Persistence of Mumps Antibodies after 2 Doses of Measles-Mumps-Rubella Vaccine

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Background. Since 1990, most US schoolchildren have received a second dose of measles-mumps-rubella vaccine (MMR2) at kindergarten entry. The objective of the present study was to evaluate the short- and long-term mumps immunogenicity of MMR2.

Methods. At enrollment in 1994–1995, children (n = 308) in a rural Wisconsin health maintenance organization received MMR2 at age 4–6 years. A comparison group of older children (n = 308) was vaccinated at age 9–11 years. Serum samples were collected over 12 years. Mumps antibody levels were evaluated by plaque-reduction neutralization (lowest detectable titer, 10).

Results. Before MMR2, the geometric mean titer (GMT) for the younger group was 33; no subject was seronegative, but 16% had the lowest detectable titer. In response to MMR2, the GMT tripled to 97, and the proportion with low titers diminished to 3%. Four-fold boosts occurred among 54%, but only 3% were positive for immunoglobulin M. Twelve years after MMR2, the GMT declined to 46, the proportion with titers ≥10 was not significantly different from the pre-MMR2 proportion, and 5% were seronegative. The older group showed similar patterns, and at age 17 years both groups had comparable antibody levels.

Conclusions. The mumps antibody response to MMR2 was vigorous, but over a 12-year period titers declined to levels similar to pre-MMR2 titers. No advantage was apparent in delaying MMR2 from kindergarten to middle school.

Mumps is an infectious viral disease, usually manifested by inflammation of the salivary glands and fever [1]. One-third of cases may be asymptomatic, but aseptic meningitis can affect 10% of cases [1], mumps is a leading cause of deafness in childhood [2], and an estimated 13% of post-pubertal males with mumps orchitis have impaired fertility [3]. In the absence of vaccination, the peak incidence of mumps occurs in the early school years, and ~90% of children are infected by age 15 years [2, 4].

A mumps vaccine was licensed in 1967, and in 1977 the Advisory Committee on Immunization Practices (ACIP) recommended universal childhood vaccination with a single dose [5]. By 1985, reports of mumps had been reduced by >98%, compared with that in the prevaccine era [6]. A resurgence occurred in the late 1980s, with sustained viral transmission in highly vaccinated school populations [7–11]. In 1989, the ACIP recommended that school-age children receive 2 doses of measles-mumps-rubella vaccine (MMR) [12]. Disease rates were markedly reduced, and the goal of mumps elimination was set for 2010 [13].

In 2006, the United States experienced a multistate outbreak involving >6500 cases, with the highest attack rate among persons 18–24 years of age, many of whom were college students [14]. Studies in affected colleges suggested that almost all persons affected had received a second dose of MMR, most >10 years previously [15, 16]. This raised concerns about long-term immunity against mumps after a second dose, of which there have been relatively few studies [17–21].
In 1994, as previously reported [22, 23], the Centers for Disease Control and Prevention (CDC) began a study of the short- and long-term immunogenicity of the second MMR dose (MMR2). We now report our findings on mumps.

**METHODS**

**Context and Setting**
All states currently require MMR2 for school attendance, most at kindergarten entry and some at middle-school entry [24]. European nations show a similar diversity in recommended ages for the second dose [25]. In 1990, the state of Wisconsin passed a law requiring MMR2 for both kindergarten and middle-school entry. This provided a setting to examine the impact of the 2 most frequently used routine MMR2 schedules. The study population was drawn from patients of Marshfield Clinic, a comprehensive health maintenance organization that is the principal health care provider for rural central Wisconsin. The clinic has a longitudinally stable patient population with a low rate of utilization of outside services.

