Mumps Antibody Levels Among Students Before a Mumps Outbreak: In Search of a Correlate of Immunity

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Background. In 2006, a mumps outbreak occurred on a university campus despite ≥ 95% coverage of students with 2 doses of measles-mumps-rubella (MMR) vaccine. Using plasma samples from a blood drive held on campus before identification of mumps cases, we compared vaccine-induced preoutbreak mumps antibody levels between individuals who developed mumps (case patients) and those who did not develop mumps (nonpatients).

Methods. Preoutbreak samples were available from 11 case patients, 22 nonpatients who reported mumps exposure but no mumps symptoms, and 103 nonpatients who reported no known exposure and no symptoms. Antibody titers were measured by plaque reduction neutralization assay using Jeryl Lynn vaccine virus and the outbreak virus Iowa-G/USA-06 and by enzyme immunoassay (EIA).

Results. Preoutbreak Jeryl Lynn virus neutralization titers were significantly lower among case patients than unexposed nonpatients (P = .023), and EIA results were significantly lower among case patients than exposed nonpatients (P = .009). Proportionately more case patients than exposed nonpatients had a preoutbreak anti–Jeryl Lynn titer < 31 (64% vs 27%, respectively; P = .065), an anti–Iowa-G/USA-06 titer < 8 (55% vs 14%; P = .033), and EIA index standard ratio < 1.40 (64% vs 9%; P = .002) and < 1.71 (73% vs 14%, P = .001).

Discussion. Case patients generally had lower preoutbreak mumps antibody levels than nonpatients. However, titers overlapped and no cutoff points separated all mumps case patients from all nonpatients.

In 2006, a multistate outbreak of mumps occurred in the United States with over 6500 reported cases. This was the largest outbreak in the United States in 19 years [1, 2]. The greatest number of cases occurred among college-aged persons, and most persons with mumps had received 2 doses of measles-mumps-rubella (MMR) vaccine as currently recommended by the Advisory Committee on Immunization Practices [3]. Waning vaccine-induced immunity may have played a role in the resurgence [1, 2]. The occurrence of a blood drive held shortly before identification of mumps cases at a Kansas university provided a key opportunity to assess the relationship between levels of vaccine-induced neutralizing antibody and protection from mumps, which currently is not known. At the university there were a total of 174 persons reported to have mumps (attack rate, 0.9% among 19 155 undergraduate students) despite ≥ 95% 2-dose MMR vaccine coverage among students [4, 5]. Mumps cases were also reported among young adults in other Kansas locations. Using plasma samples remaining from blood donations, we sought to compare vaccine-induced preoutbreak mumps antibody levels between individuals with
mumps (case patients) and those without mumps (nonpatients) to determine a correlate of immunity. If such a correlate could be identified, it may suggest there would be benefit in providing additional mumps vaccine doses to raise an antibody level above the threshold, in persons at risk of mumps (eg, young adults in crowded settings). We also sought to estimate the mumps infection rate at the university by the proportion of donors with an increase in mumps antibody titer during the outbreak.

**METHODS**

**Blood Donors**

A blood drive had been held by the Community Blood Center (CBC) and the American Red Cross (ARC) at the university 6–10 March 2006. A mumps outbreak was identified on 30 March 2006 [5] and peaked in mid-April 2006. In June 2006, investigators contacted the blood centers to obtain excess samples from the drives. Samples were frozen in storage at CBC only. Blood drives were subsequently held at the university in September 2006 and March 2007, approximately 5.5 and 11 months after the outbreak peak. Investigators attended the March 2007 drive and enrolled interested donors in-person into the study. Donors were asked for permission via written informed consent to test leftover plasma samples for mumps antibodies, to obtain their MMR vaccination records, and to obtain their medical records if they had sought medical care for symptoms possibly consistent with mumps. Enrollees were also asked to complete a written questionnaire that queried demographic factors, student status, living arrangements, history of mumps symptoms (including parotid swelling, parotid pain, jaw pain, swelling below the jaw, and testicular pain or swelling) during the period 1 January 2006 to date of questionnaire completion, date of symptom onset, mumps exposure during 2006–2007, and history of ever having physician-diagnosed mumps. Pictures showing location of parotid glands and parotid enlargement and a calendar of campus events were included. For assessing mumps exposure, donors were asked if they had been face-to-face (“as close as if you were talking with someone right in front of you”) with someone ill with mumps who was not wearing a mask.

