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Seroprevalence and durability of rubella virus antibodies in a highly immunized population

Stephen N. Crooke [a], Iana H. Haralambieva [a], Diane E. Grill [b], Inna G. Ovsyannikova [a], Richard B. Kennedy [a], Gregory A. Poland [a,*]

[a] Mayo Clinic Vaccine Research Group, Mayo Clinic, Rochester, MN 55905, USA
[b] Division of Biomedical Statistics and Informatics, Mayo Clinic, Rochester, MN 55905, USA

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ABSTRACT

Background: Although the administration of the measles-mumps-rubella (MMR) vaccine has been widespread in the United States for decades, gaps in vaccine coverage still persist for various reasons. The maintenance of herd immunity against rubella virus (RV) is important to controlling the spread and resurgence of rubella and congenital rubella syndrome.
Methods: In this study, we sought to assess the seroprevalence of RV-specific antibodies in an adult population from a defined geographic area in Olmsted County, MN, and the surrounding municipalities, with relatively high vaccine coverage and no documented evidence of circulating RV in the past 24 years. Rubella-specific IgG antibodies were measured by ELISA in a large set of serum samples ($n = 1393$) obtained from the Mayo Clinic Biobank. This cohort was 80.2% female and ranged from 20 to 44 years of age.
Results: In total, 97.8% of subjects were seropositive for rubella-specific IgG antibodies, with a median titer of 40.56 IU/mL, suggesting a high degree of immunization; however, 2.2% of subjects were found to be seronegative. Interestingly, 25.1% of subjects were seropositive but had titers lower than 25 IU/mL, indicating either a population of low responders or individuals that could potentially be at risk of waning immunity. No significant associations or differences were found between RV-specific titers and demographic variables such as age, sex, or body mass index (BMI).
Conclusions: A high rate of seropositivity for rubella was found among this young adult cohort, but a significant percent of the cohort had lower titers that may indicate poor initial vaccine response and potential risk if their antibody titers decline.

1. Introduction

Rubella virus (RV) remains a pathogen of important global health concern given the prevalence of documented infections that continue to occur despite the availability of an effective vaccine. While RV infection is typically characterized by a mild fever and rash, it can lead to serious congenital health complications in pregnant women [1,2]. The virus is capable of infecting and replicating in fetal tissues, causing systemic inflammation that can lead to a variety of birth defects (collectively termed congenital rubella syndrome) or miscarriage [3,4]. While the rate of rubella infection has been significantly reduced since the introduction of the measles-mumps-rubella (MMR) vaccine in the U.S. in 1971 [5], global estimates indicate that 100,000 cases of congenital rubella syndrome are still diagnosed each year [6].

The rubella-containing vaccine is highly effective, with clinical trials documenting >95% seroconversion in vaccinated individuals after a single dose [7]; yet, large gaps in vaccine coverage still exist. Despite its proven efficacy, waning immunity to RV has been observed among vaccinated populations [8], and considerable inter-individual variation in antibody response to the rubella vaccine is evident at the population level [9,10]. Indeed, our own studies have identified several HLA alleles and single nucleotide polymorphisms (SNPs) that are associated with this variability [11–15]. Furthermore, vaccination strategies that have failed to administer to the entire population in several countries have led to continued outbreaks and the spread of RV, as evidenced by recent occurrences in Poland and Japan [16,17]. An outbreak of rubella among young adult males in Poland during 2013 was largely attributed to a failure in vaccination policy, which only

* Corresponding author at: Mayo Clinic Vaccine Research Group, Mayo Clinic, Guggenheim Building 611C, 200 First Street SW, Rochester, MN 55905, USA.
E-mail address: poland.gregory@mayo.edu (G.A. Poland).

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mandated vaccination against RV in adolescent females from 1989 to 2004 [16]. A large-scale rubella outbreak also occurred in Japan during 2013, again reflective of an ineffective vaccination strategy whereby RV immunization was recommended but not required by the government, leading to significant gaps in coverage among the population [17].