**Subjects and Enrollment**
In 1994, the clinic’s computerized files were reviewed to identify 2 groups of children for whom MMR2 was required under state law: kindergarten (4–6 years old) and middle school (10–12 years old). For legal and ethical reasons, no child entering kindergarten had vaccine deferred until middle-school entry; both study groups were vaccinated simultaneously. Candidate study subjects were excluded if they (1) had previously had measles, mumps, or rubella disease; (2) lived in the same household with anyone who had had these diseases during the subject’s lifetime; (3) had received MMR1 other than at 12–24 months of age; (4) had received any other vaccinations within 30 days of the start of the study; or (5) had any contraindication to MMR vaccination or any condition likely to impair the immune response to the MMR vaccine, as specified in the ACIP recommendations [26]. Parents of study subjects were provided with informed permission materials, and children in middle school were additionally provided with informed assent materials. Other than reimbursement for travel expenses, no remuneration was provided to study subjects or their families. The study was approved by the human subjects protection offices of both the Marshfield Clinic and the CDC.

**Design**
Prevaccination serum samples were obtained, and the MMR2 dose (M-M-R II; Merck) was administered within 2 days by study nurses, along with any other vaccinations for which the child was eligible. Adverse events were evaluated as reported elsewhere [22]. Serum samples were collected according to a schedule (table 1) that permitted antibody levels for the 2 groups to be compared at similar ages. At each collection, families were questioned concerning mumps disease, exposures, vaccinations, and other health events. Clinic and CDC data concerning mumps disease activity were also reviewed.

**Laboratory Methods**
Antibody levels were evaluated by the plaque-reduction neutralization test [27, 28], with immunoenzymatic staining to demonstrate plaques. Testing was performed at the end of the study, and specimens from individual subjects were tested in the same run. Other than each subject’s unique identifier and serum-collection dates, the laboratory was blinded to all study information.

**Materials.** The Vero cell line and 48-well plates (Nunc) were used. All test and control serum samples were serially diluted 2-fold from 1:10 to 1:320 in Eagle’s minimal essential medium and Earl’s cell culture medium. Titers <1:10 were considered seronegative. Serum controls were high-positive, low-positive, and negative serum samples, as evaluated by EIA. The mumps virus was the Barnes strain, a 5-passage, non-adapted, wild-type strain isolated in California in the 1960s from

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**Table 1. Study design and subject retention.**

<table>
<thead>
<tr>
<th>Serum sample collection</th>
<th>Time from MMR2a</th>
<th>Year</th>
<th>Kindergarten group (n = 312)</th>
<th>Middle-school group (n = 309)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Age, years</td>
<td>Subjects, no. (%)</td>
</tr>
<tr>
<td>1</td>
<td>Before</td>
<td>1994–1995</td>
<td>5</td>
<td>308 (99)</td>
</tr>
<tr>
<td>2</td>
<td>1 month</td>
<td>1994–1995</td>
<td>5</td>
<td>301 (96)</td>
</tr>
<tr>
<td>3</td>
<td>6 months</td>
<td>1994–1995</td>
<td>5</td>
<td>295 (95)</td>
</tr>
<tr>
<td>4</td>
<td>2 years</td>
<td>1996–1997</td>
<td>7</td>
<td>242 (78)</td>
</tr>
<tr>
<td>5</td>
<td>5 years</td>
<td>1999–2000</td>
<td>10</td>
<td>174 (56)</td>
</tr>
<tr>
<td>6</td>
<td>7 years</td>
<td>2001–2002</td>
<td>12</td>
<td>160 (51)</td>
</tr>
<tr>
<td>7</td>
<td>10 years</td>
<td>2004–2005</td>
<td>15</td>
<td>154 (49)</td>
</tr>
<tr>
<td>8</td>
<td>12 years</td>
<td>2006–2007</td>
<td>17</td>
<td>144 (46)</td>
</tr>
</tbody>
</table>

**NOTE.** The study end point was antibody level at age 17 years.

a Time intervals indicate duration after the second dose of measles-mumps-rubella vaccine (MMR2).
an unvaccinated adult who had apparently acquired the infection in Europe; by genetic analysis, it is closely related to the Zagreb strain.