Some persons who had not participated in the March 2007 drive but who had donated blood in 2006 before the outbreak were contacted by email, letter, or telephone to request study participation. These included reported mumps patients, roommates/housemates of mumps patients [4], and those with pre- and postoutbreak samples available (“repeat donors”). Samples were tested for mumps virus–specific antibody by plaque-reduction neutralization (PRN) assay and a commercially available enzyme immunoassay (EIA).

Enrollees were asked if they desired their mumps EIA result and those who did were notified by letter. Those who tested negative were advised to discuss the results with their physician and consider revaccination with MMR. The study was reviewed by the institutional review boards of the Centers for Disease Control and Prevention, CBC, ARC, and the Kansas Department of Health and Environment (KDHE).

**Donor Categories**

**Case Patients**

Case patients were donors who had been reported by a clinician to KDHE as having a case of mumps and who, upon review of available records, had an illness suggestive of mumps and who had a plasma sample obtained at least 25 days (1 incubation period) before illness onset.

**Nonpatients**

Nonpatients were students at the university during the outbreak who were not reported to have mumps and who completed a questionnaire and did not report having symptoms suggestive of mumps between 1 January 2006 and the date of questionnaire completion. Although jaw pain may be a symptom of mumps, persons meeting the nonpatient criteria who reported having only jaw pain sometime during 2006–2007 but not specifically during the outbreak period (March 2006–August 2006) were considered a nonpatient.

Nonpatients were classified into 2 groups. Group 1 included those likely exposed to mumps, defined either as being a roommate/housemate of a person with mumps, reporting on the questionnaire that they had face-to-face exposure to a person with mumps during the outbreak, or repeat donors who reported no mumps symptoms but who demonstrated a $4$-fold rise in neutralizing antibody titer against either of the mumps virus strains tested (Jeryl Lynn vaccine or Iowa-G/USA06). Group 2 nonpatients were those who reported no known mumps exposure during the outbreak (and, if a repeat donor, did not have a $4$-fold rise in antibody titer).

Some donors were excluded from analysis before results were obtained because they may have been exposed to mumps before their earliest sample. During the outbreak investigation, 5 mumps cases were retrospectively identified to have occurred at the university before the blood drive [5]. We therefore excluded donors if their reported mumps exposure occurred before the donation date, if they had lived in the 1 dormitory with a case in February 2006, or if they were a member of the sorority or fraternity with a case in February–early March 2006.

Repeat donors were university students who donated a first sample before 16 March 2006 and a second sample during May 2006–May 2007, and who were not reported as having mumps. Some were also in nonpatient groups 1 or 2. Those who may have had mumps exposure before donating their first sample were excluded.
Laboratory Testing

Plasma samples were kept at –70°C at the blood centers until shipment to the US Food and Drug Administration and CDC. Laboratory scientists were blinded to donor information, including case status, until after testing was completed.

Neutralization Titors

PRN assay was used to determine virus neutralizing antibody titer in plasma as previously described [6]. Because levels of mumps virus neutralizing antibody titters detected in the PRN are a function of the challenge virus strain used [6–9], 2 different PRN assays were used: 1 using the Jeryl Lynn strain (the mumps virus component of the MMR vaccine) to measure the concentration of antibody that had been induced by immunization, and 1 using a contemporary wild-type virus isolated from the 2006 US mumps outbreak (Iowa-G/USA-06) [6] to approximate the potency of vaccine-induced antibody to the circulating wild-type strain. The neutralizing antibody titer is reported as the sample dilution capable of reducing the mean number of virus plaques by 50% or greater compared with the mean number of plaques in virus control wells using the Karber formula [10].

Enzyme Immunoassay

An indirect enzyme-linked immunosorbent immunoglobulin G (IgG) assay (Mumps IgG ELISA II, Wampole Laboratories) was used to determine mumps IgG antibody levels in plasma specimens, as described by the manufacturer. In this assay, index standard ratio (ISR) values < 0.90 are seronegative, values 0.91–1.09 are indeterminate, and values >1.10 are seropositive. All samples with seronegative or indeterminate results were retested along with an equal number of randomly selected positive specimens. The original ISR result was used in analysis if the result categories from the original and repeat run were the same, and the mean ISR result from the 2 runs was used if the original and repeat categories were different.