The long-term control and eradication of RV depends upon both the implementation of effective vaccine programs that maintain high levels of protective coverage among vaccinees as well as the durability of vaccine-induced immunity. Regular monitoring of immune status and vaccine coverage in defined geographic regions is critical for the identification of subgroups at higher risk of disease susceptibility and informing public health decisions on the design of more effective vaccination strategies. This is particularly important for women of childbearing age due to complications associated with RV infection during pregnancy. In this study, we conducted a seroepidemiological survey in Olmsted County, MN, and the surrounding municipalities to estimate the effects of age, sex, and other demographic variables on the seroprevalence of IgG antibodies against RV in an adult highly immunized population given the established two-dose childhood immunization policy for MMR in the state of Minnesota [18].

2. Methods

2.1. Sample selection

The design and recruitment criteria for the Mayo Clinic Biobank have been previously reported [19]. Individuals at Mayo Clinic (Rochester, MN) who met the following criteria were deemed eligible for inclusion in the Mayo Clinic Biobank: ≥18 years of age, United States residency, and mental capacity to consent. No restrictions on current health status were set for enrollment in the Biobank. Recruitment for the Biobank was carried out through a mailed notice to individuals scheduled for appointments in various departments at the Mayo Clinic, and informed consent was obtained from all subjects. Prior to sample collection, subjects completed a detailed questionnaire on lifestyle and medical history to compile relevant demographic information. A blood sample was collected to provide serum, plasma, white blood cells, and DNA.

For this study, 2000 subjects who were between the ages of 18 and 45 years old, and had at least 0.5 mL of serum available in the Biobank repository, were contacted to assess their interest in additional vaccine immune response studies. Interested participants who agreed to have their identifiers released to the study team were enrolled in the study. The Mayo Clinic Biobank provided serum samples for each of these subjects to the study team for analysis. The final cohort for this study was comprised of 1393 subjects (Table 1). Specifically, samples were selected from adults (20–44 years of age) who lived in Olmsted County, MN, or the surrounding municipalities. Approval of the Mayo Clinic Biobank protocol and permission to conduct this seroprevalence study were granted by Mayo Clinic's Institutional Review Board.

2.1.1. ELISA for rubella antibodies

Rubella-specific IgG antibody titers were measured using the Zeus Rubella IgG ELISA Test System (Zeus Scientific Inc.; Branchburg, NJ) using the indirect solid-phase antigen method and according to the manufacturer's instructions. Briefly, serum samples were diluted in the kit's proprietary SAVe Diluent® (dilution 1:200) and were simultaneously assayed in duplicate with the kit's provided calibrator, positive and negative control. The optical density (OD) ratio for each sample was calculated by dividing the sample OD by the cutoff OD (the mean calibrator OD value multiplied by the correction factor value, provided in the kit). This OD ratio was then multiplied by 9.091 to give the rubella-specific IgG antibody titer in IU/mL, as per the manufacturer's instructions. The coefficient of variation (CV) for this assay based on repeated measurements was 2.6% in our laboratory. Seropositivity was defined as IgG titers ≥10 IU/mL, while titers ≤8.18 were defined as seronegative. Subjects with titers ranging from 8.19 to 9.99 were defined as equivocal responders.

Table 1
Subject demographics and rubella titers summary.

Age	
N	1393
Mean (SD)	36.34 (5.33)
Median	36.82
Q1, Q3	32.55, 40.81
Range	20.41 44.66
Race	
American Indian	1 (0.07%)
Asian Indian	1 (0.07%)
Black/African/African-American	9 (0.65%)
Chinese	1 (0.07%)
Filipino	1 (0.07%)
Korean	3 (0.22%)
Caucasian-American	1327 (95.26%)
Multiple	14 (1.01%)
Other Asian	13 (0.93%)
Other	5 (0.36%)
Unknown	17 (1.22%)
Ethnicity	
Not Hispanic or Latino	1360 (97.63%)
Hispanic or Latino	19 (1.36%)
Unknown	14 (1.01%)
Sex	
Female	1117 (80.19%)
Male	276 (19.81%)
BMI	
N	942
Mean (SD)	27.03 (6.33)
Median	25.50
Q1, Q3	22.44, 30.84
Range	10.75–58.82
Number of Rubella Vaccine Doses	
1 MMR Dose on Record	216 (15.51%)
2 MMR Doses on Record	30 (2.15%)
Unknown MMR Status	1147 (82.34%)
RV Titers (IU/mL)	
N	1393
Mean (SD)	50.72 (37.9)
Median	40.56
Q1, Q3	24.57, 65.92
Range	1.15–294.06
Seropositivity	
Negative (≤8.18)	30 (2.15%)
Equivalent (8.19–9.99)	1 (0.07%)
Positive (≥10.0)	1362 (97.8%)