**Virus neutralization step.** A pretitrated cell-free dilution of mumps virus to give ~90–100 plaques per well was added to each serum dilution and incubated at 37°C in a CO₂ incubator for 90 min. Then, 40,000 freshly trypsinized Vero cells were added, and the plates were transferred to the CO₂ incubator. For positive virus controls, 6 wells were infected with virus but no serum; for negative controls, 6 wells were not infected. After incubation for 2 h, the serum–virus mixture was removed from all wells and supplemented with freshly prepared medium containing 4% fetal bovine serum. After 72 h of incubation, the cell culture medium was aspirated from all wells and dried at room temperature, and the cell monolayer was fixed with 80% cold acetone. After 10-min fixation, the acetone was removed, and the cell monolayer was dried again at room temperature.

**Immunoenzymatic staining step.** Pretitrated monoclonal antibody to mumps virus (Chemicon International) was added and incubated at 37°C for 60 min. After unbound antibody was removed, the plates were washed 3 times with PBS (0.05 mol/L PBS [pH 7.4]). Pretitrated horseradish peroxidase–conjugated rabbit anti–mouse IgG (Accurate Chemical and Scientific) was added, and the plates were incubated at 37°C for 60 min and then washed again 3 times. HistoMark (Kirkegaard and Perry Laboratory) was added, the plates were incubated at 37°C for 30 min, and the substrate solution was then removed and rinsed briefly with water to stop further color development.

**Inspection step.** All wells were inspected visually with a magnifying glass. A black-brown spot ≥1 mm in diameter was considered a plaque. An 80% reduction in plaque count, compared with the mean plaque count for the positive virus control wells, was considered indicative of neutralizing antibody.

**Analytic Methods**

Serum samples with reciprocal titers of 〈10 or 〉320 were assigned values of 5 and 640, respectively. Geometric mean titers (GMTs) were calculated using log-transformed reciprocal titers and are reported as back-transformed titers. Antibody levels falling in the lowest 2 titers (〈10, 10) were categorized as low, those falling in the highest 3 titers (80, 160, 〉320) were categorized as high, and those falling in intermediate titers (20, 40) were categorized as medium. The distribution of titers, the proportion seronegative, and the proportion with low titers were examined as primary indicators of antibody level. Using these indicators, pre- and postvaccination antibody levels were compared. The primary study end point was antibody level at age 17 years, compared with the pre-MMR2 level. Antibody levels for the 2 study groups were compared at the same serum collections and at the same ages. Risk factors examined were sex, race/ethnicity, mother’s birth year, age at MMR1, receipt of other vaccinations with MMR2, and pre-MMR2 antibody levels. In bivariable comparisons, the tests used were the Pearson χ² and Fisher’s exact tests for categorical variables; the row mean score, Jonckheere-Terpstra, and Cochran-Armitage trend tests for ordinal variables; and the Wilcoxon rank sum test for continuous variables. Linear regression analysis was used to evaluate associations for multiple risk factors.

**RESULTS**

**Study population.** The kindergarten and middle-school groups did not differ significantly by sex, race/ethnicity, or age at MMR1 (table 2). The rate of retention was 96% (597/621) during the first 6 months and 54% (333/621) by the end of the study (table 1). The 333 children who provided serum samples at every collection did not differ significantly from the 288 others with regard to demographic factors or antibody level indicators.

**Pre-MMR2 antibody levels.** Overall, before MMR2, <1% of subjects (4/616) were seronegative, but 20% (122/616) had low titers. Compared with the middle-school group (10 years after MMR1), the kindergarten group (4 years after MMR1) had higher titers (GMT, 33 vs. 26; P < .001) and fewer children with low titers (49/308 [16%] vs. 73/308 [24%]; P = .015) or negative titers (0/308 [0%] vs. 4/308 [1%]; P = .124) (figure 1).

