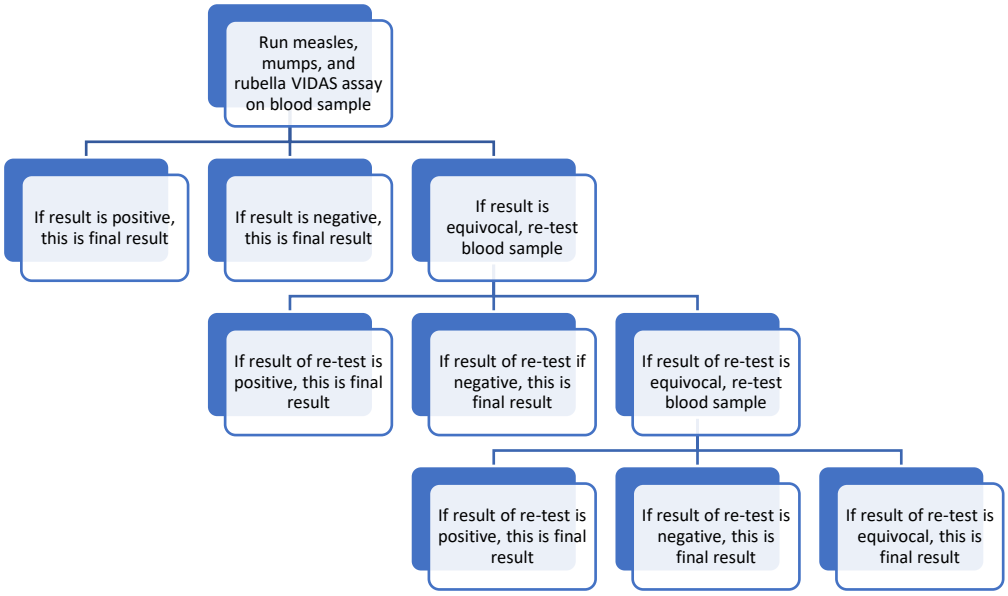
When participants were enrolled into the FSW-SM cohort baseline questionnaires were given. Some data from these questionnaires were used to perform analysis on the

SiVET data. Place of residence, how many years living in Lusaka or Ndola, number of live births, marital status, education level, local language understanding and reading, English understanding and reading, and high-risk group (FSW or SM) were obtained from the FSW-SM cohort baseline questionnaire. Date of birth was provided in the FSW-SM cohort baseline questionnaires and was used to calculate age at SiVET enrollment. Upon enrollment into SiVET, FSW-SM cohort members were asked an additional questionnaire and a vaccine education survey pre-test. BMI information was obtained from the additional questionnaire and pre-vaccine knowledge about measles, mumps, and rubella was obtained from the vaccine education pre-test. All data pertaining to serostatus to measles, mumps, or rubella was obtained from the SiVET data.

Place of residence (Lusaka or Ndola), high-risk group (Female Sex Worker of Single Mother), and pre-vaccination knowledge (Yes or No) were coded as dichotomous variables. Years of residence was coded into three categories (1-18, 19-23, and 24-36) based on tertiles. Similarly, age was categorized by quartiles into four categories (18-20, 21-23, 24-28, 29-39). The number of live births ranged from 0-6 and were divided into four categories (0, 1-2, 3-4, and 5-6). Level of education was categorized as either primary, secondary (high school), college, or none of the above. English understanding, English reading, and local language (Nyanja or Bemba) were categorized as either easily, with difficulty, or not at all. Local language understanding was coded dichotomously as easily and with difficulty or not at all since no participants stated not being able to understand their local language at all. BMI was calculated using the weight and height of the participant that was obtained upon enrollment and was then categorized as underweight (BMI < 18.5 kg/m^2), normal (BMI 18.5 to 24.9 kg/m^2), overweight (25.0 to

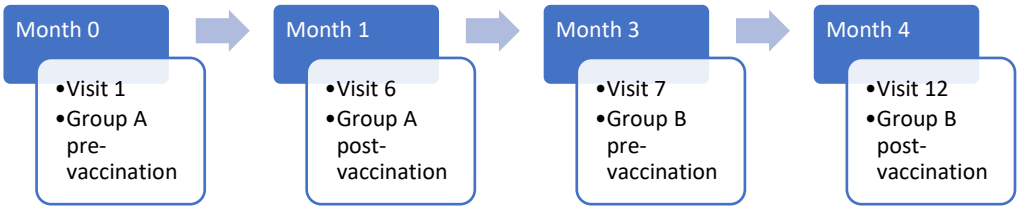
29.9 kg/m^2), or obese (BMI ≥ 30.0 kg/m^2) as indicated the Zambian Ministry of Health (19).

The final result for each visit was determined by the following algorithm:



Final results for measles and mumps are categorical (positive, negative, or equivocal) because the Vidas assay performed is a qualitative test. The Vidas assay performed for rubella provides a quantitative result that indicates level of immunity for the participant.

When a participant was missing a post-vaccine (final) result, the result from a different visit was used where appropriate. For Group A, since the MMR vaccine was administered at Visit 1, post-vaccine result could be drawn from Visit 7 or Visit 12 if Visit 6 was missing. For Group B, since the MMR vaccine was administered at Visit 7, post-vaccine result could be drawn only from Visit 12, but pre-vaccine results could be drawn from Visit 1 or Visit 6 if Visit 7 was missing. The figure below provides a timeline of participant visits.



Statistical Analyses

Inconsistencies identified during quality control checks in antibody response data were reconciled with hard copy results in country. The clean data was then matched to participant covariate data by coded ID. The data was then entered into Statistical Analysis Software (SAS 9.4, SAS Institute, Inc, Cary, NC). Descriptive analyses were used to describe the IgG seroprevalence of measles, mumps, and rubella at baseline. Logistic regression models were fitted using SAS to determine predictors for measles sero-negativity at baseline. To determine which covariates were statistically significant in predicting the outcome of negative measles antibody at baseline bivariate analyses were performed. Paired t-tests were performed to assess the change in rubella titers before and after vaccination. For Group A, titer level at visit 1, pre-vaccination, will

be compared to titer level at visit 6, post-vaccination. For Group B, titer levels at visit 7, pre-vaccination, will be compared to titer levels visit 12, post-vaccination.

Results

Participant Pre- and Post-Vaccination Results

The majority of participants had pre- and post-vaccination data available for analysis (Table 1). 94.3% of measles, 95.6% of mumps, and 83.6% of rubella pre- and post-vaccination data was available. There were 31 participants that only had pre-vaccination results available. Out of the participants missing post-vaccination results, all were positive at baseline except for a single participant who was negative for rubella IgG antibody (Appendix C). Prior to vaccination, 24.5% of measles IgG antibody, 5.7% of mumps IgG antibody, and 2.5% rubella IgG antibody results were either negative or equivocal (Table 2). It is very clear that the vaccination was effective since there are very few participants who did not become positive for measles IgG, mumps IgG, and rubella IgG after being vaccinated.

