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Measles, Mumps, and Rubella Antibody Patterns of Persistence and Rate of Decline Following the Second Dose of the MMR Vaccine

By

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Degree to be awarded: Master of Public Health

Epidemiology

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2014

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Abstract

Measles, Mumps, and Rubella Antibody Patterns of Persistence and Rate of Decline Following the Second Dose of the MMR Vaccine

By Emma Elizabeth Seagle

Previous studies of the measles, mumps, and rubella (MMR) vaccine have reported a high degree of individual variation in patterns of waning antibody immunity, yet none have defined the degree of variation that exists. We utilized data from a 12 year longitudinal study of Wisconsin children vaccinated with the second dose of MMR (MMR2) at 4-6 years of age to identify patterns of seropositivity and antibody persistence for each antigen following receipt of MMR2, compare antibody kinetic patterns across antigens, and estimate the rate of decline in antibody levels using correlated data methods. Of the 313 study participants who received MMR2, 302 had measles antibody data and 296 had mumps and rubella data. The majority of participants remained seropositive for the follow-up period for all three antigens (96% measles, 88% mumps, 79% rubella). Among the 291 individuals with defined antibody persistence trends for all 3 antigens, 41 (14%) had the same trend categorization for measles, mumps, and rubella (2 stable, 18 declining, 17 variable, and 4 other), 188 (65%) had the same trend for 2 of the 3 antigens, and 62 (21%) had discordant trends for all three antigens. Among the individuals characterized as having a "declining trend" for measles, antibodies were high post-MMR2 (1 month post GMT: 3892.7 mIU/mL), but declined an average of 9.7% per year among those with the same baseline titer and no response to MMR2 (<2 fold increase), adjusting for sex. Those with MMR2 response of ≥2 fold experienced a slower decline (6.3% per year among those with 2-4 fold increase and 7.4% per year among those with ≥4 fold increase). Mumps rate of decline was 9.2% per year, adjusting for MMR2 response and baseline titer. Rubella antibodies declined an average of 2.6% per year among those who received MMR1 at age 12-15 months, and 5.9% per year among those who received MMR1 >15 months, adjusting for baseline rubella titer and MMR2 response. The high variation in persistence trends coupled with the fast rate of decline within a subset of individuals may impact herd immunity and individual susceptibility, particularly in outbreak scenarios.

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Chapter I. Background

Measles, Mumps, and Rubella Overview

Measles (rubeola), mumps, and rubella are acute viral childhood diseases that can cause severe complications and death (1). Measles is a highly contagious disease most readily recognized by rash, accompanied by high fever, cough, runny nose, and conjunctivitis. It is transmitted by direct contact with airborne respiratory droplets, which can live in the air for up to two hours (2-4). Infected persons are infectious from four days before to four days after the rash appears, while symptoms typically appear 7-14 days after exposure (5). Roughly 90% of those exposed develop the disease, and complications include pneumonia, otitis media, diarrhea, and, in some rare instances, encephalitis (1). Infants and young children have an increased risk of death and complications (3, 4). According to the Centers for Disease Control and Prevention (CDC), there were 188 reported cases of measles spanning 24 states in 2015, down from 667 cases reported in 2014 (6). The year 2014 saw the greatest number of cases since the disease was eliminated (no continuous chain of disease transmission for ≥12 months) (7)) from the United States in 2000 (6). Since elimination, the majority of cases in the United States have occurred from importation of the virus through international travel, with spread within the United States borders primarily among intentionally unvaccinated populations (8). An estimated seropositivity proportion of 92-94% is necessary for preventing measles transmission in the United States population (5).

Rubella (German measles) presents with a rash, low-grade fever, lymphadenopathy, and malaise, yet roughly 50% of infections are subclinical (1). The virus is transmitted through direct contact or droplets from nasal secretions. Infection is

particularly dangerous in pregnant women and can result in miscarriages, stillbirths, and birth defects (congenital rubella syndrome) (1). An infected person is contagious one week prior to 7 days after onset of a rash (5). Rubella was verified as eliminated from the United States in 2004, and a median of 11 cases per year have been seen since elimination (9). All cases since 2012 were imported into the United States from other countries (9). An estimated seropositivity proportion of 85% is necessary for preventing rubella transmission (10).

Mumps presents as fever and inflammation of the salivary glands (parotitis) and is spread through droplets from nasal secretions or contact with unwashed surfaces (5). Symptoms typically appear 16-18 days after exposure (range: 12-25 days), and complications include infertility and oophoritis in adolescent and adult females, hearing loss, pancreatitis, meningitis, and encephalitis (1). Reported cases declined by 98% after the mumps vaccine was first introduced, and by 2000-2005, fewer than 350 cases per year were recorded (11). However mumps saw a resurgence in 2006 among college campuses with over 6,500 cases reported (11). Various other outbreaks ranging in size and occurring in highly vaccinated populations have also occurred since 2006, including an outbreak in New York and New Jersey among Orthodox Jewish persons (89% had received 2 doses of MMR) in 2009-2010 (12) and outbreaks on various colleges campuses in Illinois and California (13, 14). In 2016, there were 5,311 cases reported in more than 40 states (15). As of February 2017, 1,077 cases were reported in over 30 states (16). An estimated seropositivity proportion of 90% is necessary for preventing mumps transmission and providing adequate herd immunity in a population (10, 17).

The MMR Vaccine

The measles vaccine first became available in 1963 and then was improved through use of a weaker virus strain in 1968. The mumps vaccine became available in 1967, followed by the rubella vaccine in 1969 (1). The three vaccines were combined into one vaccine in 1971 creating what is today known as the Measles-Mumps-Rubella (MMR) vaccine. Currently, two live attenuated vaccines are licensed and available in the United States: trivalent MMR vaccine and quadrivalent MMRV (measles-mumps-rubella-varicella, licensed in 2005) vaccine (1).

Initial recommendations in 1963 included one dose of the measles vaccine for those 9 months of age (1). The age for the combined MMR vaccine was later increased to 15 months in 1976 because of demonstrated increased effectiveness. However, prompted by measles outbreaks in school age children (18), in 1989 ACIP (Advisory Committee on Immunization Practices) and AAFP (American Academy of Family Physicians) recommended a 2-dose regimen of the MMR vaccine with the first dose at ages 12-15 months and the second dose at ages 4-6 years (1). However, concurrently AAP (American Academy of Pediatrics) recommended the second dose be instead administered before middle school entry, citing that many of the outbreaks occurred in older school age children (1). Nonetheless in 1994, AAP changed their recommendations to match those of ACIP and AAFP (19). However, not until 2005 did all states enforce the 4-6 year old requirement (19). Revaccination with a second dose is intended to promote seroconversion among those who initially did not produce an immunologic response to the measles component of the first dose (primary vaccine failure) (1). The 2-

dose strategy led to successful elimination of measles and rubella, and also decreased the average number of mumps cases to less than 300 per year in the early 2000's (20).

Although recommended for 4-6 year olds, revaccination can occur any time after 28 days post-first dose vaccination (6). Those who have had life threatening allergic reactions to the components of the vaccine or previous dose and pregnant women should not get the vaccine. Those who have HIV/AIDS, immunosuppressive conditions, cancer, and blood disorders should consult with a doctor before receiving MMR vaccine (1).

MMR vaccine coverage has remained high for the past decade. According to the National Immunization Survey, in 2015, 91.9% (±0.8) of children aged 19-35 months had received at least 1 dose of the MMR vaccine (21). An estimated 90.7% (±0.8) of 13-17 year olds had received at least 2 doses of the MMR vaccine (22). However, vaccination coverage is not uniform across states and ethnic groups, as there exist many pockets of unvaccinated populations. For instance, Mississippi's school exemption rate of 0.1% is significantly lower than Washington's rate of 6.3% (7). Overall, it is estimated that approximately 12.5% of U.S. children are susceptible to measles, with up to 24.7% of children ages three years or younger susceptible (23). The clustering of susceptible individuals raises concerns regarding potential pockets for resurgence. Additionally, Hispanic and Asian populations have lower reported coverage estimates (88.1% and 87.5% respectively) (24). Cited barriers to vaccine uptake include concerns of safety, the incorrect assumption that the MMR vaccine is associated with autism, objections to large numbers of injections, language barriers, and lack of information and education (25).

Immunological Response and Vaccine Effectiveness

The MMR vaccine induces antibodies against all three components in most children following vaccination and has been found to be effective in preventing clinical disease (1). Vaccine immunogenicity is measured by serum collection and an analysis of antibody levels is conducted using modified plaque reduction neutralization (PRN) assays (26). Seroprotection thresholds for measles, mumps, and rubella are >120 mIU/mL, >10 mIU/mL, and ≥10 mIU/mL respectively (19, 27, 28).

The vaccine induces both antigen specific humoral and cellular immunity, however it is unclear which response plays a larger role (17, 26). Roughly 96% of children vaccinated at 12 months develop measles antibodies after the first dose, and nearly all develop antibodies following the second dose (1). Vaccine effectiveness in preventing measles was found to be 93% (range: 39-98%) after 1 dose in children 12 months of age and older, and 97% after the two dose regimen (1). Roughly 95% of those ≥12 months of age develop rubella antibodies after a single dose (1). This number increases to 99% after the second dose. Vaccine effectiveness against rubella was found to be 97% (range: 94-100%) after one dose (1). Seroconversion after 1 dose is lowest for mumps (94%, range: 89-97%). Vaccine effectiveness against mumps is reported to be 78% (range: 49-92%) after 1 dose and 88% (range: 66-95%) after 2 doses (1). Data on the effectiveness of a third dose is limited. Nonetheless, it is important to note, vaccination produces lower levels of antibodies against measles, mumps, and rubella compared to natural infection, which raises questions related to duration of protection (1).

Vaccine Failure and Outbreaks

There are currently two classifications for vaccine failure: primary and secondary failure (5). Primary vaccine failure is the lack of an immunologic response following vaccination, whereas, secondary vaccine failure is the inability to maintain appropriate protective immunity over time (29). Vaccine failure has been noted to occur during outbreaks and other exposures (12, 14).