**Table 2. Study population characteristics.**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Kindergarten group (n = 312)</th>
<th>Middle-school group (n = 309)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age group related</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at MMR2, years</td>
<td>5.1 (4.2–6.1)</td>
<td>11.2 (10.1–12.5)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Received other vaccinations with MMR2, no (%)</td>
<td>222 (71)</td>
<td>1 (0.3)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex, no. (%) female</td>
<td>153 (49)</td>
<td>150 (49)</td>
<td>NS</td>
</tr>
<tr>
<td>Race/ethnicity, no. (%) white</td>
<td>305 (98)</td>
<td>307 (99)</td>
<td>NS</td>
</tr>
<tr>
<td>Age at MMR1, months</td>
<td>15.6 (12.8–24.7)</td>
<td>15.7 (14.1–24.5)</td>
<td>NS</td>
</tr>
</tbody>
</table>

**NOTE.** Data are median (range) values, unless otherwise indicated. MMR1, first dose of measles-mumps-rubella vaccine; MMR2, second dose of measles-mumps-rubella vaccine; NS, not significant.
Initial MMR2 response. For both groups aggregated, titers rose at 1 month after MMR2 and then declined at 6 months but were still significantly higher than pre-MMR2 titers (GMT, 66 vs. 29; $P < .001$) (figure 1). In aggregate, the proportion with low titers was significantly reduced (for pre-MMR2, 122/616 [20%]; for 6 months, 23/597 [4%]; $P < .001$), and only 1 child was antibody negative. IgM positivity was detected in 2% of children (13/612), with no significant association with pre-MMR2 titer or group (for IgM-positive subjects, 9/304 [3%] in the kindergarten group vs. 4/304 [1%] in the middle-school group; $P = .173$). However, 50% of subjects (308/616) exhibited a 4-fold boost in neutralization titer, the likelihood of which was increased by lower pre-MMR2 titers; such a boost was seen in 97 (80%) of 122 subjects with low titers, in 195 (53%) of 369 with medium titers, and in 16 (13%) of 125 with high titers ($P < .001$), but there was no threshold beyond which boosts were not observed. The kindergarten group tended to have more 4-fold boosts than the middle-school group (166/308 [54%] vs. 142/308 [46%]), but this difference was not significant ($P = .307$). At 6 months, the kindergarten group had significantly higher titers than did the middle-school group (GMT, 76 vs. 58; $P < .001$) and tended to include fewer children with low titers (8/295 [3%] vs. 15/302 [5%]; $P = .202$).

Persistence of antibodies. For both groups, antibody levels rose or were unchanged during the first 5 years after MMR2 and then declined (figure 2). By age 17 years (12 years after MMR2), titers in the kindergarten group had fallen significantly ($P < .001$) but were still higher than pre-MMR2 titers (pre-MMR2 vs. 12-year GMT, 33 vs. 46; $P < .001$). However, the proportion with low titers was not significantly different from that before MMR2 (pre-MMR2 vs. 12 years, 49/308 [16%] vs. 18/144 [13%]; $P = .395$), and a significantly higher proportion were seronegative (pre-MMR2 vs. 12 years, 0/308 [0%] vs. 8/154 [5%; 95% CI, 2%–9%]; $P < .001$) (figure 3). By age 17 years (7 years after

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Figure 1. Initial response to a second dose of measles-mumps-rubella vaccine (MMR2). Each graph represents a serum collection, and the bars within each graph represent the proportion of the population with a specified titer from the mumps plaque-reduction neutralization test. The range and the 25th and 75th percentiles are indicated by the box-and-whiskers plot, the median titer by a dark vertical line, and the geometric mean titer by a triangle. Left-hand graphs represent the group that received MMR2 at kindergarten entry, and right-hand graphs represent the group that received MMR2 at middle-school entry. $P$ values are from the Jonckheere-Terpstra test, indicating the difference between the 2 groups for titer distribution.
MMR2), antibody levels in the middle-school group showed a similar pattern: overall titers were still higher than before MMR2 (pre-MMR2 vs. 7-year GMT, 26 vs. 42; \(P = .001\)), but the proportions with low titers were not significantly different (pre-MMR2 vs. 7 years, 73/308 [24%] vs. 38/189 [20%]; \(P = .376\)), and a significantly higher proportion were seronegative (4/308 [1%] vs. 17/189 [9%; 95% CI, 4%-13%]; \(P < .001\)) (figure 3).