Baseline Seroprevalence

At baseline, 118 (75%) of participants had sero-positivitity to measles, 147 (94%) of participants had sero-positivity to mumps, and 151 (97%) of participants had sero-positivity to rubella (Table 3). For those positive for measles, mumps, and rubella antibody at baseline, the majority were ages 21-23, had 1-2 live births, had lived in their place of residence for essentially their entire lives (19-23 years), had obtained a primary education, were single mothers, and had a normal BMI. The large majority of participants that had positive measles and mumps antibody had heard about the virus prior to the vaccine information session, but the large majority of participants that had positive rubella antibody had not heard about the virus prior to the vaccine. At baseline, 30 (19%) participants were negative and 9 (6%) were equivocal for measles IgG

antibody, 9 (6%) participants were negative for mumps IgG antibody, and 4 (3%) participants were negative for rubella IgG antibody. No participants had equivocal levels for mumps or rubella antibody. It was anticipated that age would impact baseline sero-status for measles, mumps, and rubella because of waning immunity. If the participant developed immunity during primary school, they would be more likely to have a decrease or loss in immunity by the time they reached 20 years of age. When assay result to measles, mumps, and rubella are stratified by age, we see a consistent trend that the highest sero-positivity for any of the viruses are in the age 21-23 group (Figure 1).

Participant Characteristics at Enrollment

A total of 160 participants were screened at baseline, and 159 were randomly assigned to Group A or Group B. Participant SVET23081 was lost to follow-up prior to receiving the MMR vaccine; therefore, they were included in baseline demographics and excluded from any pertinent analysis. Baseline demographic characteristics of the two groups were similar overall (Table 4). Overall the population has an average age of 21-23, with 1-2 live births, and they have lived in their respective city for the majority of their life. There were no statistically significant differences in these characteristics (p-value > 0.05).

Predictors of Sero-Negativity for Measles Antibody

Prior to assessing significance, Model 1 was created (see Model 1 below). To determine which predictors would remain in the final model, bivariate analyses were

performed for each covariate. None of the anticipated covariates were statistically significant predictors of being sero-negative for measles (Table 5).

Model 1: $Measles\ Result\ at\ Baseline = \beta_0 + \beta_1(age) + \beta_2(number\ of\ live\ births) + \beta_3(English\ understanding) + \beta_4(English\ reading) + \beta_5(local\ language\ understanding) + \beta_6(local\ language\ reading) + \beta_7(education) + \beta_8(BMI) + \beta_9(city) + \beta_{10}(high\text{-}risk\ group) + \beta_{11}\ (years\ in\ city) + \beta_{12}(pre\text{-}vaccination\ knowledge)$

Even though none of the predictors were statistically significant, we can use these results to determine which groups are more likely to be sero-negative by the effect measure. The odds of being sero-negative at baseline are higher if the participant lives in Ndola, has resided in the participant's place of residence for 19-23 years, is between the ages of 24 and 28, had 1-2 live births, level of education stopped at primary school, can understand English with some difficulty, cannot read English at all, can understand the participant's local language easily, cannot read the participant's local language at all, are a female-sex worker, had knowledge of the virus prior to the vaccine education session, or are obese.

Evaluate Pre- and Post-Vaccination Titer for Rubella

Unlike the measles and mumps assays, the rubella assay is quantitative, therefore pre- and post-vaccination antibody titer can be evaluated (Table 6). For Group A, the mean pre-vaccination titer was 140 Ul/mL compared to the post-vaccination titer of 159 Ul/mL. After receiving the MMR vaccine, participants rubella IgG antibody titer increased on average by 19 Ul/mL (p-value = 0.0008). If the population was given MMR, we would be 95% confident that the average change in titer would be between 6.60

Ul/mL and 24.15 Ul/mL. For Group B, the mean pre-vaccination titer was 155 Ul/mL compared to the post-vaccination titer of 170 Ul/mL. After receiving the MMR vaccine, participants rubella IgG antibody titer increased on average by 15 Ul/mL (p-value = 0.0017). If the population was given MMR, we would be 95% confident that the average change in titer would be between 6.40 Ul/mL and 26.36 Ul/mL.

When looking at an overlay frequency histogram for Group A containing Visit 1 and Visit 6 titers, the peak of the normal curves are different. The Visit 6 post-vaccine average titer is statistically significantly higher than the Visit 1 pre-vaccine average titer as demonstrated by a shift to the right of the normal curve show in Figure 2. Similarly, when looking at an overlay frequency histogram for Group B containing Visit 7 and Visit 12 titers, the post-vaccine average titer is statistically significantly higher than the pre-vaccine titer as demonstrated by a shift to the right of the normal curve show in Figure 3.

Pre- and Post-vaccine Seroprevalence of IgG to Measles, Mumps, and Rubella

As shown in Table 7, the overall MMR sero-positivity increased in both Group A and Group B (Group A: 64% to 93% and Group B: 67% to 73%). In Group A, four categories had a statistically significant change in sero-positivity coverage. Measles, mumps, and rubella (MMR) sero-positivity coverage went from 64% to 93% (p-value <0.001), measles only sero-positivity coverage went from 75% to 97% (p-value <0.0001), measles and mumps only sero-positivity coverage went from 69% to 95% (p-value <0.0001), and measles and rubella only sero-positivity coverage went from 70% to 95% (p-value <0.0001). In Group B, a statistically significant change in sero-positivity was seen in measles only, rubella only, and measles and mumps only. Measles only and

measles and mumps both saw an increase in coverage. Measles coverage went from 72% to 95% (p-value <0.0001) and measles and mumps coverage went from 70% to 92% (p-value = 0.0003). Rubella only saw a statistically significant decrease in vaccine coverage. Rubella coverage went from 96% to 76% (p-value = 0.0013).

Discussion

The primary objective of this study was to evaluate immune response to measles, mumps, and rubella before and after vaccination. This objective accomplished by assessing baseline sero-prevalence to measles, mumps, and rubella, modeling predictors for measles sero-negativity at baseline, and by analyzing pre- and post- vaccination rubella IgG titers. Vaccine coverage has important implications for preventing the resurgence of vaccine preventable viruses that could potentially lead to serious sequelae (2). Regrettably, the WHO African Region has failed to reach 80% MMR vaccine coverage and still has a high measles incidence due to these coverage gaps (6). At baseline, participants in the two groups had fairly similar demographic characteristics overall. Immunity, as indicated by IgG sero-positivity, for measles, mumps, and rubella was varied. At the time this data was collected, the Zambian Immunization Schedule did not include mumps and rubella, which makes it surprising that in this population, measles immunity was much lower than the average global coverage of 86% and was also much lower than the baseline sero-positivity of mumps and rubella (1).