Both measles and rubella have been declared eliminated in the United States, but measles outbreaks continue to occur and are typically due to importation of cases into pockets of unvaccinated populations (30). In 2015, a large multi-state outbreak was linked to a strain often seen in the Philippines; and in 2014 an outbreak occurred in unvaccinated Amish communities in Ohio following importation of measles virus from community members who traveled to the Philippines to assist in rebuilding efforts following Typhoon Haiyan (6). Although the majority of measles cases occur in unvaccinated persons, measles in vaccinated persons have been reported (1). Few rubella cases have been cited since an outbreak in the early 1990's (7). Even though these two diseases are considered eliminated, it is important to understand the patterns of antibody persistence and the potential for waning immunity that may necessitate revaccination to create appropriate recommendations and policy that ensure proper protection of individuals in a world increasingly connected through travel and trade, since both diseases remain endemic in much of the world.

Although it is understood that two doses of the vaccine provide sufficient immunity for ≥15 years for all three illnesses (1), mumps outbreaks continue to occur in populations with high 2-dose MMR vaccine coverage, indicating potentially insufficient

vaccine-induced immunity for some individuals or waning levels over time that increase the number of susceptible individuals in a population (17). For instance mumps outbreaks have occurred on university campuses and within other close-knit communities among highly 2-dose vaccinated populations (11, 12, 31, 32). During a California outbreak, 76% of the cases occurred among people previously vaccinated with 2 doses (1). Hypothesis for these vaccine failures include waning antibody levels over time and the potential for antibody levels to vary across time, as well as, high levels of exposure due to increasing spread among unvaccinated individuals that can overcome vaccine-induced immunity (33). In turn, these outbreaks raise questions in regards to persistence of antibodies, timing of vaccinations, and the need for additional booster vaccinations.

In light of recent outbreaks, questions regarding the need for a third dose have been raised (14). Historically a third dose has only been administered to healthcare workers whose serologic data indicates potential susceptibility, military recruits without regard to previous vaccinations, women potentially susceptible to rubella before or following pregnancy, adults entering college or those traveling abroad who lack previous vaccination documentation, and more recently, for mumps outbreak control (5).

Antibody Persistence Following Vaccination

Antibody levels are used as a proxy to determine immunity to measles, mumps, and rubella (29). When initially introduced, the MMR vaccine was suggested to provide lifelong protection and immunity, but a large number of studies have shown that overall titer levels vary and tend to wane over time for measles, mumps, and rubella (10, 34, 35). This decline in antibodies is faster for vaccinations compared to naturally acquired infections (10). Such is particularly the case as the circulation of natural diseases wane,

eliminating the potential natural boost they provided to antibody levels (36). Below, I describe the results of persistence studies in terms of each of the three diseases, as well as, how persistence relates to the number of doses, age, and time since vaccination.

Measles Antibody Persistence

One major question in terms of persistence is the difference in antibody levels following the first dose compared to the second dose of the vaccination series. In a study conducted by Vandermeulen et al., antibody levels in students ages 17-23 years were measured roughly 19 years after the receipt of either one (before turning 3 years old) or two (before turning 13 years old) doses (35). The proportion of measles seropositive subjects was significantly higher for those who were vaccinated with two doses compared to one dose, indicating a second vaccination is important in ensuring humoral immunity over time and increasing the level of protection conferred (35). The authors further suggested that the second dose of the vaccination should be considered a complete booster, rather than a "catch-up dose" for the proportion of the population that did not immunologically respond to the first dose (35). In contrast, a measles antibody persistence study among 4-8 year old Austrian children conducted by Paulke-Korinek et al. showed that antibody levels were not significantly affected by the number of doses of the vaccine received, after controlling for time since vaccination, yet the immunization schedule differed between the two countries (Austrian schedule recommended 2 doses during the second year of life) (34). The follow-up period was also significantly shorter and the study lacked a sufficient sample size and power among those only vaccinated once (34). Ultimately, evidence suggests that use of the two dose vaccine regimen provides the best long-term protection.

Time since previous vaccination is also a factor that has been suggested to influence antibody persistence. In Lebaron et al.'s study comparing those vaccinated with MMR2 at kindergarten vs. middle school age, time since last vaccination was the only significant factor found to be associated with higher antibody levels; however ultimately this variable explained little in regards to titer variation (27). In a 2016 study conducted in Portugal by Goncalves et al., researchers examined measles IgG antibodies in two cohorts: those who received the second dose at ages 5-6 years and those that received the second dose at ages 10-13 years (37). Significantly rapid waning immunity was observed with almost 50% of all participants falling below 150 mIU/mL 7.5 years post-MMR2 vaccination (34, 38). Nonetheless, the age of vaccination did not affect the pattern of waning antibody levels (38). Therefore, researchers concluded the importance of vaccination dosing, reinforcing Vandermeulen et al.'s results, rather than changing the age of vaccination schedules in children (38). Nonetheless, this study's results raised significant questions in terms of time since vaccination.

Such questions were further explored by Paulke-Korinek et al., whose study of Austrian 4-8 year olds found a significant negative association between antibody levels and time since last vaccination, raising questions about life-long immunity (34). In a similar study of individuals conducted in Finland, researchers followed two cohorts (14-18 months and 6 year olds at time of first vaccination) for nine years (specimens collected for baseline, 3 months post-vaccination, yearly for the first 6 years, and the 9th year of the study). Initial results showed that 95% of initially seronegative individuals enrolled became seropositive for measles after two doses of the vaccine (10). Antibody levels rose with vaccination and remained high and stable, with only small decreases, for

the first four years (39), unlike the initial rapid waning observed by Goncalves et al. (38). Yet antibody levels dropped significantly throughout the follow-up period and were 1/10 of the original levels seen in the first few years by year nine (39). In a 20 year follow-up of the same Finnish individuals, among those who were originally seropositive, levels were higher in the younger group, however, small sample size limited a complete comparative analysis (10). LeBaron et al. reported a similar decreasing pattern in the geometric mean titer (GMT) values across time following MMR2 vaccination in a cohort of 4-6 year olds and 10-12 year olds across a 10 year follow-up period (27).

Mumps Antibody Persistence

Similarly to measles, in terms of vaccination dose, according to Vandermeulen et al., the proportion of seropositive individuals is significantly higher following two doses of the MMR vaccine compared to one dose (67.5% vs. 55.6%) in 17-23 year olds (35). Paulke-Korinek et al.'s study supported these results with a final model indicating the number of vaccinations was the only significant parameter related to antibody levels (59.7% seropositive after the first vaccination and 74.4% seropositive after the second vaccination) (34).

Other more long-term studies have also indicated a significantly better humoral immune response to the two dose regimen. In the 20 year Finland follow-up study described above, results indicated antibody levels decrease rapidly following the initial dose throughout the first year post-vaccination, yet were significantly boosted with a second dose (74% of the 183 enrollees became seropositive for mumps after two doses) (36). The second dose was followed by a slower rate of decay, therefore concluding the booster vaccination is crucial for maintaining immunity across time.

In terms of age at vaccination, Davidkin et al.'s study displayed similar results to that of measles: initial antibody response was comparative between the two age cohorts (14-18 months and 6 year olds) (10). Among those who were originally seropositive, levels were higher in the younger group, however, small sample size limited a complete comparative analysis (10). Data was not available for years 10-19 of the follow-up period and, therefore, it is unclear in this study how antibody levels changed between sampling dates, although it is understood that the rate of decline was relatively slower in the final 7 years of the study (10).

In a study examining mumps long-term vaccine performance among university students during a 2006 mumps outbreak (95% of cases vaccinated), it was found that those infected were more likely to have received the second dose of the MMR vaccine ≥10 years earlier (the odds of being a case increased with each year increase in the time between second dose vaccination and infection for 18-19 year olds) indicating waning protection (31). LeBaron et al. also reported a decrease in the GMT values across time following MMR2 vaccination, with 5% seronegative by the end of the 12 year follow-up period (19). However, the potential rate of decline that occurred within these studies was not described.

Nonetheless, it is important to note that roughly 66% of those who typically do not seroconvert develop cellular immunity (memory T cells) (17). Jokinen et al. examined both humoral and cell-mediated response to the mumps component of the MMR vaccine (2 doses in childhood) by comparing vaccine-induced immunity and natural mumps infection-induced immunity in both those 22-23 years old and 27-28 years old (17). Results indicated similar IgG antibody levels, lymphoproliferative response,

antigen-specific interferon-γ production, and interleukin-10 production (17), indicating that although antibody levels sometimes differ between vaccinated and naturally infected individuals, the cellular response may not (17). Ultimately, the researchers concluded that cell-mediated immunity does not necessarily correlate with humoral immunity (17). The authors also suggested that although antibody levels may wane over time, cellular immunity may persist longer (17), potentially suggesting individuals may remain protected even when antibody levels fall. However, further research is needed.

Few studies have examined response after a third vaccination and little in known in regards to long-term persistence. In terms of seroconversion after the third dose, Date et al. found that the majority of subjects initially seronegative after 2 doses, became seropositive for mumps 1-3 months followings the third dose (29). There was no evidence of primary vaccine failure in this study, however, it is unclear the type of vaccine failure that occurred among those individuals in the study that were seronegative as young adults after completing the 2 dose series (29). Antibody response and persistence following MMR3 was also examined in a 2016 study conducted by Fiebelkorn et al. at 1 month and 1 year post-vaccination (40). Results indicated a significant association between those vaccinated 12 months to <15 months and being seronegative. All subjects saw a modest boost, but the majority of individual's titers returned to pre-MMR3 baseline values by 1 year post-vaccination, aside from those seronegative at baseline who remained seropositive (40).

Rubella Antibody Persistence

Similarly to measles and mumps, according to Vandermeulen et al., the proportion of seropositive individuals is significantly higher following two complete

doses of the MMR vaccine compared to receipt of only 1 dose (99% vs. 71.4%) (35). However, the study lacked power to detect significant differences in geometric mean titers between the one dose and two dose groups (35). Goncalves et al. further argued that instead of focusing on the age of vaccination, sustaining high coverage should take precedence (37).

In a 2016 study conducted in Portugal (37), no difference in antibody concentrations or the proportion of seronegative individuals was observed in relation to the time since vaccination. Nonetheless, the authors noted males recorded lower rubella IgG levels compared to females. However, this study was cross-sectional in nature and shows little about changes in IgG levels over time, inhibiting a formal analysis of differences by sex (37). In contrast, LeBaron et al. also reported a decrease in both sexes in the GMT values across time following MMR2 vaccination (28).