Statistical Analyses

The distributions of preoutbreak neutralization titters and EIA ISRs were compared between case patients and nonpatient groups using the Kolmogorov-Smirnov test. The preoutbreak geometric mean (with 95% confidence interval [CI]) for case patients and nonpatients was calculated for each of the 3 assays. For each assay, cutoff points for preoutbreak samples were assessed for their ability to discriminate case patients from nonpatients. Cutoff points were defined by the midpoint between a case patient’s value and the next highest value among the group 1 (“exposed”) nonpatients. The proportions of persons in each group with preoutbreak values below the cutoff points were compared using Fisher exact test, and odds ratios (ORs) with 95% CI were calculated. For all analyses, a P value < .05 was considered statistically significant. Analyses were performed using Stata software version 11.0.

RESULTS

Case Patients and Nonpatients

Eleven individuals met study criteria defining a case patient (Table 1). Three were laboratory-confirmed: patient 1 by virus isolation, patient 6 by reverse-transcription polymerase chain reaction [11], and patient 3 by a ≥ 4-fold rise in neutralizing antibody titer between pre- and postillness plasma samples. Oral swabs from the other 8 individuals were not obtained for mumps virus testing, likely because the KDHE laboratory had recommended against continued testing at confirmed outbreak locations [5]. Two of these 8 individuals (patients 9 and 11) had postillness plasma samples available. All 11 case patients had received 2 MMR doses before their preoutbreak sample (Table 1).

Twenty-two persons met criteria for a group 1 (exposed) nonpatient. Three were roommates of persons with mumps and 2 others were selected into the group because they reported no mumps symptoms but had a ≥ 4-fold rise in antibody titters. Twelve (55%) group 1 nonpatients were repeat donors (Figure 1). A total of 103 persons met criteria for a group 2 (nonexposed) nonpatient, and 58 (56%) of these were repeat donors (Figure 1). Immunization records were available on 119 (95%) of the 125 nonpatients; 118 (99%) of these had received ≥ 2 MMR doses before their preoutbreak sample and 1 (1%) had received 1 dose. No case patients or nonpatients reported a history of ever having physician-diagnosed mumps before enrollment at the university.

Anti–Jeryl Lynn Vaccine Virus Neutralizing Antibody Titters

Amoung case patients, the median preillness neutralization antibody titer against the Jeryl Lynn vaccine virus was 23.2 (Table 2). The 3 case patients with laboratory-confirmed mumps had preillness titers in the lower range (8.2, 9.5, and 23.2; Table 3). Of the 3 case patients with a postillness sample available, 1 had a 506-fold increase 2 months after illness, 1 had a nominal increase 5 months later, and 1 had a 3.9-fold drop in titer 1.1 months after illness (Table 1).

The median preoutbreak anti–Jeryl Lynn neutralization antibody titer value among group 1 nonpatients was 73.4 and the median among group 2 nonpatients was 57.6 (Table 2). The 2 persons who reported no symptoms but had a ≥ 4-fold rise in titer had preoutbreak titers of 70.8 and 73.4 (with 13.5- and 4.5-fold rises, respectively) (Table 3).

Preoutbreak Jeryl Lynn titers among case patients were significantly different from those of group 2 nonpatients (P = .023) but not group 1 nonpatients (P = .12; Table 2; Figure 2). Compared to group 1 nonpatients, case patients had 4.7 times greater odds of having a preoutbreak Jeryl Lynn titer < 31 and 5.7 times greater odds of having a titer < 41 (Table 4). Compared to group 2 nonpatients, case patients had a statistically
Table 1. Clinical Characteristics and Mumps Antibody Levels in 11 Mumps Patients