At the same serum collections, the kindergarten group tended to have higher antibody levels than the middle-school group; for example, at the last common collection, 7 years after MMR2, the kindergarten group (age 12 years) had higher overall titers than the middle-school group (age 17 years) (GMT, 63 vs. 42; \(P < .001\)) and fewer children with low titers (17/160 [11%] vs. 38/189 [20%; \(P = .018\)) or negative titers (7/160 [4%] vs. 17/189 [9%; \(P = .095\)). At the same ages (figure 4), antibody differences between the kindergarten and middle-school groups were generally not significant. For example, at the study end point of 17 years of age, the GMT was 46 for the kindergarten group versus 41 for the middle-school group (\(P = .100\)), the proportion of subjects with low titers was 18 (13%) of 144 versus 38 (20%) of 189 (\(P = .076\)), and the proportion with negative titers was 9 (6%) of 144 versus 17 (9%) of 189 (\(P = .414\)).

**Evidence of non-MMR2 boosting.** Of the 1525 specimens from beyond the period of likely MMR2 vaccination effect tested (≥2 years after MMR2), 165 (11%) demonstrated a 4-fold titer boost (mean, 7.2-fold). Of these, 150 serum samples had subsequent neutralization results, and for 63 (42%) the 4-fold rise was sustained at the next collection. The likelihood of a 4-fold boost was associated with a lower preceding titer (for low, 26/88 [30%]; for medium, 65/491 [13%]; for high 74/946 [8%; \(P < .001\)), but there was no threshold beyond which boosts were not observed. Four-fold boosts were seen at all 5 serum collections but were most frequent for 2 collections. The first was...
2 years after MMR2, when boosts were seen in 82 (16%) of 498 subjects, associated with a rise in overall GMTs (figure 2). At this 1996–1997 collection, 21% (50/236) of the kindergarten group (which had recently entered primary school) had a 4-fold rise, compared with 12% (32/262) of the middle-school group ($P = .008$). The second was 12 years after MMR2 (16/142 [11%]) in samples collected from the kindergarten group (now aged 17 years) in 2006–2007, coinciding with a nationwide resurgence of mumps in which Wisconsin was highly affected [14]. Of the 333 children with neutralization results from the final collection, 127 (38%) overall had experienced a non-MMR2 boost at some point—the kindergarten group significantly more than the middle-school group (76/144 [53%] vs. 51/189 [27%]; $P < .001$).

**Mumps disease reports.** Study subjects were born in 1981–1991 (the middle-school group in 1981–1984, the kindergarten

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**Figure 4.** Age-specific antibody levels after a second dose of measles-mumps-rubella vaccine (MMR2). Each graph represents a serum collection, and the bars within each graph represent the proportion of the population with a specified titer from the mumps plaque-reduction neutralization test. The range and the 25th and 75th percentiles are represented by the box-and-whiskers plot, the median titer by a dark vertical line, and the geometric mean titer by a triangle. Left-hand graphs represent the group that received MMR2 at kindergarten entry, and right-hand graphs represent the group that received MMR2 at middle-school entry. $P$ values are from the Jonckheere-Terpstra test, indicating the difference between the 2 groups for titer distribution.
group in 1988–1991), and the study period was from 1994 to 2007. No study subject reported mumps or exposure to mumps before or during the study period. During the study subjects’ lives before the study period (1981–1993), 64,605 US mumps cases were reported to the CDC, including 3088 from Wisconsin and 239 from Marshfield Clinic patients, but none from study subjects or their families. During the study period (1994–2007), 13,906 US mumps cases were reported to the CDC, including 937 from Wisconsin and 43 from among Marshfield Clinic patients, but none from study subjects or their families.