Due to the much lower rate of immunity to measles, predictors of waning immunity were assessed. According to previous literature, age, BMI as a proxy for nutrition, number of live births, city of residence, location, and high-risk group, education, English reading and understanding, local language reading and understanding, and pre-vaccination knowledge of each virus were probable predictors (8),(20). Unexpectedly, none of the aforementioned covariates were statistically significant predictors for being negative for measles IgG. The lower baseline measles IgG sero-positivity could indicate that some of these women were never vaccinated as children and were never exposed to wild-type virus. Interestingly, when looking at the groups combined, the overall measles

and mumps knowledge was very high, and rubella was very low. The low level of rubella titer was present even though the population had been exposed previously, as indicated by the high percent of the population that was IgG positive. Measles and mumps, pathologically, are very evident. In contrast, rubella is very mild which could impact the decreased overall knowledge.

Another cause of lower measles immunity could be due to high incidence of HIV and malaria in the general population (21). It is often recommended that a person who is severely immunocompromised due to an infection like HIV not be vaccinated against MMR. It has been shown that the vaccine can cause infection if the immune system is weakened enough, but if an HIV-infected person is asymptomatic they should be vaccinated. Regardless of severity of immunosuppression, antibody responses to MMR are variable (22). It is recommended that two doses of MMR be given to ensure immune response (23). Malaria infection impacts the transfer of measles antibody from mother to fetus. It is hypothesized that repeated malaria infections, which is common in Zambia, cause an increase in specific and non-specific stimulation of B-lymphocytes. This causes increased levels of IgG measles antibodies to be present in mothers, but the transfer of these antibodies to the fetus is impaired by malaria infection. The decrease in maternal measles antibody transferred to a newborn can impact the immune response of the newborn (24).

The population had a very high baseline immunity to mumps and rubella that was most likely due to wild-type exposure. Wild-type exposure generally provides a higher titer of vaccine induced antibodies, but due to the fact that viremic reinfection may occur in persons who have low levels of detectible antibody it was important to determine the mean titer increased after vaccination (25). The assay used only provided titer levels for

rubella. Both Group A and Group B saw a statistically significant increase in mean rubella IgG titer indicating the vaccine increased the population's rubella immunity. Previous data has shown that more than 90% of people vaccinated for rubella will have lifelong immunity from one dose, but this result is an important clinical implication that supports getting a second vaccination to prevent reinfection (25).

Overall MMR vaccination coverage increased by about 40% in Group A and 10% in Group B. Both groups had statistically significant increases in measles immunity, which is especially important due to the extremely high infectious rate of the measles virus. Measles outbreaks can occur in populations were less than 10% of persons are susceptible, making it vital to see more than 90% of a population positive for measles IgG (8). The MMR vaccination provided our entire population with 95% immunity, bringing coverage up to a protective percentage. Interestingly, Group B saw a statistically significant decrease in overall vaccine coverage, but this was most likely do to the fact that about 13.2% of the post-vaccine rubella results are missing. When only the participants with V12 rubella results are considered, the post-vaccine immunity to rubella is 100%. Additionally, one out of four participants saw a decrease in rubella titer, but very few of those cases saw greater than 10% change and they all still retained immunity to rubella IgG.

Strengths and Limitations

Strengths of the present study includes in-depth eligibility criteria, the extensiveness of demographic data gathered from baseline questionnaires, and high participant retention. The stringent eligibility criteria most likely had a large impact on the 96.2% rate of completing the one-year study, but the high completion rate does not mean all

participants now have immunity. Furthermore, five participants did not receive their second vaccine. Only one out of the five participants was randomized to Group B, so they did not receive their second vaccine (MMR). This participant was not included in post-vaccination analysis, which impacts the study results. The other four participants received MMR first and could be included in post-vaccination analysis. Additionally, the breadth of demographic data allowed for 12 covariates to be included as potential predictors for measles sero-negativity. Furthermore, equivocal results were only included as a final result after being retested twice to ensure accurate results.

A limitation of this study is the lack of generalizability to populations that are HIV-positive. Previous studies have indicated that being infected with HIV can cause adults to lose immunity at a much faster rate than those HIV-uninfected (26). Moreover, the cohort size for this study was relatively small. Additionally, those infected with HIV around the time of birth are on average, much less likely to have protective immunity against measles, mumps, and rubella (27). Another limitation of this study are the missing results for rubella post-vaccine titer level. A possible limitation to this study was recording data in excel, which could lead to incorrectly entered or transferred data that could have altered results. To limit these errors, hard copy results were obtained for data management. Finally, BMI can be a very subjective measure for nutrition especially in poorer conditions. For example, a person with kwashiorkor will have a BMI that is higher than another with marasmus, but both are severely malnourished.

Conclusions

Overall, these results indicate that there is a gap in immunity towards MMR in Ndola and Lusaka, Zambia based on the low sero-positivity to measles IgG. This gap

was most likely a leading cause of the major measles outbreak in Zambia in 2010. Even though there were no statistically significant predictors of sero-negativity for measles antibody in this population, multiple studies have shown that the covariates included are typical predictors of sero-status. The mean increase in rubella titer after receiving MMR vaccination indicates that boosters are an important factor of maintaining current immunity levels and increasing global coverage. Lastly, the statistically significant increase in measles immunity post-vaccination in both groups indicates a gap in distribution of the second measles vaccine.

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Tables and Figures

Table 1

Table 1. Participants with both pre- and post-vaccination results compared to those with only pre-vaccination results

	Both Pre-and Post-Vaccination Results (N=159)		Only Pre-Vaccination Results[a] (N=159)	
	n	%	n	%
Measles	150	94.3	6	3.8
Mumps	152	95.6	4	2.5
Rubella	133	83.6	21	13.2

[a]One participant that was missing Rubella post-vaccination results was negative pre-vaccination and all others were positive at pre-vaccination.

Table 2

Table 2. Negative and Equivocal Results at Pre- and Post-Vaccination

	Pre-Vaccination (N= 159)		Post-Vaccination (N=154)		
	n	%	n	%	p-value
Measles					
Negative	30	18.9	2	1.3	
Equivocal	9	5.7	2	1.3	
Total	39	24.5	4	2.6	<0.05
Mumps					
Negative	9	5.7	2	1.3	
Equivocal	0	0.0	0	0.0	
Total	9	5.7	2	1.3	<0.05
Rubella					
Negative	4	2.5	0	0.0	
Equivocal	0	0.0	0	0.0	
Total	4	2.5	0	0.0	0.1228

Table 3

Table 3. Baseline Seroprevalence of Measles, Mumps and Rubella lgG Antibody in Zambian Women of Childbearing Age, by Sociodemografic Factors.