2.1.2. Statistical analyses

Spearman's correlation was used to test for the association between continuous variables (e.g., BMI and age) and RV titer (IU/mL). Univariate linear regression models with log base-2 RV titer (IU/mL) as the response variable were used to test for significant differences in RV titer between sex as well as known vs. unknown dose status.

3. Results

3.1. Demographics

For this study, 1393 subjects were included based on consent and sample availability. The median age at the time of sample

collection was 36.8 years of age, with a corresponding range of 20.4–44.7 years. The majority of the participants were female (80.2%). The remaining samples (19.8%) were collected from male subjects to allow for comparison between sexes. Caucasian-Americans comprised 95.3% of the study cohort, with the remaining 4.7% of the cohort made up of a limited number of individuals identifying as other races. The majority of individuals in this study (97.6%) identified as non-Hispanic or Latino. Current body mass index (BMI) was calculated for all subjects with documented height and weight ($n = 942$). See Table 1 for a complete summary of subject demographics recorded at the time of sample collection.

3.1.1. Seroprevalence of rubella antibodies

Circulating rubella-specific IgG titers were measured in duplicate by ELISA for serum samples from each subject, with the distribution of titers presented in Fig. 1. The median titer for the cohort was 40.56 IU/mL, with 97.7% of the subjects ($n = 1362$) identified as seropositive (IgG titer $\geq$10 IU/mL). A significant portion of the seropositive population was found to have titers below 25 IU/mL ($n = 349$, 25.1%; Table 2). The remaining subjects were found to be either seronegative ($n = 30$, 2.2%) or equivocal responders ($n = 1$, 0.07%). Records for the MMR vaccination status were not available for the majority of the subjects ($n = 1147$), but the observed antibody titer distribution demonstrated that protective antibody titers were present in the majority of the population and suggested that most subjects had previously received at least one dose of the MMR vaccine (Fig. 1A). Importantly, a comparison of rubella-specific IgG titers between subjects with at least one documented dose of the MMR vaccine and those without available vaccination records revealed no significant differences ($p = 0.76$) between the two groups (Table 3, Fig. 1B), which further confirms that our cohort is representative of a highly vaccinated population.

3.1.2. Association of antibody titers with age, sex, and BMI

We next investigated if subject age, sex, or BMI was associated with RV-specific IgG antibody titer. A complete listing of age-related statistics is fully outlined in Table 1. Despite a number of well-documented studies that demonstrated waning immunity with increasing age and time since last vaccination [20–23], there was no significant correlation between rubella-specific antibody titers and age observed in this cohort (Spearman correlation analysis, $p = 0.17$; Fig. 2A).

Most of the subjects in our study cohort were female ($n = 1117$), which limits the statistical power for our comparison of RV-specific titers to the smaller group of male subjects ($n = 276$) included in this analysis. Median rubella-specific IgG titer for the male subjects was 37.8 IU/mL, while the median titer was slightly higher at 41.3 IU/mL for female subjects (Table 4, Fig. 2C). While we did observe differences in median titers, the differences did not meet statistical significance—likely due to the power issue noted above (Fig. 2D).

Lastly, we sought to determine if RV-specific titers were associated with calculated BMI for subjects with documented height and weight data ($n = 942$). Seven subjects were excluded from this analysis due to incomplete records on height and weight. The median BMI for the cohort was 25.5, with a wide range (10.7–58.8) among the population. Despite this range of BMI, there was no significant association observed between BMI and rubella-specific antibody titers by Spearman correlation analysis ($p = 0.41$; Fig. 2B).

Table 2
Positive rubella titers lower than 25 IU/mL.

