Mumps Antibody Response in Young Adults After a Third Dose of Measles-Mumps-Rubella Vaccine

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Background. Mumps outbreaks in populations with high 2-dose measles-mumps-rubella (MMR) vaccine coverage raise the question whether a third dose of MMR vaccine (MMR3) is needed. However, data on the immunogenicity of MMR3 are limited. We assessed mumps virus neutralizing antibody levels pre- and post-MMR3 in a nonoutbreak setting.

Methods. Mumps antibody titers were assessed at baseline, 1 month, and 1 year after MMR3 in subjects aged 18–28 years.

Results. At baseline, 5 of 656 (0.8%) subjects had seronegative mumps neutralizing antibody titers and 38 (5.8%) had low titers. One year post-MMR3, these numbers declined to 3 (0.5%) and 16 (2.4%), respectively. Subjects with low baseline titers were more likely to have low 1-month and 1-year titers ($R^2 = 0.81$–0.87, $P < .0001$). Compared to baseline, geometric mean titers were significantly higher at 1 month ($P < .0001$) and 1 year ($P < .01$) post-MMR3; however, reverse cumulative distribution curves showed only minimal shifts in mumps titers from baseline to 1 month and 1 year.

Conclusions. Very few subjects had negative or low baseline mumps titers. Nonetheless, mumps titers had modest but significant increases when measured 1 month and 1 year post-MMR3. This temporary increase in titers could decrease susceptibility to disease during outbreaks, but may have limited value for routine use in vaccinated populations.

Keywords. mumps; third-dose measles-mumps-rubella (MMR) vaccine; mumps immunogenicity; vaccine-preventable disease; immunization.

Mumps is an acute viral disease that classically presents with parotitis. Serious complications include orchitis, deafness, and encephalitis [1]. A monovalent mumps vaccine was licensed in 1967, and in 1977, the Advisory Committee on Immunization Practices (ACIP) recommended universal childhood vaccination with 1 dose [2]. In 1989, the ACIP recommended that school-aged children receive 2 doses of measles-mumps-rubella (MMR) vaccine for improved measles control, with the first dose at age 15 months (high-risk areas) or 12 months (non-high-risk areas) and the second dose at age 4–6 years [3]. Vaccine coverage against mumps increased, which was associated with a >99% decline in disease incidence compared with the prevaccine era [4]. Following this success, the Healthy People 2010 goal of mumps elimination was established [5]. However, unlike measles [6] and rubella [7], mumps elimination in the United States was never documented. The current Healthy People 2020 mumps goal is to reduce the number of US-acquired cases, rather than elimination [8].

Between 2006 and 2013, several large mumps outbreaks occurred in the United States and abroad, primarily among 2-dosed vaccinated school-aged children and

young adults in high-contact settings [9–16]. Although current MMR vaccination recommendations are for the first dose at age 12–15 months and the second dose at 4–6 years [17], a third dose of MMR vaccine (MMR3) was offered at school-based immunization clinics during 2 of these outbreaks as part of a public health response [10, 11]. However, serologic response was not measured. Although attack rates declined after administering MMR3 in both school-based studies, in one study, statistical significance could not be established due to the small number of cases, and in both studies, the possibility of the declines being unrelated to the intervention could not be excluded [10, 11].

A third dose of mumps-containing vaccine is also administered in some nonoutbreak settings. Healthcare personnel, military recruits, international travelers, and college students who may have been vaccinated as children but who lack documentation are routinely given an additional dose, which is often the third dose [17–19]. Pregnant women with a negative rubella titer are revaccinated after delivery even if they have had 2 previous MMR doses [20].

Despite mumps outbreaks occurring in communities with high 2-dose MMR vaccine coverage and third doses being routinely administered in some settings, data on the immunogenicity of MMR3 are limited [21, 22]. The objective of this study was to assess the magnitude and duration of aggregate mumps virus neutralizing antibody responses after MMR3 in a healthy, young adult population.

METHODS

Setting
The source population comprised patients who received care from the Marshfield Clinic, a large multispecialty group practice with 54 locations in rural central, western, and northern Wisconsin. The clinic developed and maintains an electronic vaccination registry (www.recin.org) for all immunizations administered by Marshfield Clinic providers, in addition to those given by many local public health agencies and immunization providers.

Subjects
Two cohorts comprising 685 subjects were enrolled over a 1-year period. Cohort 1 comprised 113 young adult subjects who participated in a 12-year longitudinal study at the Marshfield Clinic examining immunogenicity and adverse events following the second dose of MMR vaccine, hereafter called the "longitudinal study" [23, 24]. To achieve adequate sample size, cohort 2 was recruited. Cohort 2 comprised 572 young adults identified using Marshfield's vaccination registry who had 2 documented doses of MMR vaccine but did not participate in the longitudinal study. Invitation letters were mailed to both cohorts, and follow-up phone calls were made.