| Patient no. | Age at illness onset, y | Sex | Student at the university | History of possible exposure | Physician impression | Physician exam | Patient reported to CDC staff | Sample | Years from MMR2 to sample | Months from presample to illness onset | Months from illness onset to post sample | Anti-Jeryl Lynn neutralization titer | Anti-Iowa G/USA-06 neutralization titer | EIA result | EIA ISR |
|-------------|-------------------------|-----|---------------------------|----------------------------|-----------------------|---------------|-------------------------------|--------|---------------------------|----------------------------------------|----------------------------------------|--------------------------------------|--------------------------------------|-----------|
| 1           | 18.1                    | M   | No                        | Per student, friend’s sibling had mumps | Parotitis, lymphadenitis | Parotitis swelling, mild moderate* | Parotitis, jaw painb | pre | 12.6 | 4.1 | 8.2 | 5.3 | pos | 1.14 |
| 2           | 20.3                    | M   | Yes                       | Mumps | Parotitis swelling | Parotitis, jaw pain, testicular swelling, testicular pain | pre | 14.5 | 1.3 | 8.8 | 6.0 | neg | 0.82 |
| 3           | 19.3                    | M   | Yes                       | Wrestling opponent developed mumps shortly after match | Swollen glands, possible mumps | Submandibular swelling | Parotitis and parotid pain, jaw painb | pre | 13.9 | 1.3 | 9.5 | 7.5 | neg | 0.76 |
| 4           | 18.4                    | M   | No                        | Visited the university several weeks before illness | NA (reported to health dept as probable mumps) | NA | Parotitisc | pre | NAd | 1.0 | 14.9 | 3.9 | pos | 1.16 |
| 5           | 21.9                    | M   | Yes                       | Roommate reported to health dept as mumps case 2 weeks earlier | Probable mumps | Probable mumps | Parotitis and parotid painb | pre | 9.7  | 6.0 | 17.7 | 7.2 | pos | 1.35 |
| 6           | 21.0                    | M   | Yes                       | Mumps | Parotitis swellinga | Parotitis, jaw pain | pre | 13.3 | 2.0 | 23.2 | 5.8 | neg | 0.83 |
| 7           | 18.7                    | M   | No                        | Lived in the city where university is located | Mumps | Parotitis swelling | Parotitis, jaw painb | pre | 12.6 | 4.8 | 27.7 | 30.1 | neg | 0.66 |
| 8           | 18.3                    | F   | No                        | NA (reported to health dept as probable mumps) | Jawline swelling per nurse’s notes | Parotitis and parotid pain, jaw pain, facial swellingb | pre | 12.9 | 2.1 | 36.8 | 19.8 | pos | 2.51 |
| 9           | 18.9                    | F   | Yes                       | Mumps | Parotid tenderness only | Swelling below jaw only | pre | 13.6 | 1.4 | 76.0 | 19.4 | pos | 1.67 |
| 10          | 17.7                    | F   | No                        | Outbreak at high school | Mumps | Parotitis swelling | Parotitis, jaw painb | pre | 12.6 | 1.2 | 472.5 | 152.3 | pos | 3.74 |
| 11          | 20.0                    | M   | Yes                       | Probable mumps | Parotitis swelling, mild | Parotitis, jaw pain, testicular pain | pre | 14.9 | 1.8 | 495.7 | 84.3 | pos | 2.96 |

* Mumps virus isolated from throat washing.

** Per information provided by patient in 2007. All cases reported in 2006 to health department as having parotitis.

b Per health department interview.

c Mumps virus RNA detected from buccal swab by reverse-transcription polymerase chain reaction.

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Patients are ordered in table by anti-Jeryl Lynn neutralization titer in preillness sample.

Abbreviations: CDC, Centers for Disease Control and Prevention; EIA, enzyme immunoassay; ISR, index standard ratio; MMR2, second dose of measles-mumps-rubella vaccine; NA, not available; neg, negative; pos, positive; post, post-illness; pre, preillness.
significant 6.4 times and 4.8 times greater odds, respectively, of having a titer below those cutpoints.

**Anti–Iowa-G/USA-06 Virus Neutralizing Antibody Titers Among Case Patients Versus Nonpatients**

Among case patients, the median preillness neutralization antibody titer against the Iowa-G/USA-06 virus was 7.5 (Table 2). The 3 case patients with laboratory-confirmed mumps had preillness titers in the lower range (5.3, 5.8, and 7.5). Of the 3 case patients with postillness samples, only 1 had a ≥ 4-fold rise in titer (Table 1).