Persistence and Clinical Factors

Few studies exist that examine the effect of clinical conditions and prescribed medications on antibody levels and persistence following MMR vaccination. Nonetheless, Heijstek at al. did find that diagnosis with juvenile idiopathic arthritis (JIA) was negatively associated with geometric mean antibody concentrations against mumps and rubella, but not measles (41). Although the mechanisms are unclear, the researchers suggest primary immunodeficiency may produce lower response levels in JIA patients (41). Other immunosuppressive conditions, such as HIV/AIDS may also produce poor immunological response to vaccination, and may cause a loss of the conferred protection at a faster rate (42). Nonetheless, more research is needed to fully understand the extent

that clinical and genetic factors play in seroconversion rates, as well as, antibody persistence for measles, mumps, and rubella.

Marshfield Clinic Research Foundation Studies of Antibody Persistence

In 1994, Marshfield Clinic Research Foundation (MCRF, Marshfield, Wisconsin) and the CDC examined short and long term antibody response after the second MMR vaccine dose, and compared kindergarten vs. middle school vaccination schedules to examine differences in antibody response and persistence (19, 27, 28). Two groups of individuals (4-6 year olds and 10-12 year olds) were simultaneously vaccinated. All participants were followed until they turned 17 years old (11-13 years for the younger cohort, 5 years for the older). Serum levels were collected at intervals that allowed for time comparisons between the two groups. Titer levels were evaluated by plaque-reduction neutralization. It was found that at the same ages, there were no differences in levels between the two groups, and at the end of the study the proportion of children with low titers was similar to that before the MMR2 vaccination (19, 27, 28).

During 2009-2010, a second study (n=685) was conducted by MCRF and CDC to examine immune response and adverse events after a third dose (MMR3). Researchers administered MMR3 to young adults from two source populations (113 participants from the 1994 MMR2 study described above and 572 people with documented two prior doses recruited from the community) to assess immunogenicity over 1 year (40, 43). Serum was collected at baseline, 1 month after MMR3 vaccination, and 1 year after MMR3 vaccination. Results indicated few improvements in immune response to the measles and mumps component after receiving MMR3 (40, 43); data on response to rubella are

pending. In 2014, a 5 year assessment of long term immunogenicity of MMR3 was initiated by MCRF and CDC.

Current Analytic Methods used to Evaluate Antibody Persistence

The studies described above analyzed antibody presence and persistence using subgroups categorized according to available data on titer level (negative, low-seropositive, and high-seropositive) or GMT (geometric mean titers, calculated using log-transformed reciprocal titers) of the population that returned for the visit at each time point using techniques such as linear regression. Although this provides an informative population perspective, it says little about individual variation that may exist and the influence of this variation on an individual's susceptibility over time. Particularly given the outbreaks described above among highly vaccinated 2-dose populations, individual level analysis is important to understand patterns of susceptibility.

In turn, previous analysis of the data did not examine individual patterns nor assess characteristics of individuals with unique or variable antibody persistence patterns unlike the majority of the population. The nature of these studies also prevented a complete understanding of changes within individuals in the categorized subgroups, the associated time points of these changes, and clinical factors that may have led to such changes. Ultimately, prior studies failed to fully utilize the longitudinal nature of the data collected, preventing complete assessment of antibody persistence and patterns.

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Chapter II. Manuscript

Title

Measles, Mumps, and Rubella Antibody Patterns of Persistence and Rate of Decline Following the Second Dose of the MMR Vaccine

Authors

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Abstract

Previous studies of the measles, mumps, and rubella (MMR) vaccine have reported a high degree of individual variation in patterns of waning antibody immunity, yet none have defined the degree of variation that exists. We utilized data from a 12 year longitudinal study of Wisconsin children vaccinated with the second dose of MMR (MMR2) at 4-6 years of age to identify patterns of seropositivity and antibody persistence for each antigen following receipt of MMR2, compare antibody kinetic patterns across antigens, and estimate the rate of decline in antibody levels using correlated data methods. Of the 313 study participants who received MMR2, 302 had measles antibody data and 296 had mumps and rubella data. The majority of participants remained seropositive for the follow-up period for all three antigens (96% measles, 88% mumps, 79% rubella). Among the 291 individuals with defined antibody persistence trends for all 3 antigens, 41 (14%) had the same trend categorization for measles, mumps, and rubella (2 stable, 18 declining, 17 variable, and 4 other), 188 (65%) had the same trend for 2 of the 3 antigens, and 62 (21%) had discordant trends for all three antigens. Among the individuals characterized as having a "declining trend" for measles, antibodies were high post-MMR2 (1 month post GMT: 3892.7 mIU/mL), but declined an average of 9.7% per

year among those with the same baseline titer and no response to MMR2 (<2 fold increase), adjusting for sex. Those with MMR2 response of ≥2 fold experienced a slower decline (6.3% per year among those with 2-4 fold increase and 7.4% per year among those with ≥4 fold increase). Mumps rate of decline was 9.2% per year, adjusting for MMR2 response and baseline titer. Rubella antibodies declined an average of 2.6% per year among those who received MMR1 at age 12-15 months, and 5.9% per year among those who received MMR1 >15 months, adjusting for baseline rubella titer and MMR2 response. The high variation in persistence trends coupled with the fast rate of decline within a subset of individuals may impact herd immunity and individual susceptibility, particularly in outbreak scenarios.

Introduction

Since 1989, two doses of the measles, mumps, and rubella (MMR) vaccine have been recommended in the United States. This successful 2 dose program led to elimination of measles and rubella, and a 96% reduction in mumps cases (1). However, despite the availability of a proven safe and effective vaccine and vaccine coverage rates of over 90% (2), outbreaks of measles and mumps continue to occur (3-5). Given these continued outbreaks, it is important to better understand the patterns of antibody persistence and duration of protection to provide appropriate recommendations and ensure protection during outbreak scenarios among individuals who received two doses of MMR vaccine.

Patterns of variability and their associated factors are not well understood. Prior studies focused on antibody persistence following a second dose have recognized patterns of waning immunity and raised critical questions regarding the presumed lifelong

protection from vaccination, yet results concerning rate of decline and what factors affect the rate of decline vary (6, 7). These studies examined factors such as timing of doses, vaccination age, and time since vaccination (6, 8-13), noting that not all individuals respond the same to vaccinations and citing large differences to the degree of waning observed (7, 14, 15).

Furthermore, these studies analyzed antibody persistence using subgroups categorized according to seropositivity levels or cohort geometric mean titers (GMTs) at each follow-up time point or only had 1-2 follow-up visits (6, 7, 9, 16). Although these studies offered an informative population perspective, there was little information provided about individual variation.

In a previous study, a cohort of children who received MMR2 at age 4-6 years at Marshfield Clinic in Marshfield, Wisconsin was followed from 1994 through 2007 (10-12). As expected, antibodies waned over time among participants, but the majority of individuals were seropositive for all three antigens at the end of the follow-up period (10-12). To better understand individual variations following MMR2, we conducted an expanded analysis of this dataset. Using correlated data methods we identified patterns of seropositivity and antibody persistence following receipt of MMR2, compared antibody kinetic patterns across all three MMR antigens, and estimated the rate of decline in antibody titers over time.

Methods

Study population and procedures

Details of the study population and procedures have been previously described (10-12). Briefly, participants received MMR2, and serum samples were collected for

antibody testing prior to MMR2 (baseline) and throughout a 12 year follow-up period (post-vaccination at 1 month, 6 months, 2 years, 5 years, 7 years, 10 years, and 12 years). For this analysis, only study participants who received MMR2 at age 4 to 6 years, as currently recommended by the Advisory Committee on Immunization Practices (ACIP) (17), were included.

Antibody levels were evaluated by plaque-reduction neutralization (PRN) using immunoenzymatic staining as described elsewhere (10-12). Antibody testing for rubella and mumps was performed at the end of the 12 year study, and specimens from the same individuals were tested in the same run. For measles, specimens collected through the 10 year post-MMR2 visit were tested after completion of that visit. The 12 year post-MMR2 measles visit samples were tested after completion of that visit using the same reagents and operators as the other tests. Samples were stored at -80°C until testing.

Analysis

To assess patterns in antibody titers over time, only participants with a visit (and antibody data) before vaccination (baseline), 1 month post-vaccination, and at least 1 other visit for the antigen of interest were included to ensure ability to analyze using time series methods. A few participants (8% of all 4-6 year olds vaccinated with MMR2) did not have enough sample to test all three antigens for at least one visit.

Descriptive patterns

Participants were classified into groups based on patterns of seropositivity and antibody titer trends during the 12 year follow-up period for each antigen (measles, mumps, and rubella). Comparisons were made across all three antigens.

Seropositivity pattern. Each visit following vaccination was categorized as seropositive if titers were >120 mIU/mL for measles, ≥10 mIU/mL for mumps, and ≥10 mIU/mL for rubella (10-12). Individuals were then categorized into 3 'seropositivity' patterns based on every visit with a measured titer: seropositive, seronegative, and inconsistent. Seropositive were those with a seropositive titer at every visit throughout follow-up. Seronegative were those who became seronegative at some point during the follow-up period and remained seronegative until the subject's last study visit. This group also included those who became seronegative at their last visit. Inconsistent were those who had a seronegative titer at ≥1 visit followed by a seropositive titer.

Persistence Trends. Trends in antibody titer levels were categorized into four persistence trends defined a priori and based on trends between time points for visits ≥6 months post-MMR2 and in relation to baseline values: stable, declining, variable, and other. The stable category included those with titers at or around baseline that did not decline over time. More specifically, individuals categorized as "stable trend" had titers at all visits ≥6 months post-MMR2 vaccination within 2 fold of the titers at their previous and baseline visits; and <65% of visits ≥6 months post-MMR2 had lower titers than the previous visit (indicating a majority were not decreasing). Individuals with a "declining trend" had ≥65% of visits ≥6 months post-MMR2 with titers lower than the prior visit (<1 fold) and no visits ≥6 months post-MMR2 with >2 fold increase. For those whose titers at 6 months post-MMR2 were ≥2 fold lower than previous visit, visits ≥2 years post-MMR2 were considered for classification of the stable and declining trends since the rate of decline immediately following vaccination may vary. Those with a "variable trend" had significant increases and/or decreases in titers across time as defined by: at

least 1 visit ≥6 months post-vaccination with ≥4 fold increase from prior visit, at least 1 visit ≥2 years post-MMR2 with >4 fold decrease from prior visit, or at least 1 visit ≥2 years with >2 fold increase and at least 1 visit with >2 fold decrease from prior visit. For the latter two criteria, the titer at 6 months post-MMR2 was also considered if it was greater than or equal to the value at 1 month post-vaccination. Individuals who could not be classified into the above categories were placed in the "other trend" category. Differences in characteristics between groups were assessed using chi-square tests for categorical variables and ANOVA for continuous variables.