Although only 25 (22.1%) cohort 1 subjects had low or negative mumps titers at any point during the longitudinal study, all 93 cohort 1 subjects with at least 1 low or negative titer to any of the 3 antigens during the longitudinal study (defined as <121 mIU/mL for measles [25], ≤10 mIU/mL for mumps [23], or ≤10 mIU/mL for rubella [26]) and all cohort 2 subjects were offered a third dose of MMR vaccine (M-M-R II, Merck & Co) in this study. We combined cohorts 1 and 2 for analysis purposes as there were no statistically significant differences between the 2 cohorts in terms of sex, race/ethnicity, age, geometric mean titers (GMTs) at baseline or 1 year post-MMR3, or percentage with negative or low baseline mumps titers (Supplementary Table 1). Serum was collected from these study subjects immediately before, and 1 month and 1 year after MMR3.

Study Design
At each visit, subjects were questioned concerning mumps disease, exposures, vaccinations, and other health events. MMR vaccine was administered during the initial visit according to a standard protocol. Adverse events were evaluated and will be reported elsewhere. Informed consent was obtained by all subjects. The study was approved by the institutional review boards of the Marshfield Clinic Research Foundation and the Centers for Disease Control and Prevention (CDC).

The analysis of data for all 3 antigens was taken into consideration when determining sample size. To detect a decrease in the proportion of subjects who had low or negative titers from the last draw of the longitudinal study compared with 1 year post-MMR3, we based our sample size on a decrease from approximately 20% with low or negative titers at the last draw of the longitudinal study in 2006–2007 to 10% 1 year post-MMR3 for mumps, 5% to 1% for measles, and 50% to 30% for rubella, with 90% power and 95% confidence intervals. The target sample size of 375 was increased to 685 to account for the fact that more than one-third of the 312 subjects from the longitudinal study were ineligible to receive a third dose based on high titers for all 3 antigens throughout the longitudinal study and 53% attrition during the longitudinal study [23, 25, 26].

Exclusion Criteria
Subjects were excluded if they had a history of measles, mumps, or rubella disease, lived in the same household with anyone who had these diseases during the subject's lifetime, previously received a third MMR vaccine dose, received any vaccinations within 30 days of enrollment, had any contraindications to MMR vaccination, or had any condition likely to impair immune response, as specified in the ACIP recommendations [27].

Laboratory Methods
Although there is no established correlate of immunity for mumps, neutralizing antibody is likely essential for protection against mumps and is considered the gold standard for

mumps serology. Thus, plaque reduction neutralization (PRN) assay was used to determine virus neutralizing antibody titer in sera as described previously [28, 29]. Heat-inactivated sera were serially diluted 2-fold from 1:4 to 1:128 and mixed with an equal volume of the Jeryl Lynn vaccine virus diluted to contain approximately 80 plaque-forming units (PFU), resulting in a final serum dilution range of 1:8 to 1:256. Virus control wells were incubated with the virus preparation and an equal volume of minimal essential media (MEM) containing 5% fetal bovine serum (FBS). Reference serum "Lot 3" was included in each assay run. Following a 1-hour incubation period, half of each of the virus/serum mixtures (containing approximately 40 PFU of virus) was transferred to each of 2 wells in 24-well plates containing Vero cell monolayers and overlaid with 2% carboxy-methylcellulose (Sigma) in MEM supplemented with 10% FBS. After 5 days of incubation at 37°C, wells were stained with neutral red (Sigma), and plaques were counted the following day. The mean plaque number was determined for duplicate wells at each serum dilution. The neutralizing antibody titer was calculated as the serum dilution capable of reducing the mean number of virus plaques by ≥50% compared to the mean number of plaques in virus control wells using the Kärber formula [30]. Sera not reaching a 50% endpoint were retested in assays using a higher dilution series.

No established PRN mumps titer correlates with mumps immunity [31]. Therefore, the cutoffs used in this study for seronegative, low-positive, and high-positive were chosen based on a previous study [29]. Mumps virus neutralizing antibody titers <1:8 (limit of assay detection) were considered seronegative. For analysis purposes, we considered titers between 1:8 and <1:16 (ie, within a single dilution factor of the limit of detection) to be low-seropositive and titers ≥1:16 to be high-seropositive.

