MAJOR ARTICLE

Mumps Serum Antibody Levels Before and After an Outbreak to Assess Infection and Immunity in Vaccinated Students

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Background. Since 2009, various mumps outbreaks have occurred in the Netherlands, affecting mostly young adults vaccinated against mumps. In this retrospective study, we estimated attack rates for symptomatic and asymptomatic mumps virus infection based on mumps-specific immunoglobulin (Ig)G concentrations in paired blood samples obtained before and after the mumps outbreaks, collected in 2 university cities. We aimed to identify a serological correlate of immune protection and risk factors for mumps virus infection.

Methods. Mumps-specific IgG levels were measured by Luminex technology in paired pre- and post-outbreak samples from students from Leiden ($n = 135$) and Utrecht ($n = 619$). Persons with a 4-fold increase in mumps IgG concentrations or mumps IgG concentrations >1500 RU/mL were assumed to have had a mumps virus infection.

Results. Attack rates for symptomatic and asymptomatic mumps virus infection were 2.0% and 3.8%, respectively. Pre-outbreak mumps-specific IgG concentrations were lower among cases than among noncases ($P = .005$) despite vaccination history, but no serological cutoff for immune protection could be established. Mumps among housemates was significantly associated with serological evidence for mumps virus infection (odds ratio, 7.25 [95% confidence interval, 3.20–16.40]; $P < .001$).

Conclusions. Symptomatic and asymptomatic mumps virus infections in vaccinated persons can be identified by retrospective assessment of mumps-specific IgG antibodies in blood samples.

Keywords. asymptomatic infection; attack rates; correlate of protection; IgG antibodies; MMR vaccination; mumps virus; risk factors; serology.

Since the end of 2009, various mumps outbreaks have occurred in the Netherlands. The outbreaks affected mostly young adults, who had been twice vaccinated with the measles, mumps, and rubella (MMR) vaccine in childhood [1]. This phenomenon could be due to waning immunity in this age group, because antibody responses after vaccination last shorter than after natural infection. In the absence of mumps virus circulation, a substantial proportion of persons is seronegative 15 years after the second MMR vaccination [2, 3]. Furthermore, recent findings suggest that the MMR vaccine is not very effective in eliciting an antibody response of high avidity against mumps compared with measles and rubella [4], which also could explain the poor protection of vaccinated adolescents.

Mumps attack rates above 10% among vaccinated university students have been reported during various recent outbreaks [5, 6]. Those attack rates were based on a particular setting within a specific time frame,

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and they are therefore probably higher than overall attack rates in a nationwide outbreak. In contrast, attack rates may be underestimated because calculations are based on self-reporting of mumps symptoms, whereas many mumps virus infections run an asymptomatic course [7–9]. In theory, more reliable attack rates could be obtained from measuring mumps-specific immunoglobulin (Ig)G concentrations, because these generally increase after mumps virus infection [10]. However, a challenge is the lack of a serological correlate of protection in vaccinated individuals. Only 1 study has shown that pre-outbreak mumps antibody neutralization titers in patients with mumps were lower than in persons who were not infected with mumps virus during the outbreak, but it was not possible to set a cutoff point separating all clinical patients with mumps from nonpatients [3].

In this study, we first measured mumps-specific IgG antibody concentrations in paired pre- and post-outbreak samples from exposed students in 2 Dutch university cities to identify mumps virus infections. In this way, we could calculate the proportions of symptomatic and asymptomatic infections and determine attack rates and risk factors for mumps virus infection, irrespective of clinical outcome. Second, to identify a correlate of protection, mumps-specific IgG concentrations in pre-outbreak samples were compared between infected and non-infected persons.

METHODS

Study Design

A retrospective study was performed including 2 student cohorts from the cities of Leiden and Utrecht. The study in Leiden served as a pilot for a larger serological study in Utrecht. Questionnaires for both cohorts were comparable and included questions on MMR vaccination status, mumps history, and possible risk factors such as age, gender, membership of a student association, residence in a student house, number of housemates, and circulation of mumps in the environment. Students were included if pre-outbreak serum samples were available that were collected during their first year of (bio)medical school for posthepatitis B vaccination titer control. After informed consent was obtained from the students, these serum samples were retrospectively tested, along with post-outbreak blood samples collected as described below. Studies were approved by the medical ethics committee of the Leiden University Medical Center and the University Medical Center Utrecht.

Leiden Study

In total, 135 paired pre- and post-outbreak samples from medical students were included (Figure 1A). Pre-outbreak sera were taken between 2008 and 2010. Students were approached directly at the university by a medical team from the academic hospital to participate in this study. They filled out a questionnaire and gave permission to test their pre-outbreak serum sample retrospectively. The post-outbreak sera were taken between January and February 2011.