Estimating rate of antibody decline within "declining trend"

Repeated measures linear mixed models that allowed for within and between-subject variations were utilized to estimate the rate of decline in antibody titers after vaccination among those with a declining trend. Backwards elimination in SAS 9.4 (Cary, NC) was used to determine the most appropriate model (factors retained if p-value <0.05). Covariates examined included: sex, age at first dose of MMR (MMR1; 12-15 months vs.16-24 months), level of MMR2 response defined by dividing 1 month post-vaccination titer by pre-vaccination titer (<2 fold increase, 2 to <4 fold increase, ≥4 fold increase), log2-transformed baseline titers, and time elapsed between receipt of MMR1 and MMR2 in months.

Separate models were created for measles, mumps, and rubella using log2-transformed titers for the period 6 months post-vaccination to 12 years post-vaccination. One month post-vaccination titers was excluded to ensure most individuals were no longer experiencing significant boosts following vaccination. The 1 month visit also is represented in the model within the MMR2 response variable. Rate of decline was not

assessed for other persistence trends due to high variability and unpredictability in titers across time by definition.

Sensitivity analysis

Two sensitivity analyses were performed using the same procedures to assess the degree that missing data and variability in laboratory methods may have influenced trend categorizations or model results. Missing values were present within the dataset because not all individuals returned for every follow-up visit and for those that did, some did not have enough sample to test for all three antigens. To assess the impact of the missing data, we conducted analyses restricted to those who had data for all 8 visits. For measles, since the final MMR2 study serum collection (12 years post-MMR2) was tested separately, we conducted analyses excluding the final MMR2 visit for measles.

This study was approved by the Institutional Review Board at Marshfield Clinic Research Institute and Emory University.

Results

Of the 313 study participants who received MMR2 at age 4-6 years, 302 had measles antibody data and 296 had mumps and rubella data for the baseline pre-vaccination visit, 1 month post-vaccination, and at least 1 additional follow-up visit. The mean number of study visits was 5.9. Nearly half of the participants had antibody titers for all 8 visits (47%, 48%, and 42% for measles, mumps, and rubella respectively). Participants were primarily white (non-Hispanic) and approximately 50% female. The majority of children (59%) received MMR1 between ages 12-15 months, and mean time between MMR1 and MMR2 was 3.7 years. Prior to receipt of MMR2, 99%, 85%, and 92% were seropositive for measles, mumps, and rubella, respectively (Table 1).

Seropositivity patterns

For measles, 291 (96%) were seropositive for the entire follow-up period, 5 (2%) became and remained seronegative sometime after 6 months post-vaccination, and 6 (2%) had an inconsistent pattern (Table 2). For mumps, 260 (88%) were seropositive the entire follow-up period, 22 (7%) became and remained seronegative, and 14 (5%) had an inconsistent pattern. For rubella, 235 (79%) were seropositive, 11 (4%) became and remained seronegative, and 50 (17%) had an inconsistent pattern, including 3 individuals who were seronegative at baseline and remained seronegative until 2 years post-vaccination (Table 2).

Among the 291 individuals with defined seropositivity patterns for all three antigens, 212 (73%) were seropositive for measles, mumps, and rubella for the entire follow-up period, 67 (23%) were seropositive for 2 of the 3 antigens, and 12 (4%) had a different seropositivity pattern for each antigen (Table 2).

Seropositivity patterns excluding the 12 year follow-up visit for measles were similar to that considering all visits. However, the proportion seropositive among those who had complete data for all 8 visits was lower than the entire cohort (93% for measles, 80% for mumps, and 68% for rubella).

Persistence trends

For measles, 169 (56%) had a declining persistence trend, 50 (17%) variable, 38 (13%) stable, and 45 (15%) other. For mumps, 29 (10%) were declining, 96 (32%) variable, 63 (21%) stable, and 108 (36%) were other. For rubella, 110 (37%) were declining, 98 (33%) variable, 31 (10%) were stable, and 57 (19%) other (Table 2). Pre-vaccination titer levels were significantly different across trends for all 3 antigens (p-

values: 0.01, <0.001, <0.001 for measles, mumps, and rubella respectively; Table 3). Level of MMR2 response, as categorized above, also differed across trends for measles (p-value: 0.0002), but not mumps or rubella. No other significant demographic or clinical differences were present across trends within each antigen (Table 3).

Among the 291 individuals with defined persistence trends for all 3 antigens, 41 (14%) had the same trend categorization for measles, mumps, and rubella (2 stable, 18 declining, 17 variable, and 4 other), 188 (65%) had the same trend for 2 of the 3 antigens, and 62 (21%) had discordant trends for all three antigens (Table 2).

Persistence trends were similar when the cohort was restricted to those with data for all 8 visits. When excluding data from the 12 year follow-up visit for measles, the proportion with a variable pattern decreased from 17% to 10% and declining pattern increased from 56% to 60%.

Decline in antibody titers among those with declining trend

The rate of decline in measles antibody titers varied with response to MMR2 and baseline measles antibody levels among 169 individuals with a declining pattern (Table 4). Among those with the same baseline titer and no response to MMR2 (<2 fold increase), measles antibodies declined an average of 9.7% per year, adjusting for sex. Those with MMR2 response of ≥2 fold experienced a slower decline (6.3% per year among those with 2-4 fold increase and 7.4% per year among those with ≥4 fold increase), adjusting for sex (Figure 1A). We found that total population variation in titers among individuals was high (61.9%), yet a large portion of a single individual's variation was related to time (47.7%). However, a variance components analysis indicated unexplained within-person and baseline variation (intercept) remains after model

selection, indicating other factors not included in this analysis may influence the rate of decline.

Among 63 who had measles data for all visits, the mean rate of decline was similar: 9.1% per year among those with no response to MMR2, 6.2% per year among those with 2-4 fold increase after MMR2, and 7.8% per year among those with ≥4 fold increase after MMR2. The mean rate of decline with exclusion of year 12 follow-up visits (63 participants) was slightly faster per year (13.2%, 9.5%, and 5.6% among those with <2 fold increase, 2-4 fold increase, and ≥4 fold increase after MMR2, respectively).

Mumps rate of decline was found to be 9.2% per year among 29 individuals with a declining trend. Unlike measles, no factors examined contributed to mumps rate of decline (Table 4). We found <11% of within-person variation was explained by time (Figure 1B), leading to the conclusion that significant unexplained within-person and between-person variation remains. Insufficient data prevented construction of a model restricted to individuals who completed all 8 visits.

For 110 individuals with declining pattern for rubella, the mean rate of decline in rubella antibody titers varied based on age at MMR1 vaccination (Table 4). Antibodies declined an average of 2.6% per year among those who received MMR1 at age 12-15 months and 5.9% per year among those who received MMR1 after age 15 months, adjusting for baseline (pre-MMR2) rubella titers and MMR2 response (Figure 1C). Similar to measles, over half of total titer variation (52.2%) was found to exist between individuals. However, much less within-person variation was associated with linear time compared to measles (17.0%). Nonetheless, there remains unexplained variation, indicating factors not included in this study influence the rate of decline observed.

Among 25 with complete data for all visits, the mean rubella rate of decline was slightly higher, 3.2% and 6.9% per year among those who received MMR1 at age 12-15 and >15 months, respectively.

Discussion

We examined individual patterns of measles, mumps, and rubella seropositivity and antibody persistence, as well as, assessed the rate of decline for each antigen within a cohort of children who received MMR2 and had a declining pattern. Overall, we found that most individuals remained seropositive for all three antigens at least 12 years post-MMR2, indicating a high level of protection within the population. Percent seronegative results recorded at each individual follow-up visit are consistent with Lebaran et al.'s 12 year study (10-12) and Davidkin et al.'s 20 year study (14). However, unlike these studies that provided intermittent snapshots at the population level, our longitudinal analysis also captured an additional subset of individuals with inconsistent titers who were seropositive at the final follow-up visit, but had been seronegative at ≥1 previous visit. Identification of these individuals and their unexpected trends sheds light onto how and when 2-dose vaccinated individuals may have been at risk during outbreaks. Additionally, the high proportion of individuals with seronegative or inconsistent rubella trends also poses an issue to those traveling overseas to rubella endemic areas, especially if they are females of child-bearing age. Yet the biological reasons for becoming seronegative or fluctuating within such a short period post-vaccination remain unknown.

Although most individuals remained seropositive for all 3 antigens, persistence trends displayed significantly higher variability both among individuals within each antigen and within individuals across the 3 antigens. We found only a small subset of

persons having concordant patterns across the 3 antigens. Other studies have also recorded differences among individuals in response to vaccination (18, 19) and large variation in persistence across time (6, 7, 9, 13, 14), yet our analysis provides a quantitative picture of individual variability. It is important to understand the breadth of this variation within a population because of its potential impact on community protection. Community protection is measured by examining total number fully vaccinated within a population; however, this study reveals sufficient titer levels among those vaccinated should also be considered when modeling population susceptibility (20). The high degree of within-person variation observed also indicates that although the vaccine combines protection against 3 antigens, individuals may biologically respond to the respective components in differing ways. Nonetheless, the demographic, clinical, and genetic factors associated with these differences require further investigation.

Linear mixed model results indicated measles and mumps antibodies decline at a faster rate compared to rubella. Davidkin et al. reported similar rates of decay (calculated using GMT percent yearly change) for measles and mumps (7.1% and 9.9% respectively for the 8 years post-MMR2), but reported a meaningfully higher rate of decay for rubella (8.2%) (14). We restricted our analysis to those with a clear declining trend, and therefore, the differences observed between studies may be explained by the higher population variability in other studies that did not restrict their analysis. In our analysis, approximately one third of individuals had a variable rubella pattern, indicating they had unexpected boosts or declines, and were excluded from the analysis in order to obtain a more accurate rate among those with declining titers. Our study also provided additional information in terms of factors associated with differing rates of decline. Baseline titers

and MMR2 response level for measles and age at MMR1 for rubella were identified to influence the rate of decline. Nonetheless, it is unclear the significance of these factors. Future investigations should focus on predictive models that allow for identification of individuals whose antibody levels are expected to decline at a faster rate, potentially warranting a third dose of MMR to maintain immunity.