	Measles Result (N=157)[a,b]						Mumps Result (N=156)[c]						Rubella Result (N=155)[d]					
	Negative		Equivocal		Positive		Negative		Equivocal		Positive		Negative		Equivocal		Positive	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Total	30	19.1	9	5.7	118	75.2	9	5.8	0	0.0	147	94.2	4	2.6	0	0.0	151	97.4
Place of residence																		
Lusaka	18	11.5	4	2.5	57	36.3	9	5.8	0	0.0	67	42.9	4	2.6	0	0.0	74	47.7
Ndola	12	7.6	5	3.2	61	38.9	0	0.0	0	0.0	80	51.3	0	0.0	0	0.0	77	49.7
Age (years)																		
18 -20	6	3.8	2	1.3	22	14.0	2	1.3	0	0.0	29	18.6	0	0.0	0	0.0	31	20.0
21-23	10	6.4	2	1.3	39	24.8	1	0.6	0	0.0	49	31.4	1	0.6	0	0.0	50	32.3
24-28	6	3.8	2	1.3	29	18.5	2	1.3	0	0.0	35	22.4	0	0.0	0	0.0	37	23.9
29-39	8	5.1	3	1.9	28	17.8	4	2.6	0	0.0	34	21.8	3	1.9	0	0.0	33	21.3
Number of Live Births																		
0	3	1.9	1	0.6	8	5.1	0	0.0	0	0.0	11	7.1	2	1.3	0	0.0	10	6.5
1-2	16	10.2	4	2.5	87	55.4	6	3.8	0	0.0	102	65.4	1	0.6	0	0.0	106	68.4
3-4	9	5.7	3	1.9	19	12.1	3	1.9	0	0.0	27	17.3	1	0.6	0	0.0	28	18.1
5-6	2	1.3	1	0.6	4	2.5	0	0.0	0	0.0	7	4.5	0	0.0	0	0.0	7	4.5
Years of residence																		
1-18	13	8.3	2	1.3	36	22.9	5	3.2	0	0.0	46	29.5	2	1.3	0	0.0	48	31.0
19-23	10	6.4	3	1.9	49	31.2	1	0.6	0	0.0	61	39.1	0	0.0	0	0.0	62	40.0
24-36	7	4.5	4	2.5	33	21.0	3	1.9	0	0.0	40	25.6	2	1.3	0	0.0	41	26.5
Marital status[a]																		
Never married	23	14.7	8	5.1	78	50.0	4	2.6	0	0.0	105	67.7	3	1.9	0	0.0	107	69.5
Divorced/separated	7	4.5	1	0.6	36	23.1	4	2.6	0	0.0	39	25.2	1	0.6	0	0.0	42	27.3
Widowed	0	0.0	0	0.0	3	1.9	1	0.6	0	0.0	2	1.3	0	0.0	0	0.0	1	0.6
Education level																		
Primary	11	7.0	4	2.5	59	37.6	2	1.3	0	0.0	71	45.5	1	0.6	0	0.0	72	46.5
Secondary	16	10.2	5	3.2	50	31.8	6	3.8	0	0.0	64	41.0	1	0.6	0	0.0	69	44.5
College	1	0.6	0	0.0	2	1.3	0	0.0	0	0.0	3	1.9	1	0.6	0	0.0	2	1.3
None of the above	2	1.3	0	0.0	7	4.5	1	0.6	0	0.0	9	5.8	1	0.6	0	0.0	8	5.2
English Understanding																		
Easily	11	7.0	3	1.9	43	27.4	5	3.2	0	0.0	51	32.7	3	1.9	0	0.0	53	34.2
With Difficulty	11	7.0	3	1.9	46	29.3	2	1.3	0	0.0	58	37.2	1	0.6	0	0.0	59	38.1
Not at all	8	5.1	3	1.9	29	18.5	2	1.3	0	0.0	38	24.4	0	0.0	0	0.0	39	25.2
English Reading																		
Easily	11	7.0	2	1.3	37	23.6	4	2.6	0	0.0	44	28.2	3	1.9	0	0.0	46	29.7
With Difficulty	9	5.7	4	2.5	38	24.2	1	0.6	0	0.0	51	32.7	0	0.0	0	0.0	51	32.9
Not at all	10	6.4	3	1.9	43	27.4	4	2.6	0	0.0	52	33.3	1	0.6	0	0.0	54	34.8
Local Language Understanding																		
Easily	30	19.1	8	5.1	117	74.5	9	5.8	0	0.0	145	92.9	4	2.6	0	0.0	149	96.1
With Difficulty or Not at all	0	0.0	1	0.6	1	0.6	0	0.0	0	0.0	2	1.3	0	0.0	0	0.0	2	1.3
Local Language Reading																		
Easily	17	10.8	5	3.2	72	45.9	5	3.2	0	0.0	90	57.7	2	1.3	0	0.0	90	58.1
With Difficulty	7	4.5	3	1.9	17	10.8	0	0.0	0	0.0	27	17.3	0	0.0	0	0.0	27	17.4
Not at all	6	3.8	1	0.6	29	18.5	4	2.6	0	0.0	30	19.2	2	1.3	0	0.0	34	21.9
High-risk group																		
Female sex worker	12	7.6	3	1.9	44	28.0	4	2.6	0	0.0	54	34.6	2	1.3	0	0.0	56	36.1
Single mother	18	11.5	6	3.8	74	47.1	5	3.2	0	0.0	93	59.6	2	1.3	0	0.0	95	61.3
Pre-vaccination Knowledge from VES																		
Yes	29	18.7	9	5.8	114	73.5	8	5.2	0	0.0	137	89.5	0	0.0	0	0.0	25	16.4
No	1	0.6	0	0.0	2	1.3	1	0.7	0	0.0	7	4.6	4	2.6	0	0.0	123	80.9
BMI																		
Underweight (BMI below 18.5)	4	2.5	2	1.3	16	10.2	1	0.6	0	0.0	21	13.5	0	0.0	0	0.0	23	14.8
Normal (BMI 18.5 - 24.9)	17	10.8	4	2.5	74	47.1	7	4.5	0	0.0	88	56.4	3	1.9	0	0.0	90	58.1
Overweight (BMI 25.0 - 29.9)	8	5.1	3	1.9	23	14.6	1	0.6	0	0.0	32	20.5	0	0.0	0	0.0	33	21.3
Obese (BMI 30.0 and above)	1	0.6	0	0.0	5	3.2	0	0.0	0	0.0	6	3.8	1	0.6	0	0.0	5	3.2

[a]One study participant did not report marital status.
[b]One study participant in Group A and one study participant in Group B did not report measles pre-vaccination knowledge from VES.
[c]Two study participants in Group A and one study participant in Group B did not report mumps pre-vaccination knowledge from VES.
[d]One study participant in Group A and two study participants in Group B did not report rubella pre-vaccination knowledge from VES.

Table 4

Table 4: Baseline Demographics in Zambian Women of Childbearing Age.