Utrecht Study

Based on the results from the serological pilot study in Leiden, a larger study was performed among biomedical and medical students in Utrecht. Here, all students received a dried blot spot (DBS) self-sampling kit for post-outbreak sampling and a permission form to check vaccination status in the nationwide vaccination registration system (Praeventis), along with the questionnaire and informed consent form. All DBSs were sampled between March and June 2012. Stored sera from these students dated back to 2007–2012, depending on the year the student enrolled. Based on the reported mumps cases in Utrecht and other parts of the Netherlands in the national mandatory notification system, all sera collected between 2007 and 2010 were considered to be pre-outbreak samples. Using the inclusion criteria described below, samples from 619 students were included for analysis (Figure 1B). The vaccination status provided in the questionnaire was used for analysis, after verification of vaccination history for 498 (80.5%) of these students from the data recorded in Praeventis (data not shown). Of these 498 students, 469 students (94.2%) had received 2 MMR doses, which is in line with national MMR vaccination coverage data [11]. When students' vaccination status was not reported and could not be retrieved via Praeventis, they were not included in the analyses restricted to fully vaccinated persons.

Inclusion and Exclusion Criteria

In total, 754 of 788 students with pre- and post-outbreak blood samples were included. Besides measurement of the mumps-specific IgG concentration in the sera and DBS samples, IgG concentrations for measles and rubella were measured as external control for antibody concentration fluctuations between samples within a person over time. Persons were excluded for further analyses ($n = 29$) when their ratio of measles and/or rubella IgG concentrations of both samples was at least factor 4 [12]. In addition, all persons in the Utrecht study who had received an MMR vaccination since 2008 were excluded ($n = 5$). This latter criterion could not be applied for the Leiden cohort, because data on recent MMR vaccinations were lacking (Figure 1).

Mumps-Specific Immunoglobulin G Assay

Samples were stored at −20°C until use. For all samples, IgG antibody concentrations for MMR were determined with a fluorescent bead-based multiplex immunoassay using Luminex technology as described previously [13]. In short, 5 µL serum was 1:200 diluted in assay buffer (phosphate-buffered saline containing 0.1% Tween-20 and 3% bovine serum albumin). A punch ($r = 3.175$ mm) of each DBS sample was dissolved in 300 µL assay buffer, resulting in a solution comparable to the