Overall rates of decline observed in this study indicate waning titers leading to potential increased susceptibility, therefore, continual monitoring of declining titers for longer periods of time with larger sample sizes is required to ensure adequate population protection remains. It is also important to consider those with declining or variable patterns who may not fall below seroprotective levels, but come close, and therefore, are potentially more susceptible. The declining immunity of all 3 antigens may pose serious concerns to clusters of vaccine refusers, offering the opportunity for rapid spread following disease introduction into the cluster among the unvaccinated. Particularly for mumps, our model results indicate eventual decline below the seropositivity level for a subset of subjects, potentially implicating insufficient vaccine-induced immunity as a contributing factor in recent mumps outbreaks (3, 5, 21) where, in some, over 70% of recorded cases were within fully vaccinated individuals (3).

Sample size for the declining trend mumps model was small (70% of mumps trends were variable or could not be defined). This small sample size could be due to the nationwide mumps outbreaks (1996-1997 and 2006-2007) that impacted the Midwest during the study period (10-12). Although no study participant reported mumps infection, individuals may have experienced natural boosting from wild-type virus exposure (11). The natural boost may have contributed to the unexpected variation observed. Given the

continued mumps outbreaks, future assessments should focus on the impact of potential boosting on persistence patterns and trends among MMR2 recipients.

This study was subject to several limitations. Our analysis was conducted using a homogenous cohort of individuals living in the same geographic area and therefore, may not be representative of the U.S. population. The analytic portion of the study was also restricted to those who displayed a declining, non-variable persistence pattern, and therefore, results may not be applicable to all individuals. Although the restriction is a strength in this study, in that it allowed for a more precise qualification of rates of decline, it also limited our ability to model those with non-declining trends, which makes up a large portion of the mumps and rubella cohort. Misclassification of patterns and trend categorization likely occurred in some individuals due to missing data points or loss to follow-up. Nonetheless, sensitivity analysis using complete data revealed reasonable accuracy in original estimates. Additionally, the faster rate of decline observed after exclusion of visit 12 in the measles model was expected in that random lab errors likely drove the rate of decline towards 0 in the original model.

This additional analysis provided a greater understanding of antibody seropositivity and persistence patterns following receipt of MMR2. We confirmed the high variability in individual persistence trends and, showed how correlated data methods can be used to characterize the rate of waning immunity and its associated factors. Although the U.S. eliminated measles and rubella, and made significant reductions in mumps cases, continuous monitoring of the level of protection conferred from the MMR vaccine at the population and individual level through evaluations of individual patterns

across time could identify individuals at risk in order to assist in maintaining elimination and minimizing outbreaks.

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Manuscript Tables and Figures

Table 1. Characteristics of study participants in a longitudinal cohort of 4-6 year old children who received MMR2, by antigen

Characteristic	Measles, N=302 Mean (SD) or N (%)	Mumps, N=296 Mean (SD) or N (%)	Rubella, N=296 Mean (SD) or N (%)
Female	149 (49.3)	144 (48.7)	146 (49.3)
White, non-Hispanic	295 (97.7)	289 (97.6)	289 (97.6)
Age a first dose			
12-15 months	177 (58.6)	174 (58.7)	174 (58.8)
>15 months	125 (41.4)	122 (41.2)	122 (41.2)
Age at second dose			
4 years old	111 (36.8)	109 (36.8)	110 (37.2)
5 years old	190 (63.0)	186 (62.8)	185 (62.5)
6 years old	1 (0.3)	1 (0.3)	1 (0.3)
Time between MMR1[1] and MMR2[2] (months)	44.2 (4.4)	44.2 (4.4)	44.2 (4.4)
Average baseline titer, pre-MMR2 (mIU/mL)	2231.2 (2169.4)	44.5 (37.0)	72.4 (77.6)
Seropositive at baseline	299 (99.0)	251 (84.8)	271 (91.6)
MMR2 response[3]			
≥4 fold response	29 (9.6)	115 (38.9)	88 (29.7)
≥2 to <4 fold response	78 (25.8)	85 (28.7)	79 (26.7)
<2 fold response	195 (64.6)	96 (32.4)	129 (43.6)
Number of serum collections (visits)	5.9 (2.2)	5.9 (2.1)	5.9 (2.1)

[1] First dose of the MMR vaccine.
[2] Second dose of the MMR vaccine.
[3] MMR2 response defined by dividing 1 month post-MMR2 titer by pre-MMR2 baseline titer; categorized as ≥4 fold, 2-4 fold, <2 fold (for each antigen).

Table 2. MMR2 seropositivity patterns and persistence trends in a longitudinal cohort of 4-6 year old children, by antigen

Patterns and Trends	Measles N=302	Mumps N=296	Rubella N=296	% Concordant[3] N= 291
Seropositivity Patterns[1]				
Seropositive	291 (96.4)	260 (87.8)	235 (79.4)	212 (72.9)
Seronegative	5 (1.7)	20 (6.8)	11 (3.7)	0
Inconsistent	6 (2.0)	16 (5.4)	50 (15.9)	0
Persistence Trends[2]				
Stable	38 (12.6)	63 (21.3)	31 (10.5)	2 (0.7)
Declining	169 (56.0)	29 (9.8)	110 (37.2)	18 (6.2)
Variable	50 (16.6)	96 (32.4)	98 (33.1)	17 (5.8)
Other	45 (14.9)	108 (36.5)	57 (19.3)	4 (1.4)

[1] Seropositivity patterns defined using all 7 visits post-MMR2 (>120 mIU/mL for measles, ≥10 mIU/mL for mumps, and ≥10 mIU/mL for rubella). Seropositive: seropositive titer at every visit. Seronegative: became seronegative and remained seronegative. Inconsistent: seronegative titer at ≥1 visit followed by a seropositive titer.
[2] Stable: titers at or around baseline that did not decline over time (all visits ≥6 months post-MMR2 within 2 fold of the titers at their previous and baseline visits; and <65% of visits ≥6 months post-MMR2 had lower titers than previous visit). Declining: ≥65% of visits ≥6 months post-MMR2 with titers lower than the prior visit (<1 fold) and no visits ≥6 months post-MMR2 with >2 fold increase. Variable: significant increases/decreases as defined by at least 1 visit ≥6 months post-MMR2 with ≥4 fold increase from prior visit, at least 1 visit ≥2 years post-MMR2 with >4 fold decrease from prior visit, or at least 1 visit ≥2 years with >2 fold increase and at least 1 visit with >2 fold decrease from prior visit. Other: individuals who could not be classified.
[3] Concordant refers to individuals who had the same seropositivity pattern or persistence trend for all 3 antigens (measles, mumps, and rubella).

Table 3. Characteristics of study participants in a longitudinal cohort of 4-6 year old children who received MMR2, by antigen-specific seropositivity pattern and persistence trend

	Seropositivity Patterns[1]			Persistence Trends[2]			
Characteristic	**Seropositive**	**Seronegative**	**Inconsistent**	**Stable**	**Declining**	**Variable**	**Other**
Measles	**N=291**	**N=5**	**N=6**	**N=38**	**N=169**	**N=50**	**N=45**
Female	144 (49.5)	3 (60.0)	2 (33.3)	18 (47.4)	85 (50.3)	28 (56.0)	18 (40.0)
White, non-Hispanic	284 (97.6)	5 (100)	6 (100)	38 (100)	165 (97.6)	49 (98.0)	43 (95.6)
Age a first dose							
12-15 mo.	167 (57.4)	4 (80.0)	6 (100)	17 (44.7)	101 (59.8)	33 (66.0)	26 (57.8)
>15 mo.	124 (42.6)	1 (20.0)	0	21 (55.3)	68 (40.2)	17 (34.0)	19 (42.2)
Age at second dose							
4 years old	104 (35.7)	3 (60.0)	4 (66.7)	13 (34.2)	65 (38.5)	16 (32.0)	17 (37.8)
5 years old	186 (63.9)	2 (40.0)	2 (33.3)	25 (65.8)	103 (61.0)	34 (68.0)	28 (62.2)
6 years old	1 (0.3)	0	0	0	1 (0.59)	0	0
Time b/w MMR1[3] and MMR2[4] (mo.)	44.2 (4.4)	46.0 (5.7)	42.5 (2.9)	42.7 (4.5)	44.3 (4.2)	45.2 (4.7)	44.4 (4.5)
Average baseline titer, pre-MMR2	2286.2 (2187.3)	298.2 (122.7)	1176.8 (749.3)	2622.7 (2596.5)	2216.6 (1835.7)	1905.0 (2385.1)	2318.1 (2657.6)
Seropositive at baseline	288 (99.0)	5 (100)	6 (100)	38 (100)	168 (99.4)	49 (98.0)	44 (97.8)
MMR2 response[5]							
≥4 fold	25 (8.6)	4 (80.0)	0	0	18 (10.7)	7 (14.0)	4 (8.9)
2-4 fold	74 (25.4)	1 (20)	3 (50.0)	6 (15.8)	48 (28.4)	11 (22.0)	13 (28.9)
<2 fold	192 (66.0)	0	3 (50.0)	32 (84.2)	103 (61.0)	32 (64.0)	28 (62.2)
Number of visits	5.8 (2.2)	7.4 (1.3)	8.0 (0)	5.0 (1.9)	5.3 (2.2)	7.6 (1.2)	6.9 (1.7)
Mumps	**N=260**	**N=20**	**N=16**	**N=63**	**N=29**	**N=96**	**N=108**
Female	129 (49.6)	7 (35.0)	8 (50.0)	31 (49.2)	14 (48.3)	46 (47.9)	53 (49.1)