Data were pooled across assay runs that met the following 2 validity criteria: (1) the mean plaque number in the negative serum control wells was between 20 and 60 (a range validated in the laboratory to not influence measured neutralizing antibody titers), and (2) the neutralizing antibody titer for reference mumps serum Lot 3 was required to be within 2 standard deviations (SD) of its GMT based on historic data. Assays not meeting these poolability requirements were retested. Serum samples from individual subjects were tested in the same assay run. Other than each subject's unique identifier code and serum collection dates, laboratories were blinded to study information.

Data Analysis

Mantel-Haenszel χ^2 and Fisher exact tests were run to assess categorical variables. Wilcoxon rank-sum tests were used for continuous variables. Variables that were considered potential risk factors for low or negative mumps titers (defined as <16 mIU/mL in this study) included age at first MMR dose, time since second MMR dose (we used <15 years vs ≥15 years prior based on the average age of subjects at enrollment minus the age

when the second dose was recommended), sex, race/ethnicity, military member, post–secondary school attendance, number of household members, current illnesses, current medications, and (for post-MMR3 serum samples) the binary variable of whether the subject had low or negative baseline mumps titers. In multivariate logistic regression, a forward stepwise selection approach that used P values <.4 for inclusion and <.05 for retention identified whether any factors were independently associated with negative or low mumps antibody titer levels at baseline, 1 month and 1 year post-MMR3. GMTs were calculated from base 2 log-transformed data. Statistical significance was assigned for P values <.05. Data were analyzed with SAS software, version 9.3. Reverse cumulative distribution curves were created by SigmaPlot 12 (Systat Software, Inc) and were used to compare the shift in the curves from baseline, 1 month, and 1 year.

RESULTS

Enrollment

From the longitudinal study, we successfully contacted 194 of 200 persons attempted. Of those, 113 (58%) were enrolled, 45 (23%) refused, and 36 (19%) were ineligible (15 due to prior receipt of MMR3 and 21 for other reasons). To achieve adequate sample size, we attempted to contact an additional 1795 persons and successfully reached 1379 (77%). Of those, 572 (41%) were enrolled, 664 (48%) refused, and 143 (10%) were ineligible (4 due to prior MMR3 receipt and 139 for other reasons).

Baseline serum samples were obtained from 678 of 685 subjects enrolled from the combined group of longitudinal study participants and new recruits; 656 (95.8%) received MMR3 and completed at least 1 follow-up draw. There were 655 (99.5%) subjects who completed the 1-month draw and 612 (93.3%) who completed the 1-year draw. We excluded 20 (2.9%) subjects who were not given MMR3, because the group was too small to be considered a comparison group. An additional 2 (0.3%) were excluded because they only had baseline data. We analyzed data from 656 subjects (Figure 1); 290 (44.2%) were male and 644 (98.2%) were self-declared non-Hispanic white. Subjects ranged in age from 18 to 28 years, (mean, 20.8 [SD, 2.1] years).