	Group A (N=79)		Group B (N=80)		Total (N=159)	
	n	%	n	%	n	%
Total						
Measles Positive	58	73.4	60	75.0	118	74.2
Mumps Positive	74	93.7	73	91.3	147	92.5
Rubella Positive	74	93.7	77	96.3	151	95.0
Place of residence						
Lusaka	36	45.6	43	53.8	79	49.7
Ndola	43	54.4	37	46.3	80	50.3
Age (years)						
18 -20	16	20.3	15	18.8	31	19.5
21-23	20	25.3	32	40.0	52	32.7
24-28	20	25.3	17	21.3	37	23.3
29-39	23	29.1	16	20.0	39	24.5
Number of Live Births						
0	5	6.3	7	8.8	12	7.5
1-2	55	69.6	54	67.5	109	68.6
3-4	15	19.0	16	20.0	31	19.5
5-6	4	5.1	3	3.8	7	4.4
Years of residence						
1-18	28	35.4	24	30.0	52	32.7
19-23	26	32.9	37	46.3	63	39.6
24-36	25	31.6	19	23.8	44	27.7
Marital status[a]						
Never married	56	71.8	55	69.6	111	69.8
Divorced/separated	20	25.6	24	30.4	44	27.7
Widowed	2	2.6	1	1.3	3	1.9
Education level						
Primary	38	48.1	36	45.0	74	46.5
Secondary	31	39.2	41	51.3	72	45.3
College	3	3.8	0	0.0	3	1.9
None of the above	7	8.9	3	3.8	10	6.3
High-risk group						
Female sex worker	31	39.2	28	35.0	59	37.1
Single mother	48	60.8	52	65.0	100	62.9
Measles Pre-vaccination Knowledge from VES[a]						
Yes	77	98.7	77	97.5	154	96.9
No	1	1.3	2	2.5	3	1.9
Mumps Pre-vaccination Knowledge from VES[b]						
Yes	74	96.1	74	93.7	148	93.1
No	3	3.9	5	6.3	8	5.0
Rubella Pre-vaccination Knowledge from VES[c]						
Yes	15	19.2	11	14.1	26	16.4
No	63	80.8	67	85.9	130	81.8
BMI						
Underweight (BMI below 18.5)	8	10.1	15	18.8	23	14.5
Normal (BMI 18.5 - 24.9)	53	67.1	43	53.8	96	60.4
Overweight (BMI 25.0 - 29.9)	14	17.7	20	25.0	34	21.4
Obese (BMI 30.0 and above)	4	5.1	2	2.5	6	3.8

[a]One study participant did not report marital status or measles pre-vaccination knowledge from VES.
[b]Two study participants in Group A and one study participant in Group B did not report mumps pre-vaccination knowledge from VES.
[c]One study participant in Group A and two study participants in Group B did not report rubella pre-vaccination knowledge from VES.

Table 5

Table 5. Assessment of Potential Predictors for Sero-negativity with Bivariate Analyses

	Measles			
Covariate	**OR**	**95% CI**	**Width**	**p-value**
Place of Residence				
Lusaka	1.00			
Ndola	1.43	0.69, 2.95	2.26	0.33
Years of Residence				
1-18	1.00			
19-23	1.62	0.70, 3.79	3.10	0.26
24-36	1.35	0.55, 3.34	2.80	0.51
Age				
18 -20	1.00			
21-23	1.15	0.41, 3.21	2.80	0.79
24-28	1.31	0.43, 4.02	3.59	0.64
29-39	0.94	0.32, 2.71	2.38	0.90
Number of Live Births				
0	1.00			
1-2	2.12	0.59, 7.62	7.03	0.25
3-4	0.80	0.20, 3.20	3.00	0.75
5-6	0.72	0.11, 4.80	4.69	0.74
Education Level				
Primary	1.00			
Secondary	0.61	0.28, 1.29	1.01	0.20
College	0.45	0.04, 4.95	4.91	0.52
None of the above	0.83	0.16, 4.22	4.06	0.82
English Understanding				
Easily	1.00			
With Difficulty	1.07	0.46, 2.48	2.02	0.88
Not at all	0.88	0.35, 2.19	1.84	0.78
English Reading				
Easily	1.00			
With Difficulty	1.08	0.45, 2.62	2.18	0.86
Not at all	1.19	0.50, 2.86	2.37	0.70
Local Language Understanding				
Easily	1.00			
With Difficulty or Not at all	0.59	0.03, 10.35	10.32	0.72
Local Language Reading				
Easily	1.00			
With Difficulty	0.55	0.22, 1.36	1.14	0.20
Not at all	1.24	0.48, 3.18	2.70	0.66
High-Risk Group				
Single mother	1.00			
Female Sex Worker	1.07	0.52, 2.24	1.72	0.86
Pre-vaccination knowledge				
Yes	1.00			
No	0.60	0.057, 6.27	6.22	0.67
BMI				
Normal (BMI 18.5 - 24.9)	1.00			
Underweight (BMI below 18.5)	0.80	0.28, 2.29	2.01	0.67
Overweight (BMI 25.0 - 29.9)	0.62	0.26, 1.46	1.20	0.27
Obese (BMI 30.0 and above)	1.37	0.16, 11.82	11.66	0.78

Table 6

Table 6: Mean Rubella Titer in Ul/mL Pre- and Post-Vaccination

	Pre-Vaccination Titer (Ul/mL)	Post-Vaccination Titer (Ul/mL)	Difference	95% CI	Standard Deviation	p-value
Group A	140	159	19	6.60, 24.15	37.33	<0.001
Group B	155	170	15	6.40, 26.36	38.97	0.002

Table 7

Table 7. Seroprevalence of IgG to measles, mumps, and rubella in Zambian women of child-bearing age at pre- and post-vaccination

	Group A													Group B											
	Pre-Vaccine (N = 77)						Post-Vaccine (N = 76)							Pre-Vaccine (N=79)						Post-Vaccine (N = 78)						
	Lusaka	%	Ndola	%	Total	%	Lusaka	%	Ndola	%	Total	%	p-value	Lusaka	%	Ndola	%	Total	%	Lusaka	%	Ndola	%	Total	%	p-value
IgG antibody combination																									
MMR positive	18	23	31	40	49	64	30	39	41	54	71	93	<0.001	24	30	29	37	53	67	22	28	35	45	57	73	0.41
Measles positive	26	34	32	42	58	75	33	43	41	54	74	97	<0.001	28	35	29	37	57	72	39	50	35	45	74	95	<0.001
Mumps positive	31	40	43	56	74	96	33	43	42	55	75	99	0.32	39	49	37	47	76	96	39	50	37	47	76	97	0.66
Rubella positive	33	43	41	53	74	96	33	43	41	54	74	97	0.66	39	49	37	47	76	96	25	32	37	47	62	79	0.001
Measles + mumps positive	21	27	32	42	53	69	31	41	41	54	72	95	<0.001	26	33	29	37	55	70	37	47	35	45	72	92	<0.001
Measles + rubella positive	23	30	31	40	54	70	31	41	41	54	72	95	<0.001	26	33	29	37	55	70	23	29	35	45	58	74	0.51
Mumps + rubella positive	28	36	41	53	69	90	32	42	41	54	73	96	0.12	36	46	37	47	73	92	24	31	37	47	61	78	0.01
MMR negative	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	

Figure 1

Figure 1: Participant Randomization by Group

Group	N	Months	
		0	3
A	75	MMR	Tdap-IPV
B	75	Tdap-IPV	MMR

Figure 2

Figure 2: Comparison of Baseline Age Versus Sero-Status Stratified by Measles, Mumps, or Rubella.