White, non-Hispanic	253 (97.3)	20 (100)	16 (100)	61 (96.8)	27 (93.1)	96 (100)	105 (97.2)
Age a first dose							
12-15 mo.	149 (57.3)	11 (55.0)	14 (87.5)	37 (58.7)	14 (48.3)	59 (61.5)	64 (59.3)
>15 mo.	111 (42.7)	9 (45.0)	2 (12.5)	26 (41.3)	15 (51.7)	37 (38.5)	44 (40.7)
Age at second dose							
4 years old	96 (36.9)	8 (40.0)	5 (31.3)	20 (31.8)	9 (31.0)	36 (37.5)	44 (40.7)
5 years old	163 (62.7)	12 (60.0)	11 (68.8)	42 (66.7)	20 (69.0)	60 (62.5)	64 (59.3)
6 years old	1 (0.38)	0	0	1 (1.6)	0	0	0
Time b/w MMR1[3] and MMR2[4] (mo.)	44.2 (4.4)	43.6 (4.7)	45.5 (4.6)	44.0 (5.4)	43.8 (3.4)	44.6 (4.1)	44.0 (4.2)
Average baseline titer, pre-MMR2	47.5 (37.9)	23.5 (17.3)	21.9 (19.4)	68 .7 (40.5)	38.6 (32.5)	43.3 (35.6)	33.0 (29.3)
Seropositive at baseline	231 (88.9)	13 (65.0)	7 (43.8)	62 (98.4)	21 (72.4)	78 (81.3)	90 (83.3)
MMR2 response[5]							
≥4 fold	98 (37.7)	8 (40.0)	9 (56.3)	0	12 (41.4)	39 (40.6)	64 (59.3)
2-4 fold	82 (31.5)	1 (5.0)	2 (12.5)	20 (31.8)	12 (41.4)	28 (29.2)	25 (23.2)
<2 fold	80 (30.8)	11 (55.0)	5 (31.3)	43 (68.3)	5 (17.2)	29 (30.2)	19 (17.6)
Number of visits	5.7 (2.2)	7.0 (1.8)	7.8 (0.8)	4.4 (1.7)	4.1 (1.5)	7.8 (0.7)	5.6 (2.2)
Rubella	**N=235**	**N=11**	**N=50**	**N=31**	**N=110**	**N=98**	**N=57**
Female	117 (49.8)	3 (27.3)	26 (52.0)	16 (51.6)	55 (50.0)	50 (51.0)	25 (43.9)
White, non-Hispanic	228 (97.0)	11 (100)	50 (100)	30 (96.8)	105 (95.5)	98 (100)	56 (98.3)
Age a first dose							
12-15 mo.	135 (57.5)	7 (63.6)	32 (64.0)	17 (54.8)	63 (57.3)	59 (60.2)	35 (61.4)
>15 mo.	100 (42.6)	4 (36.4)	18 (36.0)	14 (45.2)	47 (42.7)	39 (38.9)	22 (38.6)
Age at second dose							
4 years old	87 (37.0)	4 (36.4)	19 (48.0)	17 (54.8)	36 (32.7)	37 (37.8)	20 (35.1)
5 years old	147 (62.6)	7 (63.6)	31 (52.0)	14 (45.2)	73 (66.4)	61 (62.2)	37 (64.9)
6 years old	1 (0.43)	0	0	0	1 (0.9)	0	0

Time b/w MMR1[3] and MMR2[4] (mo.)	44.1 (4.3)	44.6 (6.5)	44.6 (4.2)	42.3 (5.0)	44.1 (4.6)	44.6 (3.9)	44.5 (4.0)
Average baseline titer, pre-MMR2	81.3 (74.7)	15.0 (15.1)	43.5 (87.2)	75.8 (52.9)	73.5 (75.3)	67.0 (87.5)	77.8 (76.3)
Seropositive at baseline	225 (95.7)	6 (54.6)	40 (80.5)	31 (100)	100 (90.9)	85 (86.7)	55 (96.5)
MMR2 response[5]							
≥4 fold	63 (26.8)	8 (72.7)	17 (34.0)	7 (22.6)	35 (31.8)	28 (28.6)	18 (31.6)
2-4 fold	61 (26.0)	2 (18.2)	16 (32.0)	9 (29.0)	30 (27.3)	26 (26.5)	14 (24.6)
<2 fold	111 (47.2)	1 (9.1)	17 (34.0)	15 (48.4)	45 (40.9)	44 (44.9)	25 (43.9)
Number of visits	5.5 (2.1)	6.5 (1.5)	7.6 (0.9)	4.7 (1.6)	4.5 (2.0)	7.5 (1.0)	6.2 (1.8)

[1] Seropositivity patterns defined using all 7 visits post-MMR2 (>120 mIU/mL for measles, ≥10 mIU/mL for mumps, and ≥10 mIU/mL for rubella). Seropositive: seropositive titer at every visit. Seronegative: became seronegative and remained seronegative. Inconsistent: seronegative titer at ≥1 visit followed by a seropositive titer.
[2] Stable: titers at or around baseline that did not decline over time (all visits ≥6 months post-MMR2 within 2 fold of the titers at their previous and baseline visits; and <65% of visits ≥6 months post-MMR2 had lower titers than previous visit). Declining: ≥65% of visits ≥6 months post-MMR2 with titers lower than the prior visit (<1 fold) and no visits ≥6 months post-MMR2 with >2 fold increase. Variable: significant increases/decreases as defined by at least 1 visit ≥6 months post-MMR2 with ≥4 fold increase from prior visit, at least 1 visit ≥2 years post-MMR2 with >4 fold decrease from prior visit, or at least 1 visit ≥2 years with >2 fold increase and at least 1 visit with >2 fold decrease from prior visit. Other: individuals who could not be classified.
[3] First dose of the MMR vaccine.
[4] Second dose of the MMR vaccine.
[5] MMR2 response defined by dividing 1 month post-MMR2 titer by pre-MMR2 baseline titer; categorized as ≥4 fold, 2-4 fold, <2 fold (for each antigen).

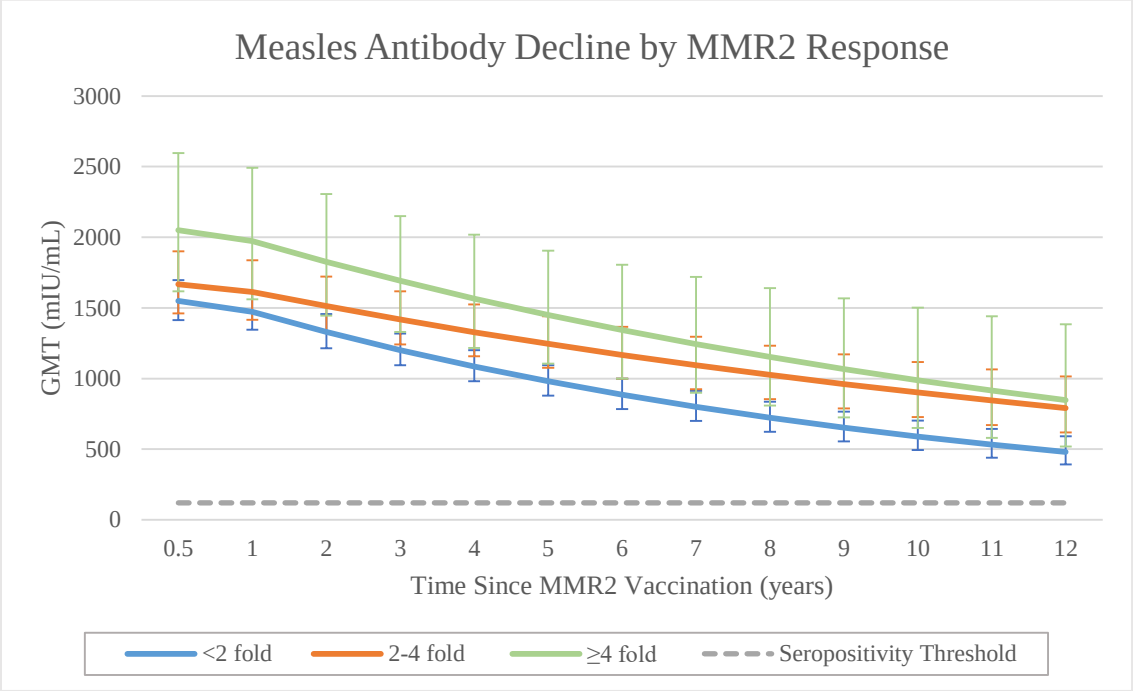
Table 4. Results of linear mixed models for declining trend in a longitudinal cohort of children vaccinated with MMR2, by antigen

Model Results	Estimate (SE)	P-value
Measles (n=169)		
Fixed Effects, initial status		
Intercept	3.89 (0.54)	<0.0001
Sex (male vs. female)	-0.26 (0.10)	0.0091
MMR2 response[1] (≥4 fold vs. <2 fold)	0.39 (0.20)	0.0528
MMR2 response[1] (2-4 fold vs. <2 fold)	0.08 (0.12)	0.5237
Log2-transformed baseline titer	0.64 (0.05)	<0.0001
Rate of Change		
Intercept (time)	-0.50 (0.11)	<0.0001
MMR2 response[1] (≥4 fold vs. <2 fold)	0.04 (0.04)	0.3191
MMR2 response[1] (2-4 fold vs. <2 fold)	0.05 (0.02)	0.0144
Log2-transformed baseline titers	0.03 (0.01)	0.0013
Variance Components		
Within person	0.31 (0.02)	<0.0001
In initial status	0.21 (0.05)	<0.0001
In rate of change	0.001 (0.001)	0.0764
Covariance	0.008 (0.006)	0.1668
ICC[2]	0.619	
Mumps (n=29)[3]		
Fixed Effects, initial status		
Intercept	2.09 (0.88)	0.0217
MMR2 response[1] (≥4 fold vs. <2 fold)	1.64 (0.44)	0.0004
MMR2 response[1] (2-4 fold vs. <2 fold)	1.38 (0.33)	<0.0001
Log2-transformed baseline titers	0.55 (0.14)	0.0004
Sex (male vs. female)	-0.63 (0.25)	0.0165
Rate of Change		
Intercept (time)	-0.14 (0.03)	<0.0001
Variance Components		
Within person	0.62 (0.12)	<0.0001
ICC[2]	invalid	
Rubella (n=110)		
Fixed Effects, initial status		
Intercept	1.76 (0.42)	<0.0001
MMR2 response[1] (≥4 fold vs. <2 fold)	0.72 (0.21)	<0.0009
MMR2 response[1] (2-4 fold vs. <2 fold)	0.28 (0.16)	0.0798
Log2-transformed baseline titers	0.61 (0.06)	<0.0001
Age at MMR1 (12-15 mo vs. >15 mo)	-0.07 (0.12)	0.5434
Rate of Change		
Intercept (time)	-0.09 (0.02)	<0.0001
Age at MMR1 (12-15 mo vs. >15 mo)	0.05 (0.02)	0.0395
Variance Components		