Mumps Titers Pre- and Post-MMR3

At baseline, 5 (0.8%) subjects were seronegative, 38 (5.8%) were low-seropositive, and 613 (93.4%) were high-seropositive (Figure 2A). Of the 613 subjects with high-seropositive baseline titers, 612 had sera drawn at 1 month and 572 had sera drawn at 1 year; all remained high-seropositive. Of the 5 subjects who were seronegative at baseline, 1 became low-seropositive and 4 became high-seropositive 1 month after MMR3. One year post-MMR3, the low-seropositive subject returned to seronegative status, while 2 high-seropositive subjects at 1 month
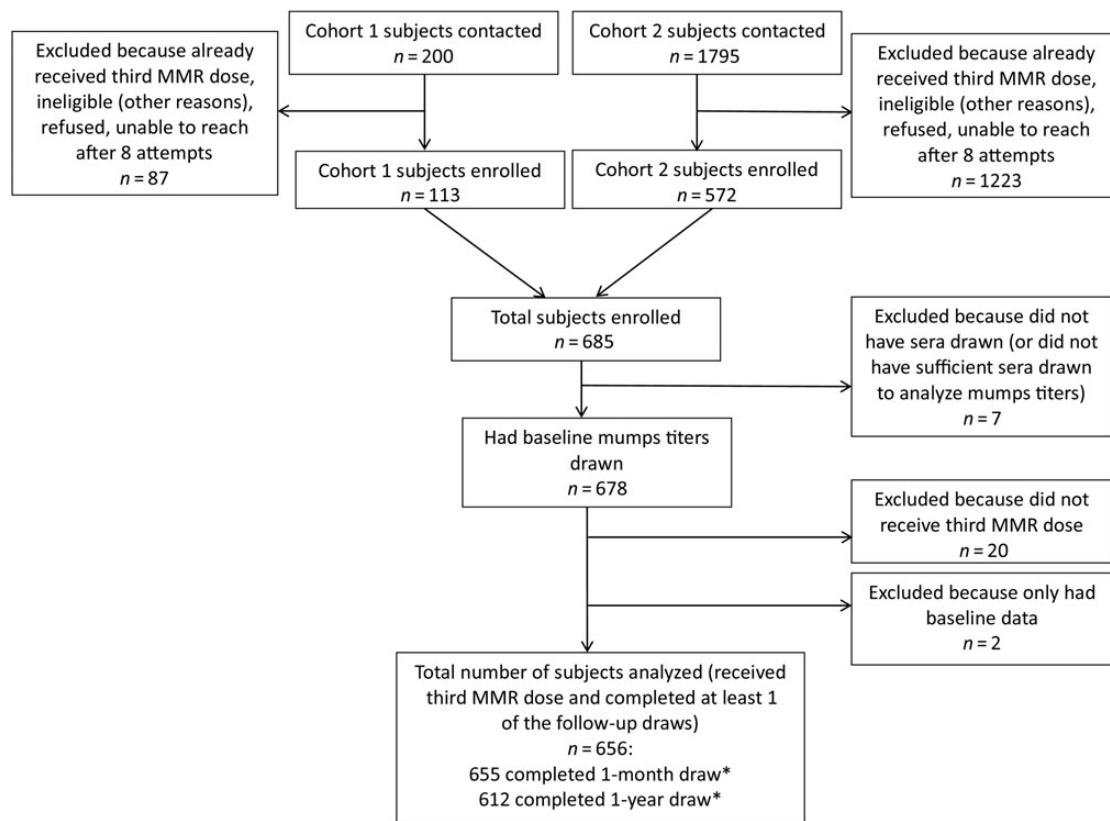
Figure 1. Flow chart for enrollment, analysis, and vaccination of subjects with a third dose of measles-mumps-rubella (MMR) vaccine.

became low-seropositive. Of the 38 low-seropositive subjects at baseline, 29 were high-seropositive 1 month post-MMR3, 9 were low-seropositive, and none were negative. One year post-MMR3, 35 of 38 had serum samples drawn, of whom 19 were high-seropositive, 14 were low-seropositive, and 2 were seronegative.

Overall, at 1 month post-MMR3, 0 of 655 subjects had negative mumps titers, 10 (1.5%) had low-seropositive titers, and 645 (98.5%) had high-seropositive titers. One year post-MMR3, 3 of 612 (0.5%) subjects had negative mumps titers, 16 (2.6%) had low-seropositive titers, and 593 (96.9%) had high-seropositive titers. A majority of subjects with high-seropositive titers were in the upper end of the titer distribution at baseline, 1 month, and 1 year (Figure 2B).

GMTs and Reverse Cumulative Distribution Curves

GMTs were statistically different between baseline and 1 month post-MMR3 (104.1 vs 159.2; $P < .0001$), as well as between baseline and 1 year post-MMR3 (104.1 vs 125.9; $P < .01$). However, as shown in the reverse cumulative distribution curves (Figure 3), the shift in mumps titers from baseline to 1 month to 1 year was minimal. The shape and distribution of the curves at all 3 time points was nearly identical when using untransformed mumps titers and transformed titers on a logarithmic scale.

Four-Fold Rises

Forty of 655 (6.1%) subjects had 4-fold rises from baseline to 1 month post-MMR3, of whom 4 had negative baseline titers, 11 had low baseline titers, and 25 had positive baseline titers. Thirteen of 612 (2.1%) subjects had 4-fold rises from baseline to 1 year post-MMR3, of whom 2 had negative baseline titers, 3 had low baseline titers, and 8 had positive baseline titers. There were no subjects with a 4-fold rise in titer from 1 month to 1 year.

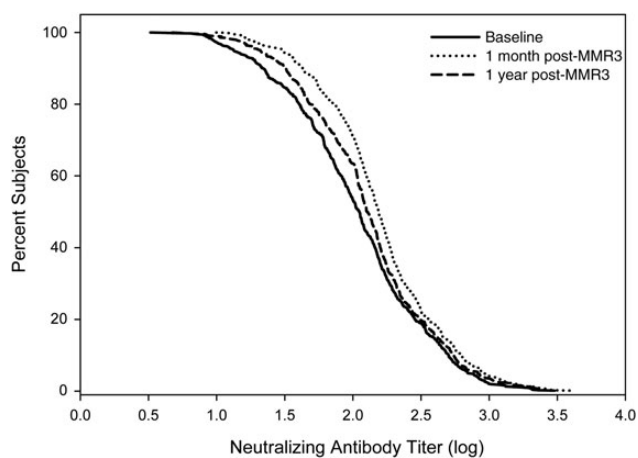### Risk Factors for Negative or Low-Seropositive Mumps Titers

By χ^2 analysis, sex, race/ethnicity, military member, post–secondary school attendance, number of household members, current illnesses, and current medications were not associated with negative or low-seropositive mumps titers at baseline, 1 month, or 1 year post-MMR3 (Table 1).