Risk factors for antibody levels. For pre-MMR2 antibody levels, shorter time since MMR1 was the only factor significantly associated with higher titers ($R^2 = 0.018; P < .001$). For each post-MMR2 collection, the dominant factor was pre-MMR2 titer ($R^2 = 0.134–0.244; P < .001$). No other factor examined (sex, race/ethnicity, mother’s birth year, age at MMR1, receipt of other vaccinations with MMR2) was consistently associated with post-MMR2 titers.

DISCUSSION

In summary, we found that, 4–9 years after the first dose of MMR administered at age 1 year, <1% of 616 children were seronegative to mumps but 20% had low antibody titers. Subjects in the kindergarten group, who had received the first dose 4 years previously, tended to have higher antibody levels than those in the middle-school group, who had received it 10 years previously. Response to the second dose was vigorous, with 50% overall exhibiting a 4-fold boost, although only 2% were IgM positive (3% in the kindergarten group and 1% in the middle-school group). The proportion with low titers was reduced to from 20% to 4%, with no significant difference in this reduction between groups (for the kindergarten group, from 16% to 3%; for the middle-school group, from 24% to 5%). During the 12-year study period, 53% of the kindergarten group and 27% of the middle-school group were apparently exposed to wild disease, as inferred from 4-fold boosts outside the vaccination period, although no cases were diagnosed clinically. Despite these apparent boosts, antibody levels declined for both groups. Twelve years after the second dose, the kindergarten group’s overall titers were approximately half those observed shortly after the second dose (GMT, 46 vs. 97), the proportion of children with low titers was similar to that observed immediately before the second dose (13% vs. 16%), and a higher proportion were seronegative (5% vs. 0%). Seven years after the second dose, the middle-school group showed similar patterns, and post-MMR2 differences between groups were generally not significant.

The antibody threshold that provides disease protection is not well defined for mumps. Two small prospective studies performed in the 1960s found that plaque-reduction neutralization titers of at least 4–8 were needed for protection, but a graded response was observed in the relationship between titer and protection [28, 29]. EIA results can vary greatly by assay and by operator [30, 31]. For a panel of serum samples found to be negative by neutralization, rates of negative detection according to the most commonly used commercial EIA ranged from as low as 45% to as high as 100%, depending on which European national laboratory was performing the assay [30]. EIA positivity may not always represent protection. A 2008 study in the US military found that, of 43 recruits who acquired mumps in the service, 41 had previously been EIA seropositive within an average period of 18 months [32].

Assessing protection is complicated by difficulties in diagnosing mumps in highly vaccinated populations. IgM results are frequently negative even in the presence of classic symptoms [33–35]. Viral shedding may be reduced or transient [36]. Findings of a study in a highly affected college population during the 2006 resurgence suggested that mild or atypical disease presentations were predominant and that asymptomatic transmission may have accounted for >85% of spread [16]. Such findings raise the possibility that mumps vaccination may be more effective against severe or typical disease than against mild disease or asymptomatic infection.

Data from our study may shed light on this situation. Before the second dose, <1% of subjects were seronegative, and <2% generated an IgM response in response to the second dose. Thus, primary vaccine failure after the first dose, in the sense of an absent humoral response, was not prevalent in the study population. Nevertheless, a 4-fold antibody boost was generated in response to the second dose in 50%, suggesting frequent viral replication. This supports the notion that, although antibody positivity alone does not prevent virus replication, the higher the antibody level, the less likely it is that replication will occur. The lack of detectable vaccine adverse events, as previously reported [22], suggests that mumps viral replication need not produce recognizable clinical symptoms.