The median preoutbreak Iowa-G/USA-06 titer was 20.3 among the group 1 nonpatients and 19.5 among the group 2 nonpatients. The 2 nonpatients demonstrating ≥ 4-fold rise had preoutbreak titers of 28.5 and 37.0 (with a 7.9- and 8.2-fold rise, respectively).

The preoutbreak anti-Iowa-G/USA-06 virus neutralizing antibody titers among case patients were not significantly different from those of group 1 ($P = .12$) or group 2 nonpatients ($P = .13$) (Table 2; Figure 2). Case patients had a statistically significant 7.6 times greater odds of having a preoutbreak anti–Iowa-G/USA-06 virus titer < 8 compared with group 1.
nonpatients and 4.7 times greater odds compared with group 2 nonpatients (Table 4).

EIA ISR Results Among Case Patients Versus Nonpatients

Preoutbreak EIA ISR values among case patients differed significantly from those of group 1 ($P = .007$) and group 2 ($P = .009$) nonpatients (Table 2; Figure 2). Thirty-six percent of case patients (including 2 of 3 with laboratory-confirmed mumps) were EIA-negative or indeterminant preoutbreak, compared with 5% of group 1 nonpatients and 9% of group 2 nonpatients. Three EIA cutoffs (1.24, 1.40, and 1.71) discriminated case patients from nonpatients (eg, case patients had a statistically significant 17.5 times greater odds of having a preoutbreak EIA ISR, 1.40 compared with group 1 nonpatients, and 8.3 times greater odds compared with group 2 nonpatients (Table 4).

### Table 2. Preoutbreak Mumps Neutralization Antibody Titers and EIA ISR Results of Case Patients and Nonpatients

<table>
<thead>
<tr>
<th>Case patients (n = 11)</th>
<th>Group 1 nonpatients (n = 22)</th>
<th>Group 2 nonpatients (n = 103)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jeryl Lynn neutralization antibody titer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median titer (IQR)</td>
<td>23.2 (9.5–76.0)</td>
<td>73.4 (22.5–124.2)$^b$</td>
</tr>
<tr>
<td>GMT (95% CI)</td>
<td>34.3 (12.8–91.8)</td>
<td>59.6 (38.8–91.8)</td>
</tr>
<tr>
<td>Iowa-G/USA-06 neutralization antibody titer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median titer (IQR)</td>
<td>7.5 (5.8–30.1)</td>
<td>20.3 (12.2–58.2)$^b$</td>
</tr>
<tr>
<td>GMT (95% CI)</td>
<td>14.5 (6.4–32.6)</td>
<td>22.1 (14.7–33.1)</td>
</tr>
<tr>
<td>EIA ISR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>1.16 (0.82–2.51)</td>
<td>2.40 (1.81–3.54)$^b$</td>
</tr>
<tr>
<td>Geometric mean (95% CI)</td>
<td>1.36 (.91–2.01)</td>
<td>2.52 (2.06–3.08)</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; EIA, enzyme immunoassay; GMT, geometric mean titer; IQR, interquartile range; ISR, index standard ratio.

* Exact $P$ value by Kolmogorov–Smirnov test for nonpatient group compared with case patients.

* The 3 roommates/housemates of persons with mumps had Jeryl Lynn neutralization antibody titers, Iowa-G/USA-06 neutralization antibody titers, and EIA ISR results, respectively, as follows: roommate A: 11.8 (13.9 6.2 months after reported exposure), 10.7 (13.1 6.2 months after reported exposure) and 2.61; roommate B (to a polymerase chain reaction–positive case patient): 18.3, 6.6, and 2.09; roommate C: 261.8, 78.2, and 4.04.