Within person	0.31 (0.03)	<0.0001
In initial status	0.14 (0.05)	0.0022
In rate of change	0.001 (0.001)	0.1272
Covariance	-0.003 (0.006)	0.5591
ICC[2]	0.522	

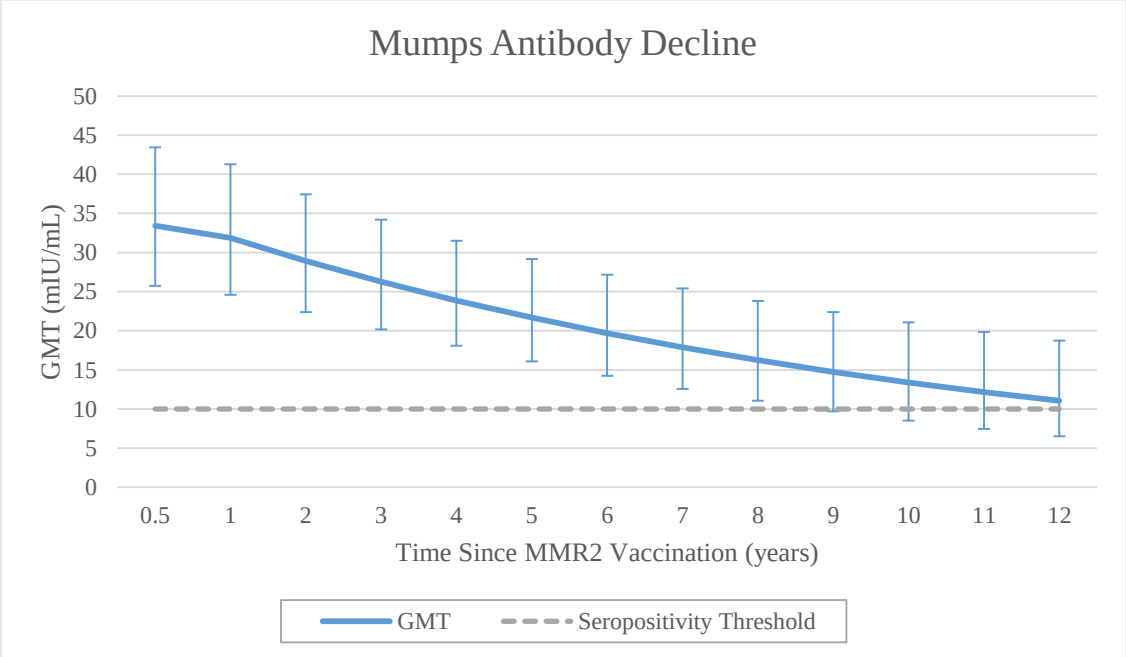
[1] MMR2 response defined by dividing 1 month post-MMR2 titer by pre-MMR2 baseline titer; categorized as ≥4 fold, 2-4 fold, <2 fold (for each antigen).
[2] ICC: intraclass correlation coefficient (correlation of measurements between individuals).
[3] Unlike measles and rubella, the mumps model did not include a random effects statement.

Figure 1A. Measles antibody decline 6 months post-MMR2 to 12 years post-MMR2 by MMR2 response, adjusting for sex and baseline titer



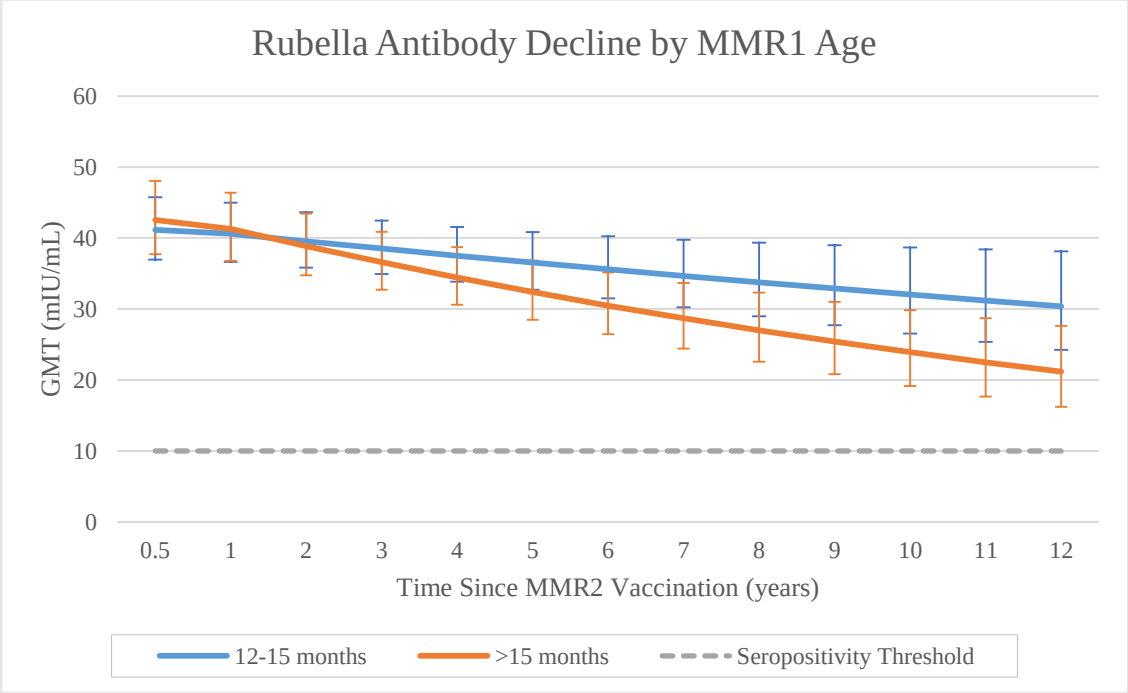
[1] Figure represents a male individual with median baseline antibody titer (1722.2 mIU/mL).
[2] Rate of decline per year: 9.7% among individuals with <2 fold response, 6.3% among those with 2-4 fold response, and 7.4% among those with ≥4 fold response.
[3] MMR2 response defined by dividing one month post-MMR2 titer by pre-vaccination (baseline) titer.

Figure 1B. Mumps antibody decline 6 months post-MMR2 to 12 years post-MMR2, adjusting for MMR2 response and baseline titer



[1] Figure represents a male individual with median baseline antibody titer (28.6 mIU/mL).
[2] Rate of decline per year: 9.2% (no significant factors associated with the rate of decline).

Figure 1C. Rubella antibody decline 6 months post-MMR2 to 12 years post-MMR2 by MMR1 age, adjusting for MMR2 response and baseline titer



[1] Figure represents a male individual with median baseline antibody titer (45.6 mIU/mL).
[2] Rate of decline per year: 2.6% among individuals vaccinated 12-15 months, 5.9% among those vaccinated >15 months

Chapter III. Public Health Implications

Implications of Pattern and Trend Analysis

To our knowledge, this was the first study to operationalize definitions that sort individuals into different seropositivity patterns and persistence trends relative to time.

This study revealed that a small subset of individuals become seronegative soon after vaccination or have an inconsistent pattern shifting between seronegative and seropositive titer values across time. An individual is assumed to have a higher risk of infection when their titer values are around or below the seropositivity threshold. Therefore, in light of recent mumps outbreaks among highly vaccinated (≥2 doses) individuals, this study is particularly important in that it indicates the potential that infected individuals in these outbreaks had either seronegative or inconsistent patterns, as defined in this study. Therefore, future studies should be conducted to understand methods to best identify individuals at risk of having these types of patterns, identify reasons for variation in patterns, and determine clinical and demographic factors that may be associated with changes in titers across time that lead to seroconversion and increased susceptibility in order to ensure appropriate vaccination recommendations are created.

Our study also provides an in depth picture of the degree of individual variation in persistence patterns and allows for comparisons of these patterns both across individuals and antigens. The range in variation found within the persistence trends has significant public health implications. As noted in Davidkin et al. (1), a certain level of immunity is required to appropriately protect a population, however, these estimates often do not account for the fact that some vaccinated individuals have titers that wane faster or are more variable across time than others. In turn, future studies are required to understand

how these patterns of persistence and differing rates of waning immunity impact the level of protection within a population.

Results also indicated that although individuals are given the MMR vaccine at the same time, the majority of individuals do not biologically respond to each of the individual antigens in the same manner. Such results reiterate the need to study each of the three antigens separately when evaluating the MMR vaccine, to understand clinical and demographic factors associated with antigen-specific persistence patterns.

Implications of Waning Immunity

While other studies have noted waning immunity and differences in GMTs between years (1-6), this is the first study to characterize the rate of decline among persons with a declining trend and report average percent decrease per year. These statistics provide a more accurate picture of waning immunity and the factors associated with this rate of change. In turn, this and future models should be used to monitor annual changes and predict future antibody levels to ensure community protection within a population and make appropriate vaccine recommendations. This portion of the analysis is also key in identifying subgroups of individuals who display faster rates of decline than others, and in turn, potentially require a third dose of the MMR vaccine. Future analysis with larger cohorts should focus efforts in predicting titers in individuals who do not have a declining trend, as defined in our analysis.

Moving Forward

In conclusion, this study reveals the need for constant vigilance and further investigations into the patterns, trends, and rates of decline observed in this study. Future studies should also focus on more geographically and demographically diverse

populations to assess the differences in rates of decline and persistence trends within differing populations. Although the United States eliminated measles and rubella, and made significant reductions in mumps cases, continuous monitoring of the level of protection conferred from the MMR vaccine at the population and individual level over time will assist in maintaining elimination and minimizing outbreaks.