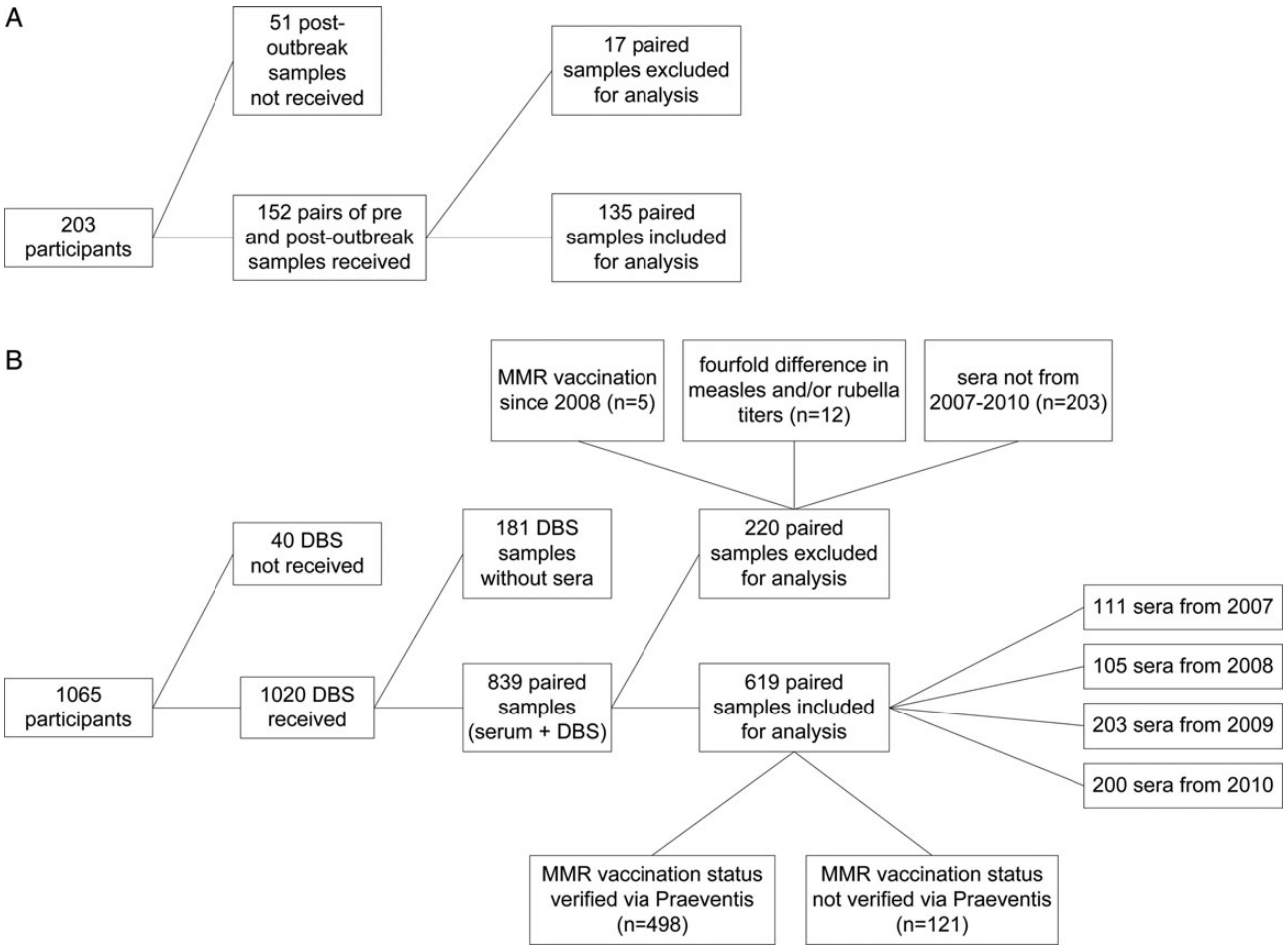

Figure 1. A flowchart for inclusion of samples is shown. (A) The flowchart for the Leiden cohort is illustrated. In total, 135 paired samples were included for analysis. The paired samples that were excluded ($n = 17$) were all excluded on the basis of the measles and rubella concentration differences between the pre- and post-outbreak samples. (B) The flowchart for the Utrecht study is shown. In total, samples from 619 persons were included for analysis. Years in the right column are the years in which serum samples were drawn. All dried blot spots (DBS) were obtained between March and June 2012. Abbreviation: MMR, measles, mumps, and rubella.

1:200 dilution of serum samples. When the 1:200 dilution fell outside the range of the reference serum curve, the results of a 1:4000 dilution were used for analysis.

On each plate, the WHO International Standard Anti Rubella Immunoglobulin RUBI-1-94 (The National Institute for Biological Standards and Control), controls, and blanks were included. The fluorescent intensity of the samples was interpolated in the reference serum curve to obtain antibody concentrations, which were expressed in RIVM units per milliliter (RU/mL) for mumps. The RIVM units for mumps used in this assay were previously standardized against other mumps standards, in which mumps IgG-positive test results were equivalent to values higher than 45 RU/mL [14, 15]. RUBI-1-94 has a mumps-specific IgG concentration of 4384.512 RU/mL and was selected as alternative serological standard for mumps, thus enabling comparison and bridging of our results to other studies. For measles and rubella, IgG concentrations were expressed as international units per milliliter (IU/mL).

Definition of Mumps Virus Infection

The period between the 2 blood samples varied between 2 and 5 years. Because no major outbreaks of measles and rubella were reported between 2007 and 2012, most subjects were assumed not to have been exposed to measles or rubella in this time period. Therefore, mumps-specific IgG antibody concentration rises were normalized against the concentration changes for measles and rubella, to correct for possible differences due to quality issues and technical differences related to sample storage and recovery of antibodies from DBS. The mumps-specific IgG concentrations were individually corrected using the average ratios of both measles- and rubella-specific IgG concentrations between the 2 consecutive blood samples.

Two criteria were set for the detection of mumps virus infections. First, a 4-fold increase or more of mumps-specific IgG in the 2 consecutive blood samples, acknowledged as the most specific criterion to confirm mumps virus infection, was used [12]. Second, a single-point cutoff criterion was calculated by the use of a receiver operator characteristics (ROC) curve. The positive reference group for this analysis consisted of laboratory-confirmed mumps cases who had been vaccinated twice with the MMR vaccine in childhood ($n = 15$). These persons were identified through enhanced surveillance of mumps in the Netherlands and were contacted in the context of a medically ethically approved clinical study to collect samples between 6 and 10 months after mumps virus infection. The negative control group consisted of 451 twice MMR vaccinated age-matched individuals (between 18 and 25 years of age) from a large Dutch serosurveillance study in 2006/2007 [16]. Persons who fulfilled at least 1 of the 2 serological criteria and had reported clinical mumps in the questionnaire were regarded as symptomatically infected, whereas persons who had not reported clinical mumps in the questionnaire were regarded as asymptomatically infected.