### Table 3. Comparison of Preoutbreak Mumps Neutralization Antibody Titers and EIA ISR Results Between Case Patients and Nonpatients

<table>
<thead>
<tr>
<th>Case patients (n = 11)</th>
<th>Group 1 nonpatients (n = 22)</th>
<th>Group 2 nonpatients (n = 103)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proportion (n) below this level</td>
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<tr>
<td>Jeryl Lynn neutralization antibody titer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;16</td>
<td>36.4 (4)</td>
<td>9.1 (2)</td>
</tr>
<tr>
<td>&lt;19</td>
<td>45.4 (5)</td>
<td>22.7 (5)</td>
</tr>
<tr>
<td>&lt;31</td>
<td>63.6 (7)</td>
<td>27.3 (6)</td>
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<tr>
<td>&lt;41</td>
<td>72.7 (8)</td>
<td>31.8 (7)</td>
</tr>
<tr>
<td>&lt;83</td>
<td>81.8 (9)</td>
<td>54.5 (12)</td>
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<tr>
<td>Iowa-G/USA-06 neutralization antibody titer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;7</td>
<td>36.4 (4)</td>
<td>9.1 (2)</td>
</tr>
<tr>
<td>&lt;8</td>
<td>54.5 (6)</td>
<td>13.6 (3)</td>
</tr>
<tr>
<td>&lt;21</td>
<td>72.7 (8)</td>
<td>50.0 (11)</td>
</tr>
<tr>
<td>&lt;34</td>
<td>81.8 (9)</td>
<td>63.6 (14)</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; EIA, enzyme immunoassay; ISR, index standard ratio; OR, odds ratio.

* Comparison of the proportions below the cutoff point by Fisher exact test.

* Comparison of the proportions below the cutoff point by Fisher exact test.
Figure 2. Preoutbreak antibody levels by patient category. Line indicates a cutpoint value presented in Table 3. **Top panel:** Anti–Jeryl Lynn virus neutralizing antibody titers. **Middle panel:** Anti–Iowa-G/USA-06 virus neutralizing antibody titers. **Bottom panel:** Enzyme immunoassay index standard ratio (EIA ISR) results.
Increases in Neutralizing Antibody Titers Among Repeat Donors

A total of 113 persons were repeat donors (Figure 1). Of the 31 who had a first postoutbreak sample obtained during May 2006–July 2006, 2 (6.5%) had a ≥ 4-fold rise in Jeryl Lynn or Iowa-G/USA-06 titers. One was a group 1 nonpatient described earlier and the other did not complete the symptom questionnaire so symptom information was unavailable (preoutbreak Jeryl Lynn titer, 39.9; Iowa-G/USA-06 titer, 8.0). Of the 45 students with a first postoutbreak sample obtained in September 2006, 3 (6.7%) had a ≥ 4-fold rise in titer. One was a group 1 nonpatient described earlier, 1 did not complete the questionnaire so symptom information was unavailable (preoutbreak Jeryl Lynn titer, 134.1; Iowa-G/USA-06 titer, 34.1), and 1 reported mumps exposure during the outbreak and developed jaw pain (preoutbreak Jeryl Lynn titer, 30.1; Iowa-G/USA-06, 12.2; values approximating the cutoffs used in previous analysis) (Table 3). Overall, of the 47 persons who donated their post sample during May 2006–September 2006 and completed the symptom questionnaire, 3 (6.4%) had the specified titer rise. None of the 37 students whose first postoutbreak sample was obtained February 2007–May 2007 had a ≥ 4-fold rise. One student with a March 2008 postoutbreak sample had a 5.3-fold rise in Jeryl Lynn titer (preoutbreak Jeryl Lynn titer, 57.1; Iowa-G/USA-06, 18.1; EIA ISR, 2.21). He had been a resident of a dormitory where mumps cases were reported and in mid-2008, he reported no mumps symptoms during the outbreak or subsequently.

DISCUSSION

In our very highly vaccinated population of young adults, pre-outbreak Jeryl Lynn vaccine virus neutralization titers were lower among case patients than among group 2 nonpatients, and EIA ISR results were lower among case patients than among both nonpatient groups. We identified cutoff points in each assay that statistically discriminated case patients from nonpatients; however, titers overlapped and there were no cutoff points that separated all case patients from all nonpatients. Of note, the data presented in this report were based on testing of plasma samples. While it is likely that similar results would be obtained had sera been available for testing, differences cannot be ruled out.