Significant risk factors for negative or low baseline mumps titers by χ^2 analysis were age at first MMR dose (odds ratio [OR] = 2.58; 95% confidence interval [CI], 1.08–6.13; $P = .03$)
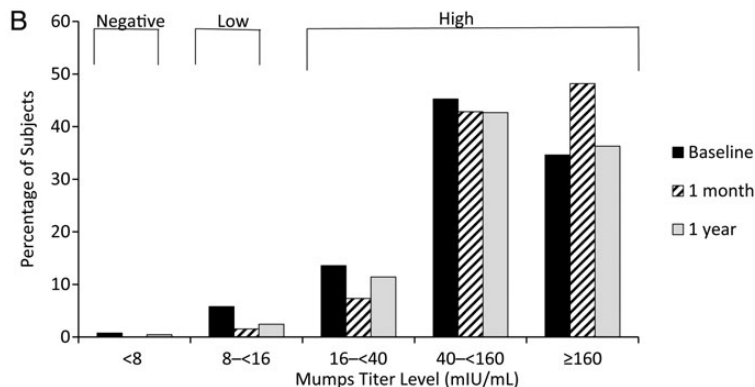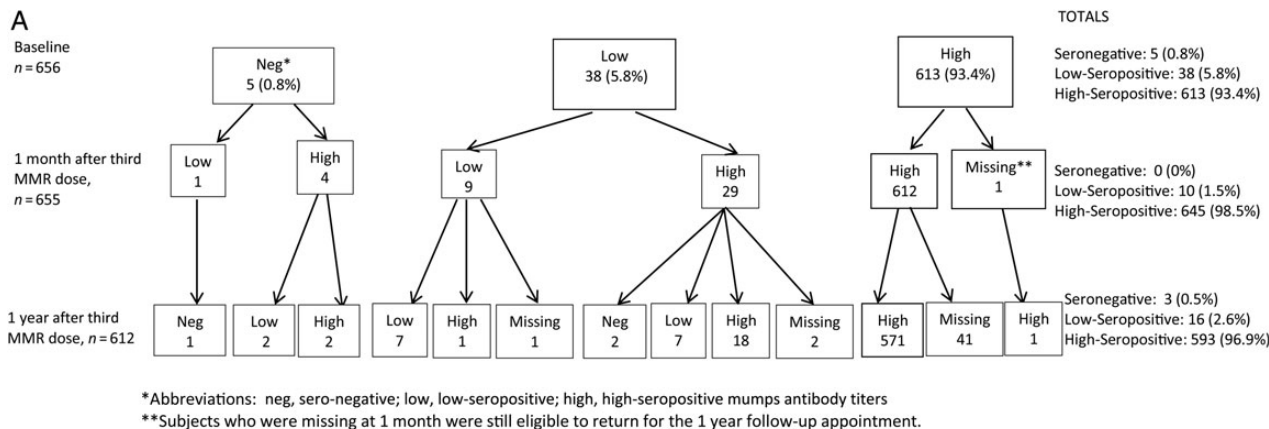
Figure 2. A, Flow chart of mumps antibody titer levels at baseline, 1 month, and 1 year. B, Percentage of subjects who had negative, low-seropositive, and high-seropositive mumps antibody titer levels at baseline, and 1 month and 1 year following a third dose of measles-mumps-rubella (MMR) vaccine.

Figure 3. Reverse cumulative distribution curve using log-transformed titers by percent of subjects who had neutralizing mumps antibody titers at baseline and 1 month and 1 year following a third dose of measles-mumps-rubella (MMR3) vaccine.

and time since second MMR dose (OR = 0.38; 95% CI, 0.16–0.92; P = .03). Of the 50 (7.6%) subjects who received their first dose at age 12 to <15 months, 7 (14%) had negative or

low baseline mumps titers, compared with 36 of 606 (5.9%) subjects who were vaccinated with their first dose at age ≥15 months. Of the 189 (28.8%) subjects who received their second dose <15 years prior, 6 (3.2%) had negative or low titers, whereas, of the 467 (71.2%) subjects who received their second dose ≥15 years prior, 37 (7.9%) had negative or low titers.