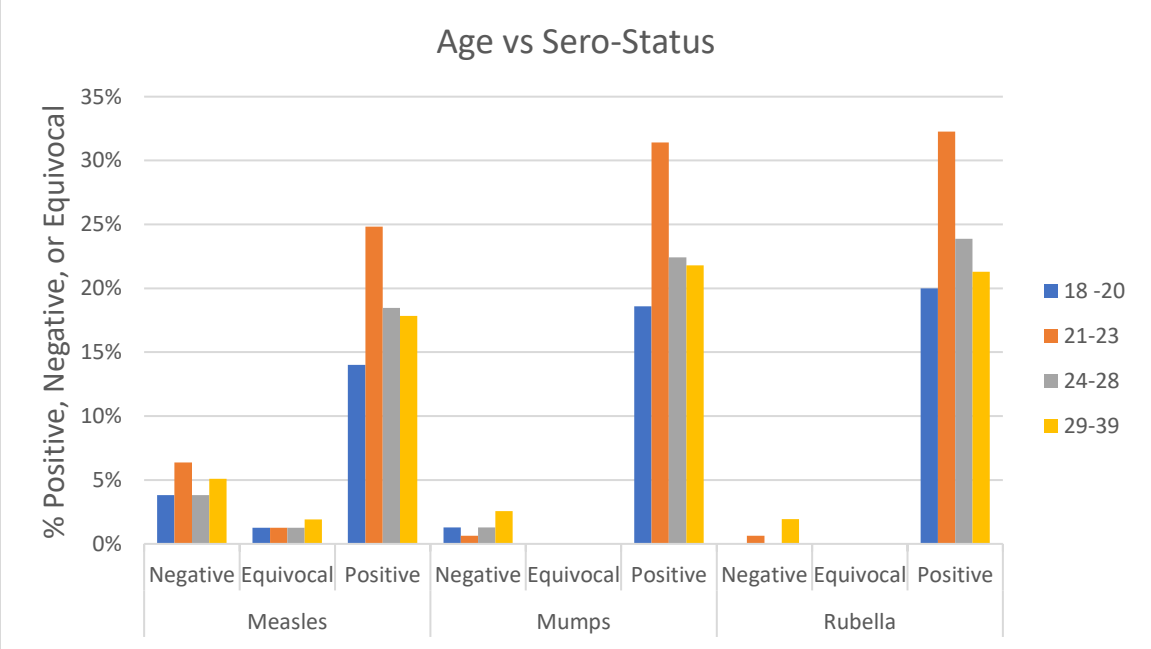


Figure 3

Figure 3: Comparison of Group A Pre-Vaccine (Visit 1) and Post-Vaccine (Visit 6) Rubella Titers

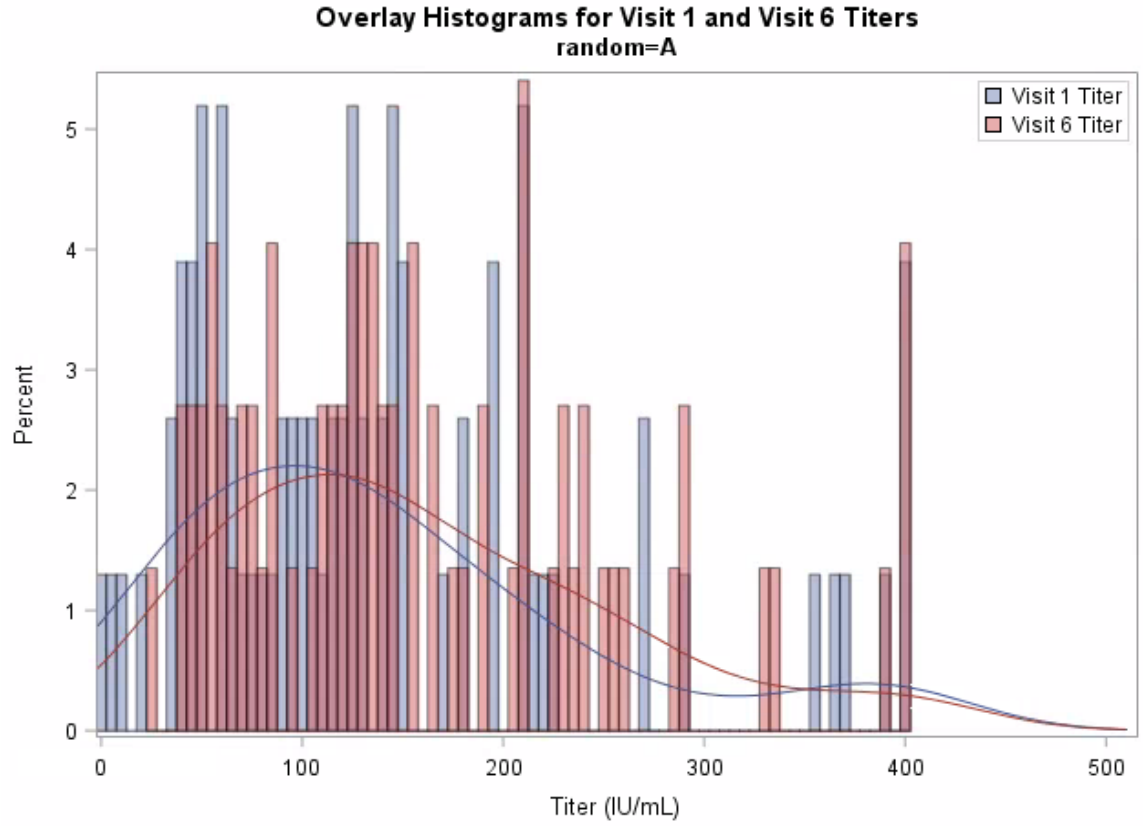
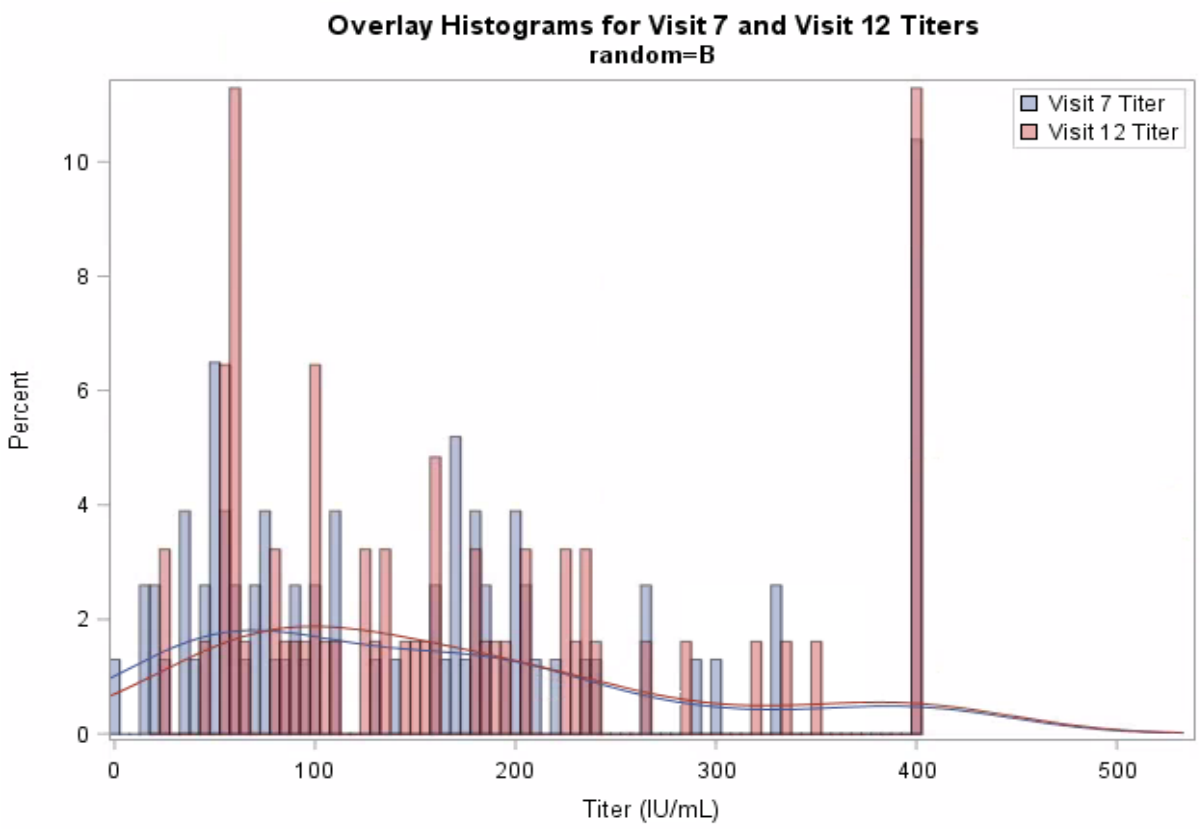