Interestingly, the 1:8 cutoff identified in this study using a contemporary wild-type isolate (Iowa-G/USA06) is similar to that reported by others in studies conducted in the prevaccine era [12–15]. No such studies have been conducted subsequently. Notably, levels of virus neutralizing antibody titers measured in vitro are dependent on the challenge virus strain used in the assay [6–9], highlighting the importance of defining a serological correlate of protection based on appropriate selection of the challenge virus strain, among other considerations. In our study, the minimal level of antibody correlating with protection is best investigated using the vaccine virus strain, given that antibodies detected in pre-exposure plasma are vaccine derived. Unfortunately, a robust correlate of protection using the Jeryl Lynn virus could not be identified in our study. Despite the difficulty in establishing a correlate of protection, anti-Iowa-G/USA06 neutralizing antibodies were present in preexposure plasma obtained from all case patients, suggesting that susceptibility to disease was not due to the inability of vaccine-induced antibody to neutralize the outbreak strain (ie, immune escape). In addition, the detection of neutralizing antibodies in preexposure plasma from all case patients suggests that their susceptibility to mumps was unlikely to have been a result of primary vaccine failure. It is important to acknowledge, however, that measurements of virus neutralizing antibody in vitro may not be fully predictive of immunological activity in vivo given that Fc-mediated phagocytosis, antibody-dependent cell-mediated cytotoxicity, and other processes that occur in the host are not reflected in the assays used to measure virus viability in vitro.

Several aspects of our study limited our ability to identify an antibody-based correlate of immunity, if one indeed exists. The
most significant limitation was that preexposure samples were available from only 11 case patients. Given the small number of case patients, misclassification of even a few nonpatients with high preoutbreak titers into this group would impact our results. We wanted to compare case patients to a group of nonpatients who had truly been exposed to mumps virus. Short of demonstrating an increase in antibody titer in ideally timed samples (our convenience samples were not ideally timed), identifying such persons is inherently difficult. The greater the enrichment of our nonpatient groups with truly unexposed persons, the less likely we would have been to identify an immune correlate. Additionally, the epidemic curve indicates there was limited mumps virus circulation before the first blood drive. It is therefore possible that some samples from nonpatients that we considered preexposure samples were actually postexposure samples, inflating the differences between case patients and nonpatients. To address this, we excluded donors if the information available raised a possibility of mumps exposure before the first donation.

It is possible that the level of immunity required to protect against classic clinical mumps illness depends on the inoculum of virus one is exposed to, so that protection at a particular antibody titer is not absolute. This would also make identifying a protective level more difficult. As in our university outbreak overall [5] and other similar outbreaks [16, 17], most of the 11 case patients in our study could not identify a specific exposure. This may indicate that transmission occurred from persons asymptomatic at the time of exposure, and therefore quantifying exposure intensity (as a marker for viral inoculum) would be very difficult for most case patients in these outbreaks.

The infectiousness of asymptomatic or minimally symptomatic 2-dose vaccinees is not known, but it is possible such persons play an important role in outbreaks in highly vaccinated populations. Viral shedding from unvaccinated, minimally symptomatic persons has been documented [15, 18]. Among our repeat donors who did not develop clinical mumps, the minimum mumps infection rate was 6.4% (3 of 47 repeat donors who did not develop clinical mumps had a ≥ 4-fold rise in titer). This is a minimum rate because most post samples were from routine blood donations several months after the outbreak peak and hence transient titer rises would have been missed. Furthermore, it is not known if all mumps infections in 2-dose vaccinees are associated with a ≥ 4-fold rise in antibody titer. While we do not know how representative our blood donors are of the entire student body with regard to mumps exposure, by dividing this percentage (6.4%) of repeat donors who had a ≥ 4-fold rise in titer but no or minimal mumps symptoms by the percentage of March 2006 donors who developed clinical mumps (1.6%; 5 of 313, Figure 1), we estimate that there may have been at least 4 individuals with asymptomatic or minimally symptomatic infections during the university outbreak for every reported case. This proportion (4 of 5; 80%) of asymptomatic or minimally symptomatic persons appears higher than the 20%–40% generally reported in the literature from the pre-vaccine era [15, 19, 20], suggesting, perhaps not surprisingly, that subclinical or mild infections may be more common in vaccinated persons.

Identifying a vaccine-induced correlate of immunity for protection against mumps disease and infectiousness would be of great benefit to immunization programs. We assessed only 1 component of the immune response to the Jeryl Lynn vaccine, and while there are limitations to using samples obtained from blood donations around a mumps outbreak, our results should be useful for additional investigations to help identify the best correlate.

**Notes**

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