By χ^2 analysis, a significant risk factor for negative or low mumps titers 1 month post-MMR3 was whether a subject had low or negative baseline mumps titers (OR, 384.0; 95% CI, 22.0–6692.9; P < .0001). Significant risk factors for negative or low mumps titers 1 year post-MMR3 were whether a subject had low or negative baseline mumps titers (OR, 1038.5; 95% CI, 60.7–17 773.6; P < .0001) and the time since the second MMR dose (OR, 0.38; 95% CI, 0.16–0.92, P = .02). When mumps titer levels were assessed as a continuous variable, baseline MMR3 titers were significantly associated with individual log-transformed mumps titer levels at 1 month and 1 year. Subjects with lower baseline titers were more likely to have lower titers at 1 month and 1 year, whereas subjects with higher baseline titers were more likely to have higher titers at 1 month and 1 year (R^2 = 0.81–0.87; P < .0001; Figure 4).

A logistic regression model showed that age at first MMR dose and time since second MMR dose remained independently

Table 1. Risk Factors for Negative or Low Mumps Titers at Baseline and 1 Month and 1 Year After Receiving the Third Measles-Mumps-Rubella Vaccine Dose

Risk Factor	Baseline (n = 656)		Adjusted Baseline (n = 656)		1 Month Post-MMR3 (n = 655)		1 Year Post-MMR3 (n = 612)	
	Unadjusted OR (95% CI)	P Value*	Adjusted OR (95% CI)[a]	Adjusted P Value	Unadjusted OR (95% CI)	P Value	Unadjusted OR (95% CI)	P Value
Sex								
Female	0.82 (0.44–1.52)	.53	. . .	. . .	0.79 (0.23–2.76)	.71	1.04 (0.41–2.62)	.94
Male	Reference							
Race/ethnicity								
White, non-Hispanic	1.81 (0.11–31.05)	.35	. . .	. . .	0.41 (0.02–7.47)	.66	0.77 (0.04–13.54)	.55
All other races	Reference							
Military member								
Yes	1.27 (0.07–23.38)	.55	. . .	. . .	5.55 (0.29–106.86)	.78	4.33 (0.22–86.66)	.76
No	Reference							
Post–secondary school attendance								
Yes	0.71 (0.38–1.32)	.27	. . .	. . .	0.61 (0.17–2.17)	.44	0.41 (0.15–1.09)	.06
No	Reference							
No. of other persons in household								
≥1 person	0.49 (0.20–1.22)	.12	. . .	. . .	0.76 (0.09–6.09)	.79	1.59 (0.21–12.13)	.65
None	Reference							
Current medical conditions								
≥1 condition	0.31 (0.04–2.29)	.22	. . .	. . .	0.63 (0.04–10.90)	.39	0.75 (0.10–5.74)	.78
None	Reference							
Current medications								
≥1 medication	0.80 (0.43–1.50)	.49	. . .	. . .	0.58 (0.17–2.03)	.39	1.21 (0.45–3.22)	.71
None	Reference							
Age at 1st MMR dose								
12 to <15 mo	2.58 (1.08–6.13)	.03*	2.85 (1.18–6.85)	.02*	3.11 (0.64–15.05)	.14	2.34 (0.66–8.34)	.18
≥15 mo	Reference							
Time since 2nd MMR dose								
<15 y	0.38 (0.16–0.92)	.03*	0.36 (0.15–0.87)	.02*	0.27 (0.03–2.15)	.19	0.13 (0.02–0.98)	.02*
≥15 y	Reference							
Baseline titers								
<16 mIU/mL	. . .	. . .	. . .	. . .	384.0 (22.0–6692.9)	<.0001*	1038.5 (60.7–17 773.6)	<.0001*
≥16 mIU/mL	. . .	. . .	. . .	. . .	Reference			

Abbreviations: CI, confidence interval; MMR, measles-mumps-rubella vaccine; MMR3, third dose of measles-mumps-rubella vaccine; OR, odds ratio.

[a] Adjusted ORs are not reported for 1 month post-MMR3 and 1 year post-MMR3 because the models that included the significant variables from χ^2 analysis were poor fits. When the variable "baseline titers" was excluded from the 1 month and 1 year models, no significant results were found.

* Statistical significance at $P < .05$.

associated with a subject's baseline mumps titer levels (OR, 2.85; CI, 1.18–6.85 [P = .02] and OR, 0.36; CI, 0.15–0.87 [P = .02], respectively) (Table 1). No factors were independently associated with a subject's mumps titers 1 month or 1 year post-MMR3. The 1 month and 1 year post-MMR3 models that included the significant variables from χ^2 analysis were poor-fitting models. Even when the significant variable "baseline titers" was excluded from the 1-month and 1-year models because of its instability due to a zero-cell in bivariate analysis, and other variables were included, no significant results were found.