Figure 4

Figure 4: Comparison of Group B Pre-Vaccine (Visit 7) and Post-Vaccine (Visit 12) Rubella Titers



Appendices

Appendix A: Zambian Immunization Schedule

ROUTINE CHILDHOOD IMMUNISATION SCHEDULE

AGE OF CHILD	VACCINE	DOSE	ROUTE	SITE
AT BIRTH	BCG	0.05mls	INTRADERMAL INJECTION	INSERT OF THE DELTOID MUSCLE RIGHT ARM
	POLIO VACCINE	2 DROPS – DOSE 0	ORAL	MOUTH
	VITAMIN A	200 000IU	AMINISTERED TO THE MOTHER	ORAL
6 WEEKS OLD	POLIO VACCINE	2 DROPS – DOSE 1	ORAL	MOUTH
	DTP/HIB VACCINE	0,5mls	DEEP INTRA-MUSCULAR INJECTION	LEFT THIGH
	HEP B VACCINE	0,5mls	DEEP INTRA-MUSCULAR INJECTION	RIGHT THIGH
10 WEEKS	POLIO VACCINE	2 DROPS – DOSE 2	ORAL	MOUTH
	DTP/HIB VACCINE	0,5mls	DEEP INTRA-MUSCULAR INJECTION	LEFT THIGH
	HEP B VACCINE	0,5mls	DEEP INTRA-MUSCULAR INJECTION	RIGHT THIGH
14 WEEKS	POLIO VACCINE	2 DROPS – DOSE 3	ORAL	MOUTH
	DTP/HIB VACCINE	0,5mls	DEEP INTRA-MUSCULAR INJECTION	LEFT THIGH
	HEP B VACCINE	0,5mls	DEEP INTRA-MUSCULAR INJECTION	RIGHT THIGH
6 MONTHS	VITAMIN A	100 000IU	ORAL	MOUTH
9 MONTHS	MEASLES	0,5mls	DEEP INTRA-MUSCULAR INJECTION	RIGHT THIGH
1 YEAR	VITAMIN A	200 000IU	ORAL	MOUTH
18 MONTHS	POLIO VACCINE	2 DROPS – DOSE 4	ORAL	MOUTH
	DTP VACCINE	0,5mls	DEEP INTRA-MUSCULAR INJECTION	LEFT UPPER ARM
	MEASLES	0,5mls	DEEP INTRA-MUSCULAR INJECTION	RIGHT UPPER ARM
	VITAMIN A	200 000IU	ORAL	MOUTH
2 YEARS	VITAMIN A	200 000IU	ORAL	MOUTH
2 AND A HALF YEARS	VITAMIN A	200 000IU	ORAL	MOUTH
3 YEARS	VITAMIN A	200 000IU	ORAL	MOUTH
3 AND A HALF YEARS	VITAMIN A	200 000IU	ORAL	MOUTH
4 YEARS	VITAMIN A	200 000IU	ORAL	MOUTH
4 AND A HALF YEARS	VITAMIN A	200 000IU	ORAL	MOUTH
5 YEARS	VITAMIN A	200 000IU	ORAL	MOUTH
	DT VACCINE	0,5mls	DEEP INTRA-MUSCULAR INJECTION	LEFT UPPER ARM

BCG	BACILLUS	CALMETTE	GURIN	VACCINE AGAINST TURBERCULOSIS
DTP	VACCINE	AGAINST	DIPHTHERIA, TETANUS	AND PERTUSSIS (WHOOPING COUGH)
Hib	VACCINE	AGAINST	HAEMOPHILUS	INFLUENZAE TYPE B
HBV	VACCINE	AGAINST	HEPATITIS B	
DT	VACCINE	AGAINST	DIPHTHERIIA AND TETANUS	

CATCH UP IMMUNIZATIONS FOR OLDER CHILDREN

9 MONTHS – 2 YEARS	Give TOPV, Measles, DTP and HBV. All other doses are given with normal time intervals
2 YEARS – 10 YEARS	Give TOPV, Measles, DT at the same time. All other doses are given with normal time intervals

IF A CHILD IS WELL ENOUGH TO GO HOME, THAT CHILD IS WELL ENOUGH TO GO HOME IMMUNIZED.