RV Titers (IU/mL)	
N	318
Mean	17.44
Median	17.51
Q1, Q3	13.84, 20.83
Range	10.45–24.57
10–15 IU/mL	99 (31.13%)
15–20 IU/mL	117 (36.79%)
20–25 IU/mL	102 (32.08%)

Table 3
Comparison of seropositivity and MMR dose.

	1 Dose of MMR	2 Doses of MMR	Unknown MMR Status
Negative ($\leq$8.18)	5	1	24
Equivalent (8.19–9.99)	0	0	1
Positive ($\geq$10.0)	211	29	1122

4. Discussion

The generation and maintenance of protective antibody titers among the population is the primary goal of public health vaccination programs, and the success of these programs is dependent upon monitoring and subsequent alterations in policy. In this study, we sought to assess the seroprevalence of rubella-specific IgG antibodies among a contemporary, large population of healthy adults from a geographically defined region with known high vaccine coverage in southeastern Minnesota. We also investigated the relationship between rubella-specific antibody titers and

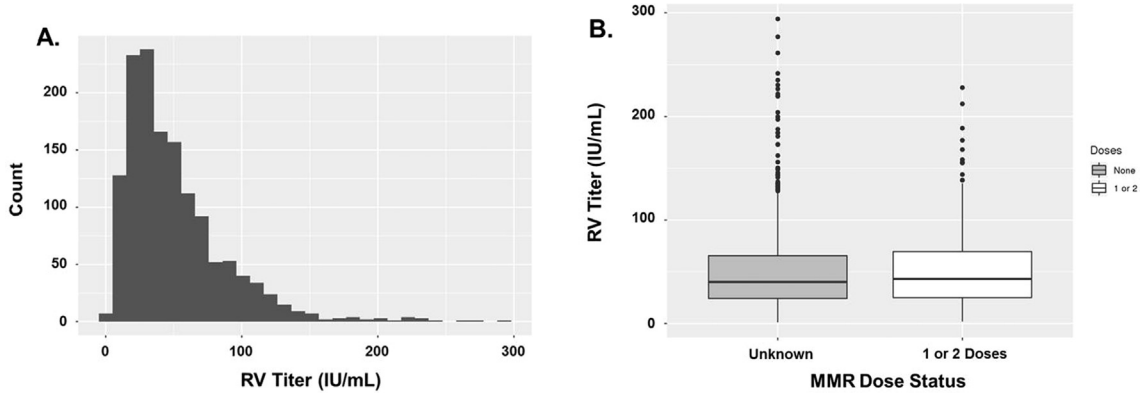

Fig. 1. Rubella-specific IgG titers. (A) Distribution of RV titers in the population. (B) Comparison of rubella-specific IgG titers between individuals with unknown MMR vaccination records ($n = 1147$) and those with 1 ($n = 216$) or 2 ($n = 30$) documented MMR doses. No significant differences were observed for titers between individuals with or without vaccination records.