DISCUSSION

Almost all subjects were mumps virus seropositive prior to receiving MMR3. Virus neutralizing antibody titers had a modest but significant increase following MMR3 when measured 1 month after vaccination. Of 43 subjects with low or negative baseline titers, 33 (76.7%) increased to high titers 1 month after receiving a third MMR dose. This increase in neutralizing titers could facilitate outbreak control by temporarily boosting mumps titers, particularly for those on the cusp of protection.
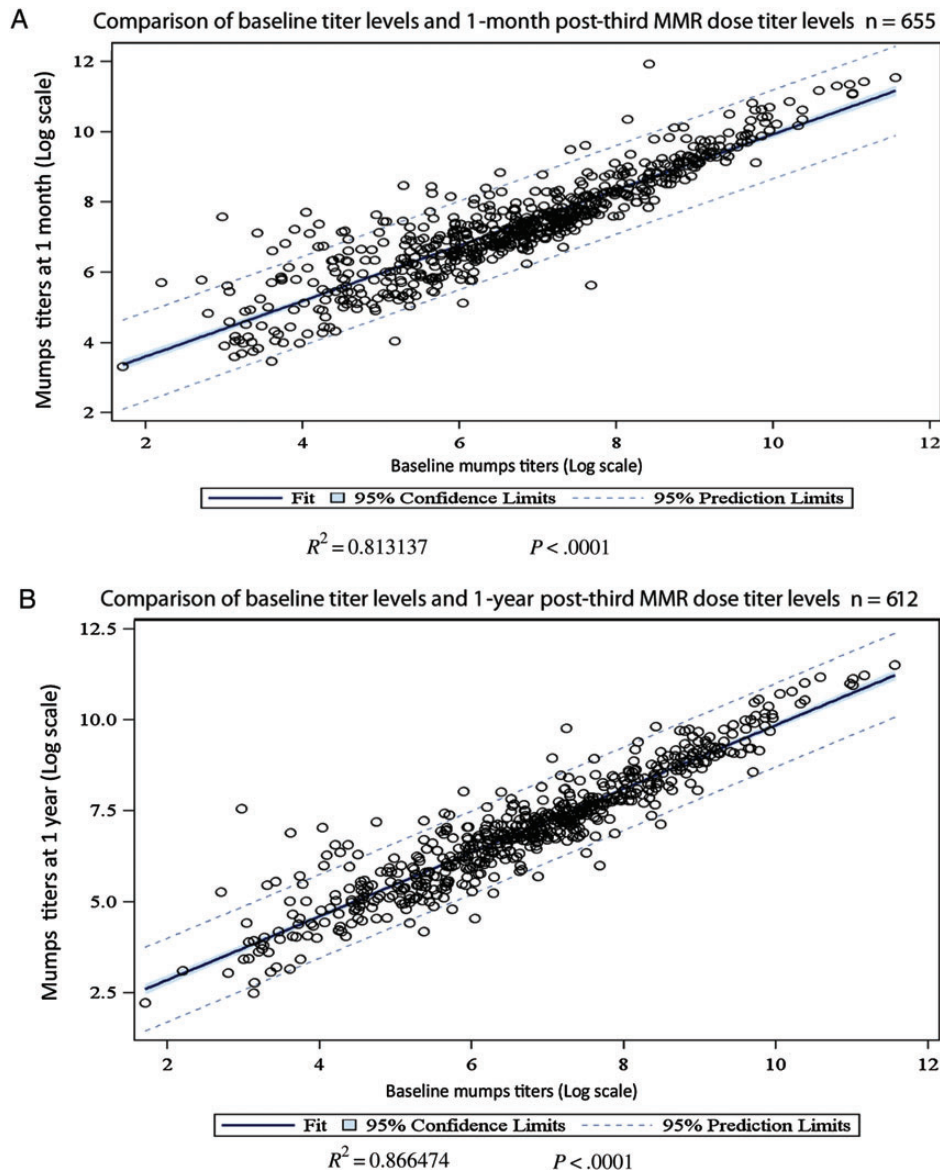
Figure 4. A, Comparison of individual mumps titer levels at baseline and 1 month following a third dose of measles-mumps-rubella (MMR) vaccine. Circles represent individual titer levels. The dark solid line represents the linear regression of the best-fit of the comparison. The light shading around the line represents the 95% confidence interval. The dotted lines represent the 95% confidence limit. B, Comparison of individual mumps titer levels at baseline and 1 year following a third dose of MMR vaccine. Circles represent individual titer levels. The dark solid line represents the linear regression of the best fit of the comparison. The light shading around the line represents the 95% confidence interval. The dotted lines represent the 95% confidence limit.