TETANUS TOXOIDE IMMUNISATION SCHEDULE FOR PREGNANT WOMEN

First Pregnancy	3 doses of TT, starting at first antenatal visit, with minimum of 4 weeks between each dose even if these doses are given in the postnatal period.
Later Pregnancies	1 TT booster dose on first visit for antenatal care, unless there is no record of antenatal care in previous pregnancies, in which case the woman should be immunised as if it is her first pregnancy

TT can safely be given in the first trimester

Appendix B: Group A and B Schedule of Procedures

Schedule of Procedures-Groups A & B													
Trial Month (M)	**Screening [1]**	**M0 Enrolment [1]**			**M1**	**M3**			**M4**	**M6**	**M9**	**M12**	**Early Termination**
Study Day (D)		**0**	**3**	**7**	**28**	**84**	**87**	**91**	**112**	**168**	**252**	**336**	
Visit Number (V)	**V00.0**	**V01.0**	**V02.0**	**V03.0**	**V06.0**	**V07.0**	**V08.0**	**V09.0**	**V12.0**	**V13.0**	**V14.0**	**V15.0**	**V99.0**
Visit Type (V=Vaccine, C=Cohort, H=Home contact; L=Lab only)	**C**	**V**	**H**		**L**	**V+C**	**H**		**L**	**C**	**C**	**C**	
Vaccination													
MMR-(Tdap-IPV) or (Tdap-IPV)-MMR [2]		**X**				**X**							
Consent/Counselling													
Informed Consent and Assessment of Understanding		X											
Eligibility checklist		X											
HIV Risk Reduction Counseling	X					X				X	X	X	X
Pre-/Post HIV-test Counseling	X					X				X	X	X	X
Family Planning Counseling	X					X				X	X	X	X
FSW-U5 Cohort Procedures													
FSW or U5 FollowUp Questionnaire (HIV Risk Assessment)	X					X				X	X	X	
Clinical Safety Assessments													
Vital Signs		X				X							
Comprehensive Medical History		X											
Concomitant Medications		X				X				X	X	X	X
General Physical Exam (Wt & Ht) with gynecologic exam		X											X
Symptom-Directed Physical Exam	X					X				X	X	X	
Post Vaccination Contact (within 72hours of vaccination) [3]			X				X						
Local and systemic reagenocity		X		X		X		X					
Adverse events		X	X	X	X	X	X	X	X				
Serious Adverse events [4]													
Lab Samples and Tests													
HIV Tests	X[5]	X[5]				X				X	X	X	X
Syphilis	X[5]					X				X	X	X	
Urinalysis		X				X				X	X	X	
Microscopic STI screening	X					X				X	X	X	
Pregnancy	X[5]	X[5]				X				X	X	X	X
Measles, Mumps, Rubella IgG ELFA		X			X	X			X				
Sample storage-plasma		X				X				X	X	X	
Sample storage-serum		X			X	X			X	X	X	X	
Total Blood Volume (ml)-approximately		15	0	0	10	15	0	0	10	10	10	10	5

[1] Enrollment will be conducted within 3 days of screening visit.

[2] Participants will be randomized to either MMR-(Tdap-IPV) or (Tdap-IPV)-MMR

[3] Post Vaccination Contact by ZEHRP; participant is not required to come to study site.

[4] Serious adverse events will be monitored and recorded anytime they occur for duration of the study

[5] Serology testing will be performed using fingerprick at screening. If screening and enrollment procedures do not occur same day, HIV tests and pregnancy tests MUST be repeated.

Appendix C

Supplemental Table 1: Pre-vaccination results for participants missing post-vaccination results.

	Pre-Vaccination Result
Measles	
SVET23013	Positive
SVET23016	Positive
SVET30013	Positive
SVET30024	Positive
SVET30058	Positive
SVET30077	Positive
Mumps	
SVET23013	Positive
SVET23016	Positive
SVET23040	Positive
SVET30013	Positive
Rubella	
SVET23013	Positive
SVET23014	Positive
SVET23016	Positive
SVET23026	Negative
SVET23069	Positive
SVET23071	Positive
SVET23072	Positive
SVET23073	Positive
SVET23074	Positive
SVET23076	Positive
SVET23078	Positive
SVET23080	Positive
SVET23083	Positive
SVET23084	Positive
SVET23085	Positive
SVET23086	Positive
SVET23087	Positive
SVET23088	Positive
SVET23090	Positive
SVET30013	Positive
SVET30058	Positive

Chapter III: Summary, Public Health Implications, Possible Future Directions

Summary

The primary goal of this research was to evaluate immune response to measles, mumps, and rubella before and after receiving MMR vaccination. We hypothesized that receiving the vaccine would cause an increase in IgG seropositivity to measles, mumps, and rubella in this population. At baseline, 94.2% and 97.4% of the population were IgG positive for mumps and rubella respectively. This was much higher than the 75.2% IgG positive for measles at baseline.

The lower immunity to measles, the only virus out of the three that Zambia vaccinates against, indicates that immunity can wane. Thus, a predictive model was created using covariates previous literature indicated was more like to contribute to waning immunity. We found that none of the anticipated predictors were significant in predicting being IgG negative to measles, which was unexpected.

The very high baseline immunity to rubella was most likely caused to wild-type exposure to the virus. Since viremic reinfection is likely in persons with low levels of detectible antibody, it was important to see if average rubella IgG antibody titers increased after vaccination. Both Group A and Group B saw a statistically significant increase in mean rubella IgG titer. This indicates that the vaccine was effective in increasing the overall titer levels and the populations overall immunity to rubella.

Overall, the results indicate that there is a gap in immunity in regards to measles, mumps, and rubella in Ndola and Lusaka, Zambia, especially when it comes to measles coverage. This gap is most likely the cause of the major measles outbreak the country saw in 2010.

Public Health Implications

The findings of this study have implications in regard to global routine vaccination coverage. The data found supports previous findings that there are major gaps in vaccination coverage, especially in the African Region. It would be prudent to continue to expand vaccination programs in this region to increase the total population protected from measles, mumps, and rubella. Another finding of importance is the overall increase of rubella IgG titer after vaccination. This suggests retaining life-long immunity for rubella might not always be the case.

If MMR vaccine coverage declines, even slightly, there could be a dramatic impact on public health due to an increased risk of large outbreaks. If vaccination coverage drops below 90%, the protection of herd immunity will disappear leaving populations very vulnerable towards these vaccine preventable diseases. As shown in this study's population, vaccination coverage in Zambia is too low to rely upon herd immunity but providing the MMR vaccine caused coverage to increase. More vaccinations must be given to increase coverage across the African region.

Future Directions

There is currently a gap in understanding of immune responses to MMR vaccination in African populations. This study has provided some interesting findings, but there is still a substantial amount of work that should be done. Additional research should be conducted in varied populations, such as men or HIV-infected peoples. Further research should be done to try and determine predictors for negative IgG measles, mumps, and rubella antibody to try and assess what characteristics make someone more or less likely to retain immunity after vaccination.

Furthermore, increased vaccination surveillance in the African region would help determine where extra efforts should be provided to reach higher levels of coverage. As stated previous, measles, mumps, and rubella are extremely virulent and outbreaks can only be prevented if the large majority of the population is immunized. Ultimately, continued work towards providing vaccinations to all is crucial for the decrease in vaccine preventable illness and death.

Lastly, further evaluation of the type of immune response secondary to vaccination should be evaluated. This would allow for a better understanding of why some people do not develop life-long immunity after exposure to wile-type virus or vaccine. Additionally, increased knowledge will provide insight into variation in response types that may influence a populations immunity.

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