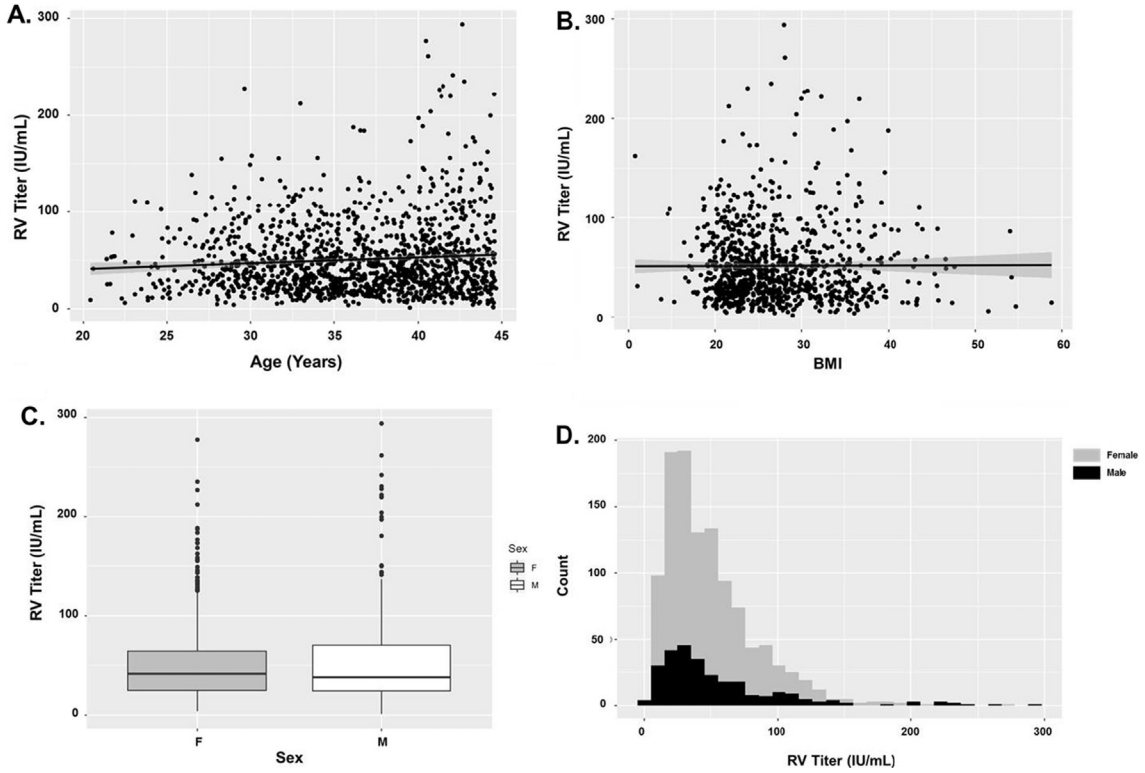

Fig. 2. Comparison of rubella-specific IgG titers with demographic variables. (A and B) Spearman correlation analysis of rubella-specific titers with subject age (A) and BMI (B). There were no significant relationships between subject age ($p = 0.17$) and BMI ($p = 0.41$) with RV titer. (C) Box-and-whisker plot comparing RV titer between male and female subjects. (D) Rubella-specific titer distribution for male and female subjects.

Table 4
Comparison of rubella titers and sex.

Male	
N	276
Mean (SD)	55.35 (49.44)
Median	37.82
Q1, Q3	24.16, 70.69
Range	1.15–294.06
Female	
N	1117
Mean (SD)	49.58 (34.4)
Median	41.30
Q1, Q3	24.73, 64.84
Range	3.72–277.01

demographic variables (age, sex, and BMI), as vaccine responses have been previously shown to be influenced by such factors [24–27].

The study included 1393 subjects with preexisting samples collected from the Mayo Clinic Biobank. As no health or demographic restrictions were placed on the individuals contacted for initial recruitment into the Biobank, the selected cohort used for this study should be reflective of the general population. We found that 97.7% of the subjects were seropositive for rubella-specific antibodies, which is in agreement with previous studies reporting seropositivity rates against RV between 90 and 100% [20,28–32]. A small but notable percentage of our study cohort were found to be either seronegative (2.2%, $n = 30$) or equivocal (0.07%, $n = 1$) responders, in good agreement with the rates observed in these previous reports [20,28–32]. Some studies have reported lower rates of seroprevalence for rubella-specific antibodies [33–36], but these differences may be reflected in the regional variation of childhood immunization policies and/or the demographics of the individual study cohorts. Our study cohort was predominantly female (80.2%), and by extension, a large number of the seronegative individuals ($n = 18$) were also female; these individuals are at particular risk for complications during pregnancy if infected with rubella. Furthermore, a significant portion of seropositive individuals in our study cohort were found to have titers below 25 IU/mL (22.8%, $n = 318$), with multiple individuals having titers below 15 IU/mL (7.1%, $n = 99$). These individuals potentially represent a group of poor vaccine responders and could be at risk of becoming susceptible to RV infection should their immune responses wane below the protective level (i.e., 10 IU/mL) over time. Although rare, limited cases of breakthrough rubella infections in vaccinated individuals—particularly pregnant women—have been documented, [37,38] and this phenomenon has also been observed for other diseases [39,40]. Waning immunity has also been implicated as an underlying cause for the increased number of mumps outbreaks in recent years [23]. The large proportion of individuals in our cohort with low antibody titers may be at risk for future RV infection should their antibody titers further decline, highlighting the need for a personalized approach to vaccination in order to provide robust protective immunity. Personalized vaccinology is an approach that tailors immunizations for the individual; given current practice, subjects who fail to develop robust immune responses under standard vaccination policies may benefit from receipt of an additional dose or an adjuvanted vaccine [41–44].