However, only 52.5% of subjects with low or negative baseline titers sustained high titers 1 year post-MMR3. Overall, titers returned to near-baseline levels 1 year later, which does not support routine administration of a third MMR dose.

Even though the mumps component of the MMR vaccine is the least effective of the 3 antigens, with a 1-dose and 2-dose vaccine effectiveness ranging from 49% to 91% [32–36] and 66% to 95% [34, 35, 37], respectively, 2 MMR doses are generally sufficient to prevent large-scale transmission. During 2006–2012, a median of 454 cases was reported in the United States annually; when outbreaks occurred, they were primarily contained to the affected group (eg, school-aged children, college students, insulated religious communities, inmates), with minimal spread to the broader community.

Although timing of the administration of the first and second doses of MMR vaccine significantly affected mumps titer levels later in life, these findings represented only a small proportion of the population. Nonetheless, individuals who received their first MMR dose at the earlier end of the recommended age range spectrum (12 to <15 months) had nearly 3 times the odds of low or negative baseline mumps titers compared with those who had their first dose at ≥15 months. However, unpublished

data from previous outbreaks in New York and Guam did not find that those who received their first MMR dose at age 12 to <15 months vs ≥15 months were more likely to become infected with mumps (CDC, unpublished data). We found that subjects who received their second dose more recently had a protective effect. Research shows conflicting findings regarding an association between vaccine failure and increasing time since vaccination, with a positive correlation found in some studies [32, 37] and no association found in others [36, 38].

Subjects with high baseline antibody levels tended to stay high over time, and those with lower levels tended to stay lower. This finding suggests there may be an inherent trajectory for mumps antibody production based on an individual's baseline titers (ie, some individuals may be predisposed to lower mumps titers or higher mumps titers, irrespective of the number of additional doses of mumps-containing vaccine they receive).

Although the antibody threshold that provides protection against mumps disease has not been established [31], a prospective study published in 1969 found that clinical mumps during an outbreak did not occur in individuals with titers ≥8 mIU/mL; however, even lower titers offered protection to some individuals [29]. In a recent outbreak-related study conducted among MMR vaccinees, significantly higher neutralizing antibody titers appeared to be associated with protection, although no antibody titer unambiguously discriminated cases from non-cases [31]. Without a correlate of immunity, we cannot assume that the presence of antibodies below the arbitrary cutoff is insufficient to offer protection, nor can we postulate that the presence of antibodies at or above the cutoff necessarily provides protection from mumps infection.

Similar to results previously reported in the longitudinal study [23], <1% of subjects in the current study had negative baseline titers. In contrast, whereas 20% of subjects in the longitudinal study had low neutralizing baseline antibody titers, only 5.8% of subjects in the current study possessed low neutralizing baseline titers. This likely reflects that in the longitudinal study, subjects at baseline had previously received 1 MMR dose, whereas, in our current study, subjects at baseline had previously received 2 MMR doses. This also likely explains why only 2.1% of subjects in the present study vs 50% in the longitudinal study demonstrated a 4-fold rise in titer from baseline to 1 year postvaccination [23]. Also, numerous studies have found mumps virus neutralizing antibody titers to be dependent on the challenge virus strain used in the assay [31, 39]. Whereas the Barnes challenge virus strain was used in the neutralization assay during the longitudinal study, the Jeryl Lynn strain was used during the current study.

Our study has limitations. Subjects resided in predominantly rural areas and self-declared as non-Hispanic white. Thus, they are not representative of the US population. Selection bias may have been present in cohort 1, because MMR3 was only offered to those who had a low or negative measles, mumps, or rubella titer during the longitudinal study. The number of subjects not receiving MMR3 was small, which prevented us from having an adequate comparison group. Although the refusal rate among subjects was high, participation bias based on baseline titer category was unlikely, because individuals were unaware of their baseline titer levels.

Overall, mumps virus neutralizing antibody titers initially increased in response to MMR3 but declined to near-baseline levels 1 year later. Nonetheless, the temporary boosting at 1 month might be sufficient to help control outbreaks if the appropriate population is targeted. Although these quantitative findings show limited application of a third dose of MMR vaccine for routine use, future studies on qualitative aspects of the mumps immune response (eg, antibody avidity, B-cell memory, or cellular-mediated immune responses) are necessary to determine whether MMR3 might be beneficial in nonoutbreak settings.

Supplementary Material

Supplementary material is available online at *Open Forum Infectious Diseases* (http://OpenForumInfectiousDiseases.oxford journals.org/).

Notes

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