This boosting pattern contrasts with the second-dose response to measles vaccine among the same study subjects, as previously reported [23]: only 4% demonstrated a 4-fold boost, which was largely restricted to those with the lowest titers. Unlike mumps, for which 2-dose failure has been prominent [14], outbreaks of measles have not been reported among 2-dose recipients in the United States [37, 38]. Mumps and measles virus, despite belonging to the same paramyxovirus family and having vaccines that are injected in the same syringe, may interact with the human immune system in very different ways.

Mumps antibody levels declined with time since the second dose. By age 17 years, the proportion of subjects with low titers did not differ significantly from that before the second dose, and a significantly higher proportion were seronegative. Because wild-virus boosting apparently occurred in the population, the decline in antibody levels we observed may well be an underestimate relative to that which would be seen in a population with no wild-virus exposure. Davidkin et al. [18] also observed de-
clining mumps antibody levels and rising negativity rates in their longitudinal study of Finnish children who had received MMR2 at age 6 years. Cohen et al. [39] found data suggesting progressively declining vaccine effectiveness with time since vaccination during the 2004–2005 mumps epidemic in the United Kingdom.

Mumps outbreaks have been common among 1-dose recipients [7–11]. If, in the decade after the second dose, protection declines to a level similar to that after a single dose, population resistance to infection might not be sufficient to prevent disease transmission in crowded settings, such as dormitories and classrooms. During the 2006 mumps resurgence, 2-dose vaccine effectiveness among college students (most of whom had received the second dose 10–15 years earlier) was found to be approximately equivalent to that of 1 dose [16]. Among those with prolonged exposure to an infectious roommate, attack rates for clinically apparent mumps ranged from 3% to 9% [15, 16], approximately equivalent to the 5% (95% CI, 2%–9%) negativity rate we found by neutralization among persons who had received the second dose 12 years earlier. Nevertheless, it is important to note that, had the population never received a second dose, antibody levels would certainly have been lower and attack rates higher.

Despite declining antibody levels in both study groups, we did not identify a clear advantage of deferring the second dose until middle school. In collection-specific comparisons, antibody levels tended to be higher for the kindergarten group, and in age-specific comparisons differences between groups were not significant. Among the possible explanations are the greater chance for wild-virus boosting among kindergartners or enhanced vaccine responsiveness in younger cohorts of children.

Our study has a number of limitations. Study subjects were healthy, rural, non-Hispanic whites and were thus not representative of US children. The study began with the second dose, and it is possible that the study population might have experienced unrecognized prior wild-virus exposures. Because of state requirements, the 2 study groups were of different ages, rather than a single cohort randomly allocated to different ages for vaccination. Attrition reduced the study population by 46%. We did not confirm the possibility of attenuated virus proliferation after vaccination by attempting viral isolation. The Barnes virus strain used in our neutralization assay was neither the Jeryl Lynn strain with which the study subjects were vaccinated nor the genotype G wild-virus strain that circulated during the 2006 US mumps resurgence; findings of neutralization studies by Rubin et al. [40] suggest that titers may be affected by the virus strain used. We did not test high-titer specimens to the end point, so our GMTs may be underestimates. We did not investigate the role played by cell-mediated immunity, an area of active investigation by Gas and colleagues in the United States [19] and by Davidkin and colleagues in Finland [17].

These limitations suggest the need for further investigations into the persistence of mumps immunity. Unlike the situation for measles [41] and rubella [42], hemispheric control of mumps has not yet occurred, 43% of the world’s nations do not have routine mumps vaccination programs [43], and even nations with mumps vaccination programs have large outbreaks [44, 45]. Hence, ongoing wild mumps virus challenges to US herd immunity appear inevitable. Theory and experience suggest that sustained high 2-dose MMR childhood coverage is sufficient to control and even eliminate measles and rubella [46–48]. Whether the same is true for mumps requires further study.

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**References**