Sex-based differences in immune response outcomes have also been observed in response to several vaccines, with females typically displaying higher antibody titers following immunization [24,45,46]. Our study cohort was primarily comprised of female subjects of child-bearing age, which is the group that would be at the highest risk of complications from RV infection during pregnancy. We found that female subjects had slightly higher median titers (41.3 IU/mL) compared to male subjects (37.8 IU/mL), although the difference in titers was not statistically significant. The overall distribution of titers among our study population was

similar for both male and female subjects, although it should be noted that only 276 male subject samples were included for analysis, which limited our ability for statistical comparisons. These observations were consistent with a 2014 study of healthcare workers in Spain that found no significant differences in rubella-specific responses based on sex [47]. A large study of the U.S. population from 1999 to 2004 also found that rubella seropositivity among adults was statistically equivalent, but also slightly higher in women (91.5%) than men (88.0%) [48]. In contrast, an age-stratified study using data collected from the National Health and Nutrition Examination Survey (NHANES) during 2009–2010 found that rubella seropositivity was significantly higher in females (97.2%) compared to males (93.5%) [49]. Other studies have also reported significant differences in humoral responses in women compared to men following vaccination against both rubella and other diseases [24,50,51]. The results of our analyses are consistent with previous reports for larger cohorts, suggesting that our observations are representative of the general population.

We also sought to evaluate the effects of age on the seroprevalence of rubella-specific IgG titers. It is well known that the function of the immune system declines with age and time since last vaccination [52–54], and waning immunity has been observed for rubella as well as other vaccine-preventable diseases as the time since the last immunization increases [20–23,33]. It should be noted that most studies investigating waning immunity following MMR vaccination have focused on mumps and measles, as many seroprevalence studies report 90–100% seropositivity against rubella several years after vaccination [20,28–32]. Unfortunately, MMR immunization records were not available for the majority of the subject samples, and the few subjects that did report the number of doses received ($n = 246$) did not provide the dates for those immunizations. We elected to analyze the correlation between titer and age as a surrogate comparison for time since the last vaccine dose, as age-matched individuals should have received their final dose of MMR within 1–2 years of each other under current US vaccination policy. This analysis did not identify any significant correlation between age and rubella-specific titer ($r = 0.04$, $p = 0.17$), suggesting that waning immunity against rubella may not be a major concern at the population level.

Due to the lack of vaccination records for most of our subjects, we compared the rubella antibody distribution between those with immunization records ($n = 246$) and those without ($n = 1147$). Both groups had a similar distribution of rubella antibody titers. This suggests that the vaccination histories of the two groups are similar, which is not surprising as our population was born and raised in the United States under a consistent and uniform MMR vaccination policy. We also note that there have been no documented cases of rubella in Olmsted County, MN, for the last 24 years [55]; therefore, our antibody results reflect this population's vaccine-induced, rather than disease-induced, immunity to rubella.

Finally, we sought to investigate the relationship between subject BMI and rubella-specific antibody titers. While existing data is limited and often conflicting, studies have suggested that obesity is a contributing factor to poor vaccine responses as well as declining antibody titers [25]. A report by Hui and colleagues found that higher BMI was associated with rubella seronegativity in a young cohort (<25 years of age) from Hong Kong [56]; alternatively, Siberry et al. found no association between obesity and rubella vaccine responses in a study of children in the U.S. with perinatal exposure to HIV [57]. Interestingly, a comparative study of H1N1 influenza vaccine responses in healthy, overweight, and obese individuals found that influenza-specific antibody titers declined in the year following vaccination, and this decline was associated with higher BMI [27]. While we did not have access to subject's BMI at the time of vaccination, we did have BMI at the time of entry into the Biobank (several years after vaccination). We did not identify any significant correlation between rubella antibody titers and BMI ($r = -0.03$, $p = 0.41$) in our study cohort of healthy young adults.