Statistical Analysis

SPSS version 19 and GraphPad Prism version 6 were used for data analyses. The attack rates for symptomatic and asymptomatic mumps virus infection were calculated for the entire outbreak period, assuming that students were exposed since January 2010 and that the exposure period was similar for all students included. Because the time frame and geographical region differed between the 2 student cohorts, attack rates were calculated separately. Distributions of pre-outbreak mumps-specific IgG concentrations in serum samples were compared using the Mann–Whitney U test. Median IgG concentrations, ROC analysis, and mixture modeling were used to identify a correlate of protection against mumps virus infection. For all analyses, P values $< .05$ were considered as statistically significant. Risk factors in the Leiden and Utrecht student cohorts were compared with multilevel analysis. Thereafter, possible risk factors for symptomatic and asymptomatic mumps virus infection were determined with logistic regression analysis. Factors with a P value $\leq .10$ were included in the multivariate analysis to calculate odds ratios (OR) and 95% confidence intervals (CIs).

RESULTS

Cohort Description

In total, 135 students in Leiden and 619 students in Utrecht were included (Figure 1). The majority of students were female ($n = 606$; 80.4%) and median year of birth was 1989 (interquartile range [IQR], 1988–1990). Of 498 students of whom vaccination status could be checked, 469 (94.2%) had received 2 MMR doses. This is in line with MMR vaccination coverage data in these birth cohorts [11], and it was therefore assumed that most of the students in Leiden and Utrecht with unknown vaccination status were vaccinated twice in childhood according to the National Immunization Program. Data on vaccination status of the students are shown in Table 1.

Table 1. Vaccination Status for the 2 Separate Cohorts and the Total Cohort

Cohort Description	MMR Vaccinations	Number of Participants N (%)
Leiden[a]	At least 2× MMR	47 (34.8)
	1× MMR	5 (3.7)
	Vaccinated, but unknown doses	76 (56.3)
	No MMR	2 (1.5)
	Unknown vaccination status	5 (3.7)
Utrecht[b]	At least 2× MMR	534 (86.3)
	1× MMR	14 (2.3)
	Vaccinated, but unknown doses	52 (8.4)
	No MMR	14 (2.3)
	Unknown vaccination status	5 (0.8)
Total	At least 2× MMR	581 (77.1)
	1× MMR	19 (2.5)
	Vaccinated, but unknown doses	128 (17.0)
	No MMR	16 (2.1)
	Unknown vaccination status	10 (1.3)

Abbreviation: MMR, measles, mumps, and rubella.

[a] Based on self-reported vaccination history. Five students (3.7%) did not know whether they were vaccinated. Seventy-six students (56.3%) indicated that they were vaccinated, but they did not know the number of MMR doses.

[b] Vaccination status of 121 students (19.5%) could not be verified via Praeventis.

Identification of Mumps Virus Infections

The median mumps-specific IgG concentrations in the reference group sampled 6–10 months after proven mumps virus infection were 6648 RU/mL (IQR, 5923–8136 RU/mL), whereas the median concentrations in the negative control group were 139 RU/mL (IQR, 82–256 RU/mL). Receiver operator characteristics analysis showed that at 1500 RU/mL, sensitivity and specificity were 100% and 99.6%, respectively, with an area under the curve (AUC) of 0.99 (95% CI, 0.99–1.00; $P < .001$). From the negative controls, 0.4% of vaccinated persons had a mumps-specific IgG concentration higher than 1500 RU/mL (Figure 2A). The majority of pre-outbreak samples from the Utrecht study cohort had IgG concentrations below 1500 RU/mL, except for 3 students for whom the first serum samples were obtained in 2010 (Figure 2B).

When applying our criteria for infection, defined as a 4-fold or more increase in IgG concentration or a post-outbreak IgG concentration higher than 1500 RU/mL, 44 of 754 students (5.8%) had a mumps virus infection, and 15 of these persons had a symptomatic infection, whereas 29 persons had an asymptomatic infection (Table 2). The cutoff of 1500 RU/mL