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Appendix

Supplemental Manuscript Materials and Tables

Supplemental Table 1. Bivariate associations among predictors for declining trend in a longitudinal cohort of children vaccinated with MMR2, by antigen

Variables for Respective Antigens	P-Value for Association[2]		
	Measles	**Mumps**	**Rubella**
Sex vs. MMR1[3] age	0.5725	0.5726	0.8472
Sex vs. MMR2 response[1]	0.5463	0.3083	0.0117
Sex vs. log2-transformed baseline titers	0.5658	0.1273	0.0529
Sex vs. time between MMR1[3] and MMR2[4] (months)	0.3773	0.9527	0.7909
MMR1[3] age vs. MMR2 response[1]	0.0431	0.1022	0.0975
MMR1[3] age vs. log2-transformed baseline titers	0.4440	0.1273	0.4120
MMR1[3] age vs. time between MMR1[3] and MMR2[4] (months)	0.0003	0.9666	<0.0001
MMR2 response[1] vs. log2-transformed baseline titers	<0.001	0.0305	<0.0001
MMR2 response[1] vs. time between MMR1[3] and MMR2[4] (months)	0.1641	0.2827	0.3577
Log2-transformed baseline titers vs. time between MMR1[3] and MMR2[4] (months)	0.1086	0.5789	0.1349

[1] MMR2 response defined by dividing 1 month post-MMR2 titer by pre-MMR2 baseline titer; categorized as ≥4 fold, 2-4 fold, <2 fold (for each antigen).
[2] Differences in characteristics between groups were assessed using chi-square tests for categorical variables and ANOVA for continuous variables.
[3] First dose of the MMR vaccine.
[4] Second dose of the MMR vaccine.

Supplemental Table 2. Unconditional means and unconditional growth model results for declining trend in a longitudinal cohort of children vaccinated with MMR2, by antigen

Model Results	Unconditional Means model[1], Estimate (SE)	Unconditional Growth model[1], Estimate (SE)
Measles (n=169)		
Fixed Effects, initial status	10.27 (0.08)	10.67 (0.09)
Rate of Change		-0.12 (0.01)
Variance Component		
Within person	0.58 (0.04)	0.33 (0.02)
In initial status	0.94 (0.13)	1.04 (0.13)
Mumps (n=29)[2]		
Fixed Effects, initial status	5.34 (0.14)	5.65 (0.17)
Rate of Change		-0.11 (0.04)
Variance Component		
Within person	1.20 (0.22)	1.08 (0.20)
In initial status	not given	not given
Rubella (n=110)		
Fixed Effects, initial status	0.52 (0.08)	5.38 (0.09)
Rate of Change		-0.06 (0.01)
Variance Component		
Within person	0.45 (0.04)	0.37 (0.04)
In initial status	0.49 (0.10)	0.56 (0.10)

[1] All estimates and variance components have a p-value <0.0001.

[2] Unlike measles and rubella, the mumps model did not include a random effects statement.

Supplemental Table 3. Complete data[1] sensitivity analysis results of linear mixed models for declining trend in longitudinal cohort of children vaccinated with MMR2, by antigen[2]

Model Results	Estimate (SE)	p-value
Measles (n=63)		
Fixed Effects, initial status		
Intercept	3.11 (0.94)	0.0010
Sex (male vs. female)	0.08 (0.12)	0.5135
MMR2 response[3] (≥4 fold vs. <2 fold)	0.07 (0.29)	0.8004
MMR2 response[3] (2-4 fold vs. <2 fold)	0.16 (0.17)	0.3490
Log2-transformed baseline titers	0.70 (0.08)	<0.0001
Rate of Change		
Intercept (time)	-0.34 (0.12)	0.0049
MMR2 response[3] (≥4 fold vs. <2 fold)	0.02 (0.04)	0.5732
MMR2 response[3] (2-4 fold vs. <2 fold)	0.05 (0.02)	0.0349
Log2-transformed baseline titers	0.02 (0.01)	0.0661
Variance Components		
Within person	0.28 (0.02)	<0.0001
In initial status	0.11 (0.05)	0.0147
In rate of change	0.001 (0.001)	0.0561
Covariance	0.008 (0.005)	0.0993
Rubella (n=25)		
Fixed Effects, initial status		
Intercept	0.36 (0.93)	0.0016
MMR2 response[3] (≥4 fold vs. <2 fold)	0.35 (0.46)	0.4518
MMR2 response[3] (2-4 fold vs. <2 fold)	-0.02 (0.32)	0.9398
Log2-transformed baseline titers	0.41 (0.11)	0.0019
Age at MMR1[4] (12-15 mo vs. >15 mo)	0.03 (0.34)	0.9402
Rate of Change		
Intercept (time)	-0.10 (0.02)	0.0003
Age at MMR1[4] (12-15 mo vs. >15 mo)	0.06 (0.03)	0.0911
Variance Components		
Within person	0.41 (0.06)	<0.0001
In initial status	0.46 (0.21)	0.0132
In rate of change	0.002 (0.002)	0.1162
Covariance	-0.03 (0.02)	0.1155

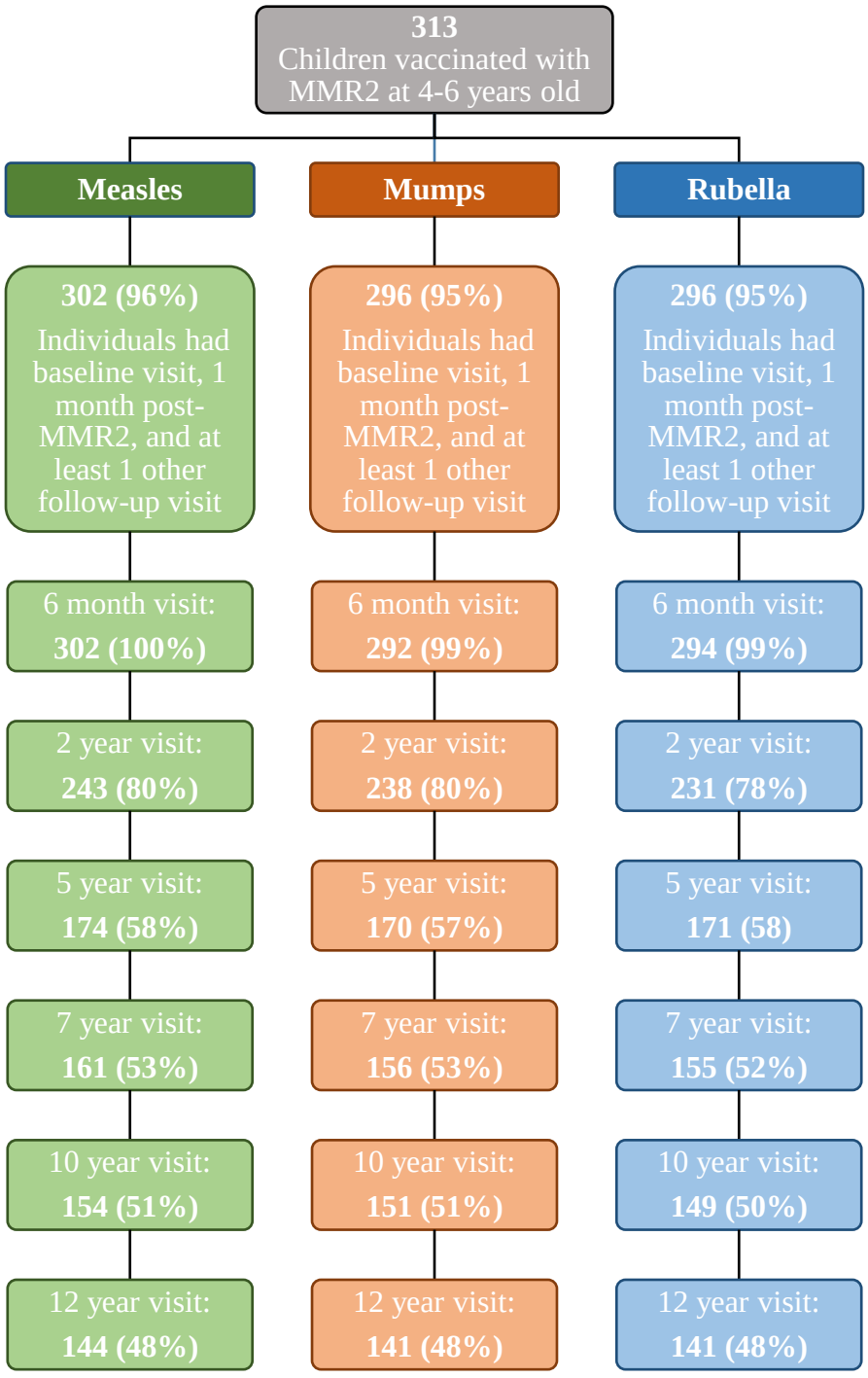
[1] Complete data analysis restricted to individuals who completed all 8 study visits.
[2] Insufficient sample size for mumps analysis.
[3] MMR2 response defined by dividing 1 month post-MMR2 titer by pre-MMR2 baseline titer; categorized as ≥4 fold, 2-4 fold, <2 fold (for each antigen).
[4] First dose of the MMR vaccine.

Supplemental Table 4. Exclusion of year 12 sensitivity analysis results of linear mixed models for measles declining trend in longitudinal cohort of children vaccinated with MMR2

Measles Model Results (N=63)	Estimate (SE)	p-value
Fixed Effects, initial status		
Intercept	3.99 (0.53)	<0.0001
Sex (male vs. female)	0.26 (0.10)	0.0087
MMR2 response[1] (≥4 fold vs. <2 fold)	0.51 (0.18)	0.0065
MMR2 response[1] (2-4 fold vs. <2 fold)	0.14 (0.12)	0.2430
Log2-transformed baseline titer	0.64 (0.05)	<0.0001
Rate of Change		
Intercept (time)	-0.64 (0.14)	<0.0001
MMR2 response[1] (≥4 fold vs. <2 fold)	0.06 (0.04)	0.1565
MMR2 response[1] (2-4 fold vs. <2 fold)	0.06 (0.03)	0.0345
Log2-transformed baseline titer	0.04 (0.01)	0.0018
Variance Components		
Within person	0.28 (0.02)	<0.0001
In initial status	0.23 (0.05)	<0.0001
In rate of change	0.005 (0.002)	0.0023
Covariance	0.01 (0.01)	0.3497

[1] MMR2 response defined by dividing 1 month post-MMR2 titer by pre-MMR2 baseline titer; categorized as ≥4 fold, 2-4 fold, <2 fold (for each antigen).

Supplemental Figure 1. Study population flow chart of participants enrolled in initial study, who met inclusion criteria, and percent who completed serum collection visits.



* The denominator for visits starting 6 months and after is the number that met the inclusion criteria (measles: 302, mumps and rubella: 296).