Our data indicate that the majority of our study population is seropositive for protective titers of rubella-specific antibodies, particularly among females of child-bearing age, though we identified a sizable proportion with lower than anticipated antibody titers—potentially suggesting poor initial vaccine responses or waning immunity. While we did not find any significant associations between the titer and the demographic variables of age, sex, and BMI, studies among more diverse populations are warranted to fully understand how these factors may broadly influence vaccine responses. One of the main limitations in this study was the lack of ethnic diversity among the cohort, which was 95.26% Caucasian-American and 97.63% non-Hispanic; the cohort was also predominantly female (80.2%), which allowed for a clinically relevant population but limited our ability to conduct statistical analyses based on sex. In future reports evaluating the seroprevalence of protective antibodies, broader demographic inclusion will allow for a more complete assessment of vaccine efficacy among all ethnic groups in the population. Evaluating antibody titers in an equally distributed population of men and women will also allow for a more rigorous analysis of sex-based differences. The lack of available vaccination records for the subjects also limited our ability to more closely examine relationships between antibody titers and time since the last vaccine dose. Our surrogate analysis of age and antibody titers suggests that waning immunity is not a major concern among the population, but further studies that control for the receipt of vaccine doses should be considered.

Seroprevalence studies such as this one are critical for monitoring the overall effectiveness of vaccination programs and informing changes in healthcare policies in order to prevent recurrent outbreaks of disease. Our study population demonstrated high rates of immunity against RV (97.8%); however, a small portion (2.2%) of the cohort were seronegative and a larger portion (22.8%) were seropositive but had titers below 25 IU/mL. These data demonstrate that vaccination efforts may benefit from a personalized vaccinomics approach [41,42,58], as these individuals likely have a poor initial vaccine response or suffer some degree of waning immunity over time. Vaccinomics approaches allow an understanding of the underlying mechanism for non- or poor-vaccine responses and inform new candidate vaccine development. Our data suggest the former, and it would be highly informative to investigate the underlying immunogenetic mechanisms governing responses to rubella vaccination in these individuals who exhibit lower antibody titers. In any case, those individuals with rubella-specific titers approaching or below 10 IU/mL might benefit from receipt of a third dose of the MMR vaccine. There have been no documented cases of rubella in Olmsted County, MN, in the past 24 years, which is likely explained by the high prevalence of protective antibody titers among this population. Moreover, only seven cases of rubella have been documented in the entire state of Minnesota during that same timespan [55]. In 2016, the National Immunization Survey found that MMR vaccination coverage among children in Minnesota (age 19–35 months) was 94.3% [59], suggesting that the majority of the population received at least one dose of vaccine in their lifetime. Our comparative analysis in this study found that anti-rubella IgG titers were similar for adults both with and without documented vaccination records, which is in agreement with these high coverage rates and suggests that this level of vaccine coverage is contributing to effective herd immunity among this population. While the results of this study suggest the current rubella vaccination policy is effective at maintaining protective levels of antibodies among the population, it also highlights the inter-individual variability of vaccine responses and potentially indicates a group of individuals that should be

considered for further study in order to fully characterize the factors governing long-term immunity to rubella.

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Conflicts of Interest

Dr. Poland is the chair of a Safety Evaluation Committee for novel non-rubella investigational vaccine trials being conducted by Merck Research Laboratories. Dr. Poland offers consultative advice on vaccine development to Merck & Co. Inc., Avianax, Adjuvance, Valneva, Medicago, Sanofi Pasteur, GlaxoSmithKline, and Emergent Biosolutions. Drs. Poland and Ovsyannikova hold three patents on measles and vaccinia peptide research. Dr. Kennedy holds a patent on vaccinia peptide research. Dr. Kennedy has also received funding from Merck Research Laboratories to study waning immunity to mumps. All other authors declare no competing financial interests. These activities have been reviewed by the Mayo Clinic Conflict of Interest Review Board and are conducted in compliance with Mayo Clinic Conflict of Interest policies. This research has been reviewed by the Mayo Clinic Conflict of Interest Review Board and was conducted in compliance with Mayo Clinic Conflict of Interest policies.

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