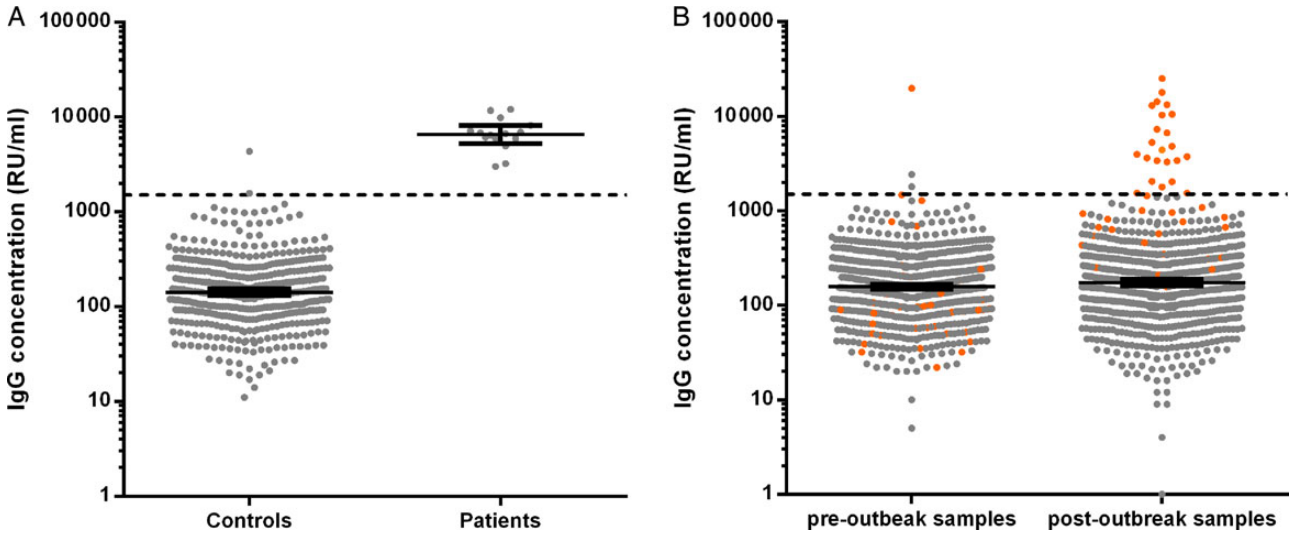

Figure 2. The graphic illustrates determination of a cutoff for mumps virus infections. (A) Based on a receiver operator characteristics (ROC) analysis, a cutoff of 1500 RU/mL (range, 1384–2288 RU/mL) was calculated for mumps virus infection (dashed line). Patient samples were from fully measles, mumps, and rubella (MMR)-vaccinated mumps patients, sampled between 6 and 10 months after infection ($n = 15$). For the control group, we used immunoglobulin (Ig)G levels from vaccinated age-matched participants in a Dutch national serosurveillance study carried out in 2006/2007 ($n = 451$). (B) The graphic shows mumps-specific IgG concentrations of pre- and post-outbreak samples from participants included in the Utrecht and Leiden cohort ($n = 754$). Orange dots represent the pre- and post-outbreak IgG concentrations in individuals infected with mumps virus ($n = 44$). Dashed line indicates the cutoff of 1500 RU/mL. Median IgG concentrations did not significantly differ between pre-outbreak samples and post-outbreak samples (158 vs 167 RU/mL; $P = .166$).

led to the identification of 4 additional mumps virus infections that did not result in a 4-fold or more increase in IgG concentration. With respect to symptomatic mumps virus infections, 13 of 15 blood samples fulfilled both serological criteria (Figure 3). However, for asymptomatic mumps virus infections, only 6 persons fulfilled both serological criteria, whereas a

Table 2. Attack Rates for Symptomatic and Asymptomatic Mumps Virus Infection for the 2 Separate Cohorts and the Total Cohort, Stratified by Vaccination Status*

		Mumps Virus Infections N (%)		
Cohort Description	Number of Participants	Symptomatic	Asymptomatic	Total
Leiden[a]				
At least 2× MMR	47	2 (4.3)	0 (0.0)	2 (4.3)
At least 1× MMR	128	3 (2.3)	4 (3.1)	7 (5.5)
All students	135	3 (2.2)	5 (3.7)	8 (5.9)
Utrecht[b]				
At least 2× MMR	534	11 (2.1)	19 (3.6)	30 (5.6)
At least 1× MMR	600	12 (2.0)	22 (3.7)	34 (5.7)
All students	619	12 (1.9)	24 (3.9)	36 (5.8)
Total				
At least 2× MMR	581	13 (2.2)	19 (3.3)	32 (5.5)
At least 1× MMR	728	15 (2.1)	26 (3.6)	41 (5.6)
All students	754	15 (2.0)	29 (3.8)	44 (5.8)

Abbreviations: Ig, immunoglobulin; MMR, measles, mumps, and rubella.

* Mumps virus infections were defined as either a 4-fold increase or more in mumps-specific IgG concentrations in the 2 consecutive blood samples or an IgG concentration higher than 1500 RU/mL in the post-outbreak sample.

[a] Based on self-reported vaccination history. Five students (3.7%) did not know whether they were vaccinated. Seventy-six students (56.3%) indicated that they were vaccinated, but they did not know the number of MMR doses.

[b] Vaccination status of 121 students (19.5%) could not be verified via Praeventis.

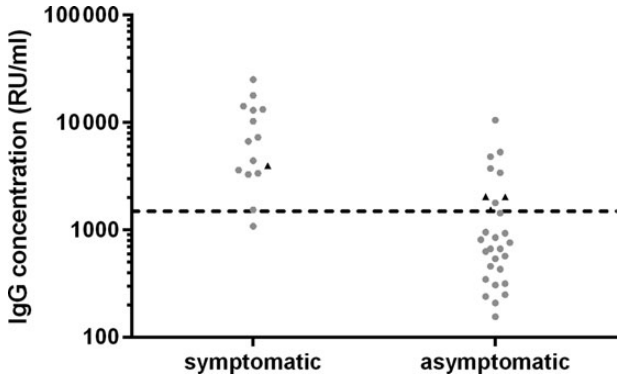

Figure 3. Distribution of the post-outbreak mumps-specific immunoglobulin (Ig)G concentrations in persons with symptomatic and asymptomatic mumps virus infections from the Leiden cohort ($n = 135$) and Utrecht cohort ($n = 619$). Mumps-specific IgG concentrations were higher in the post-outbreak samples of persons with a symptomatic mumps virus infection compared with persons with an asymptomatic mumps virus infection. Gray dots represent post-outbreak samples with a 4-fold or more increase in IgG concentration. Black triangles represent post-outbreak samples with no 4-fold increase in IgG concentration. Dashed line indicates the single-point cutoff at 1500 RU/mL.

4-fold or higher increase in IgG concentration could be detected in 26 students (Figure 3). This result indicates that the mumps-specific IgG concentrations after symptomatic mumps virus infections are higher than after asymptomatic infections. In addition, 3 persons with asymptomatic mumps virus infection had an IgG concentration in their post-outbreak blood sample above 1500 RU/mL but no 4-fold increase (Figure 3). Of the 25 persons who reported clinical mumps in the questionnaire, samples from 10 persons did not meet the serological criteria. Post-outbreak IgG concentrations in these 10 persons varied between 32 and 787 RU/mL.

Attack Rates

Eight students from the Leiden cohort ($n = 135$) had serological evidence for mumps virus infection, resulting in an attack rate of 5.9% (Table 2). Three of those students had symptomatic mumps (attack rate 2.2%), diagnosed by a physician in 1 case. In the Utrecht cohort, 36 of the 619 students had a mumps virus infection during the outbreak based on their IgG concentrations, resulting in an attack rate of 5.8% (Table 2). Twelve of these 36 students (attack rate 1.9%) had a symptomatic mumps virus infection according to the questionnaires, and 6 of these were diagnosed by a physician. Attack rates in students who had received at least 2 MMR doses ($n = 534$) were comparable with the total Utrecht cohort (Table 2).

Correlate of Protection for Vaccinated Students

To determine whether individuals with low mumps-specific IgG concentrations have an increased risk for mumps virus

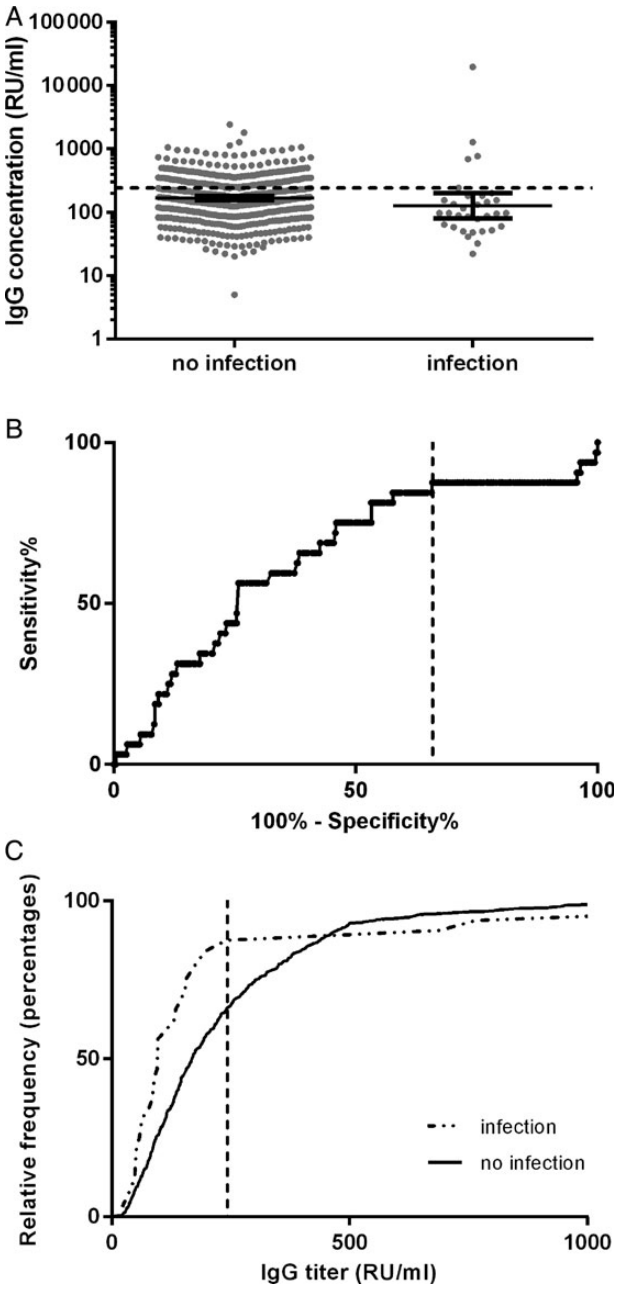

Figure 4. Comparison of pre-outbreak antibody levels between persons who were infected with mumps during the outbreak and persons who were not infected. All persons from the Leiden and Utrecht cohort with at least 2 measles, mumps, and rubella vaccinations were included ($n = 571$). (A) Distribution of pre-outbreak mumps-specific immunoglobulin (Ig)G concentrations in persons with and without a mumps virus infection. Median IgG concentrations were lower in infected persons ($P = .005$). Sensitivity and specificity were 87.5% and 34.1%, respectively, with a cutoff at 243 RU/mL (dashed line). (B) Receiver operator characteristics analysis of the mumps-specific IgG pre-outbreak concentrations of persons with and without a mumps virus infection. Dashed line indicates the cutoff at 243 RU/mL. (C) Relative frequency distribution of pre-outbreak mumps-specific IgG concentrations in persons with and without a mumps virus infection. Dashed line indicates the cutoff at 243 RU/mL.

Table 3. Univariate and Multivariate Analysis of Risk Factors for Mumps Virus Infection[a]

	Univariate Analysis Results		Multivariate Analysis Results			
Characteristic	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value
Year of birth		.501		.377		.166
1958–1985	0.00	.979	0.00	.978	0.00	.977
1986–1988	0.35 (0.09–1.35)	.127	0.29 (0.07–1.15)	.079	0.20 (0.05–0.81)	.024
1989–1991	0.45 (0.13–1.61)	.218	0.35 (0.10–1.29)	.114	0.28 (0.08–1.05)	.058
1992–1994	Ref.	–	Ref.	–	Ref.	–
Gender						
Female	Ref.	–	Ref.	–	Ref.	–
Male	1.22 (0.59–2.53)	.594	1.37 (0.65–2.88)	.409	1.49 (0.69–3.22)	.309
MMR vaccination						
No	Ref.	–				
≥1 dose	0.96 (0.12–7.38)	.965				
Membership of student association						
No	Ref.	–	Ref.	–	Ref.	–
Yes	2.18 (1.06–4.49)	.034	1.78 (0.84–3.76)	.130	1.53 (0.71–3.32)	.277
Living in a student house						
No	Ref.	–	Ref.	–	Ref.	–
Yes	2.42 (1.11–5.28)	.027	2.04 (0.91–4.61)	.085	1.56 (0.66–3.66)	.311
Number of housemates		.319				
1–3	Ref.	–				
4–6	0.98 (0.39–2.42)	.959				
7–10	1.68 (0.61–4.65)	.319				
11–19	2.46 (0.81–7.50)	.115				
20 or more	2.34 (0.45–12.10)	.311				
Circulation of mumps in the environment		<.001				<.001
No	Ref.	–			Ref.	–
Yes, in the social environment	1.35 (0.61–2.99)	.463			1.11 (0.49–2.51)	.808
Yes, among housemates	8.49 (3.95–18.20)	<.001			7.25 (3.20–16.40)	<.001

Abbreviations: CI, confidence interval; Ig, immunoglobulin; MMR, measles, mumps, and rubella; OR, odds ratio; Ref., reference group.

[a] All persons with a 4-fold or more increase in mumps-specific IgG concentration or pre-outbreak IgG concentration ≥1500 RU/mL were considered to have had a mumps virus infection ($n = 44$).

infection, serological data from pre-outbreak samples from Utrecht (2007–2010) and Leiden (2008–2010) were merged for all persons who had received 2 MMR doses ($n = 571$). Thirty-two persons (5.6%) had been infected with mumps virus based on serological analysis. Sera from the others ($n = 539$) were considered negative controls. Median mumps-specific IgG concentrations in the pre-outbreak sera of the infected students were significantly lower than median concentrations in the control group (97 RU/mL [IQR, 59–175 RU/mL] vs 169 RU/mL [IQR, 94–304 RU/mL]; $P = .005$; Figure 4A). A ROC analysis showed an AUC of 0.65 (95% CI, 0.54–0.75). However, no clear pre-outbreak cutoff could be identified that separated infected persons from noninfected persons. Mixture modeling did not substantiate this difference. The cutoff value that discriminated best between the pre-outbreak IgG concentrations from infected and noninfected persons was 243 RU/mL, resulting in a sensitivity and specificity of 87.5% (95% CI, 71.0%–96.5%) and 34.1% (95% CI, 30.1%–38.3%), respectively. However, specificity percentages have to be interpreted with caution, because probably not all persons were exposed to mumps (Figures 4B and C). Among persons exposed to mumps, the IgG concentrations between infected and noninfected persons overlapped as well (data not shown), which indicates that pre-outbreak IgG concentrations are not the only protective factor against mumps virus infection. There was no significant difference in pre-outbreak concentrations between persons with symptomatic and asymptomatic infection (data not shown).

Risk Factors for Mumps Virus Infection

Risk factors for mumps virus infection were determined from analysis of the questionnaire responses. Questionnaires used in Leiden and Utrecht were comparable, and because multilevel analysis did not result in significant differences between the

2 student cohorts, the data were merged in logistic regression analysis. The risk factor significantly associated with mumps virus infection in both univariate and multivariate analyses was circulation of mumps among housemates (OR, 7.25 [95% CI, 3.20–16.40]; $P < .001$) (Table 3).

DISCUSSION

This study has shown that serological analysis can be used to define mumps virus infection in vaccinated persons during outbreak situations with high sensitivity and specificity. Approximately two thirds of these serologically confirmed mumps virus infections were asymptomatic, judged from the fact that those persons had not reported clinical mumps in the questionnaire. This percentage is comparable to the percentage asymptomatic mumps virus infections estimated previously [8]. Besides the 4-fold increase in mumps-specific IgG concentrations that serves as the gold standard in serological studies, we have added a single-point cutoff value of 1500 RU/mL to discriminate antibodies acquired through vaccination from antibodies induced by mumps virus infection. Although this cutoff value is very conservative, some individuals classified as being infected with mumps virus would have been missed on the basis of solely a 4-fold increase in mumps-specific IgG concentration. When less conservative serological criteria were applied, more asymptomatic infections compared with symptomatic infections were identified, thereby changing the ratio between symptomatic and asymptomatic infections (data not shown). In total, 10 clinical mumps cases could not be confirmed as such based on our serological approach. Because these cases had indicated in the questionnaire that mumps was not laboratory confirmed by either polymerase chain reaction or IgM serology during period of disease, it is possible that the symptoms were not caused by a mumps virus infection. Four of these mumps cases were diagnosed by a physician, but no further information was provided regarding whether samples of these 4 cases tested negative for infection or whether there were no laboratory tests were performed. The other 6 mumps cases were not diagnosed by a physician and therefore no laboratory tests were performed.

Attack rate calculations were based on the assumption that mumps had not circulated among these cohorts before January 2010. However, it cannot be excluded that some students had been exposed to mumps earlier. Mumps outbreaks have occurred in the Netherlands in 2004 at an international university of hospitality management and between 2007 and 2009 in an orthodox religious community with low vaccination coverage [17, 18]. Still, the latter outbreak involved another age group and genotype mumps virus (D), and surveillance data showed no evidence for previous mumps virus infections in our study cohort. Three persons in the Utrecht cohort had pre-outbreak IgG concentrations higher than 1500 RU/mL, and they potentially had a mumps virus infection before the pre-outbreak serum was drawn. Sera from these 3 persons had been banked at the beginning of 2010.

In a previous study, no cutoff point could separate all mumps patients from nonpatients based on pre-outbreak mumps neutralization titers [3]. In this study, a potential explanation for the lack of a cutoff is that it remains unknown who was exposed, and lack of exposure in part of the study population will result in an underestimation of the specificity. Furthermore, the Luminex assay uses purified whole-virus antigens, and therefore the assay will also detect nonneutralizing IgG antibodies, which do not prevent the virus from entering the cells [19]. The failure to define a specific concentration of mumps-specific antibodies that is protective against mumps virus infection suggests that effective protection against mumps virus infection is governed by host immune mechanisms other than IgG concentrations in serum.

Median IgG concentrations in pre-outbreak samples from persons who became infected with mumps after serum sampling were 97 RU/mL. This concentration is higher than the 45 RU/mL, which is used as a measure to confer immune protection [13–15]. When applying these cutoffs to mumps-specific IgG concentrations from 451 vaccinated age-matched individuals included in a large Dutch serosurveillance study, the IgG concentration was below 97 RU/mL in 147 persons (32.6%) and below 45 RU/mL in only 42 (9.3%) persons [16].

To conclude, serological analysis enabled us to calculate attack rates for both symptomatic and asymptomatic mumps virus infection and to determine risk factors for mumps virus infection. This study shows the usefulness of serological analysis in addition to questionnaires and the possibility to retrospectively identify mumps virus infections based on mumps-specific IgG concentrations in paired pre- and post-outbreak samples.

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