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Assessment of serological evidence for mumps virus infection in vaccinated children

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ABSTRACT

It is estimated that at least one-third of mumps virus infections in non-vaccinated individuals are asymptomatic. Little information is available whether this proportion is the same among those vaccinated. We validated a commercial oral fluid mumps IgG-specific Enzyme Immunoassays (EIA) with vaccinated control groups to identify symptomatic and asymptomatic mumps virus infections in vaccinated individuals during a mumps outbreak in The Netherlands. A vaccinated control group was required to define a new cutoff value for the assay, because of the presence of low but significant levels of IgG antibodies in oral fluid as a result of mumps vaccination in the past. With a new cutoff, calculated using receiver operator characteristic analysis, we identified an attack rate of 7–10% compared to 2.7% based on clinical symptoms among vaccinated children. This finding has important implications when studying transmission patterns, strain virulence, as well as mumps vaccine effectiveness to protect from infection rather than disease.

1. Background

Mumps is a viral infection caused by an enveloped RNA virus of the family *Paramyxoviridae* that usually manifests in a mild illness with the most common symptom being parotitis and orchitis [1]. Complications can include encephalitis, aseptic meningitis and permanent sensorial hearing loss. In about 30% of the cases, the disease is asymptomatic [2,3]. Although death due to mumps is very rare, the disease burden caused by a mumps epidemic in an unvaccinated community justifies vaccination.

Mumps vaccination, as part of the measles–mumps–rubella (MMR) vaccine was introduced in the Netherlands in 1987. Since its introduction, the overall vaccination coverage has increased to more than 95% [4], except in municipalities with orthodox reformed communities, where vaccine coverage can drop as low as 40% among primary school children (unpublished data). Between mid 2007 and 2009 a mumps outbreak took place in the Netherlands. Here, most of the laboratory confirmed cases were living in the low vaccination coverage area [5]. The increase in mumps cases is not a Dutch phenomenon, and not restricted to non-vaccinated individuals; outbreaks of mumps, not only in unvaccinated communities, all over the world are on the rise [3,6–8]. By studying epidemiological links between mumps cases, Marin et al. suggests that asymptomatic transmission by vaccinated individuals contributed a great deal to the spread within a college population. Similar observations by others, and the isolation of virus from asymptomatic children for up to 9 days after first detection [10] confirm the potential role of asymptomatic shedders in spread of the infection [9,10]. The mumps IgG-specific capture Enzyme Immunoassays (EIA) from Microimmune is a commercial assay to detect mumps IgG antibodies in sera and oral fluids. We believe the cutoff IgG value defined by the manufacturer is not suitable when mumps IgG levels in previously vaccinated patients are under investigation, since it was validated using unvaccinated groups. This is the case because vaccinated individuals present with residual IgG mumps antibodies due to the vaccination rather than an infection. To account for this vaccine induced IgG antibodies we set out to define a more suitable cutoff value. Such a cutoff can be a useful tool to (1) retrospectively identify infections among vaccinated individuals during a mumps outbreak; (2) potentially assess the numbers of asymptomatic spreaders in the given population and (3) estimate the effectiveness of the vaccine to protect from mumps infection rather than disease. An additional aspect was the use of oral fluid samples, as they represent one of the easiest and least invasive sampling strategies in large, retrospective population studies. Due to the retrospective nature of the study only IgG

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Table 1
Descriptive data on the control and study group.

	Control population		School study population
	Mumps virus negative	Mumps virus positive	
Gender	$n = 113$	$n = 17$	$n = 1175$
Male (% total)	63 (55.8)	13 (76.5)	594 (50.6)
Female (% total)	49 (43.4)	3 (17.6)	581 (49.4)
Unknown (% total)	1 (0.9)	1 (5.9)	0 (0.0)
Age	$n = 113$	$n = 16$[a]	$n = 1175$
Mean (95% CI)	5.6 (5.1–6.2)	12.8 (9.6–16.0)	7.6 (7.5–7.7)
Minimum	1	5	3
Median	5	12.5	8
Maximum	17	24	13
Vaccination	$n = 113$	$n = 17$	$n = 1175$
0 × MMR (% total)	0 (0.0)	0 (0.0).	318 (27.1)
1 × MMR (% total)	98 (86.7)	8 (47.1)	489 (41.6)
2 × MMR (% total)	15 (13.3)	9 (52.9)	299 (25.4)
Vaccination unknown	0 (0.0)	0 (0.0)	69 (5.9)

Mumps virus negative persons represent those recruited from MMR vaccinated but unexposed individuals. Mumps virus positive persons are persons with clinical symptoms and laboratory confirmed by RT-PCR. The control population represents all patients that were used for the final calculation of the assay parameters. The vaccination status of the study group is based on the official records from the Dutch vaccination-registration system "PRAEVENTIS".

[a] Samples were included despite the missing age data since selection was done on the basis of vaccination status.

antibody detection was deemed useful since sample taking was in most cases too long after the suspected day of onset to make IgM or direct detection with PCR useful tools.

Here, we report the cutoff adaptation of the IgG mumps assay, using receiver operator characteristic (ROC) analysis, in order to identify mumps infections in vaccinated individuals. The cutoff were defined and validated using two control groups containing vaccinated individuals with and without a recent mumps infection in contrast to the original validation of the manufacturer, were non-vaccinated individuals were used to assess the specificity. By using this adapted assay, we study the frequency of asymptomatic infections in a highly vaccinated population during the 2007–2009 mumps epidemic in Dutch schools.

2. Material and method

2.1. Defined control populations

The mumps negative-control group consisted of MMR vaccinated but mumps virus infection negative individuals, from whom clinical samples were sent to the National Laboratory for Infectious Diseases at the RIVM from 2003 to 2006 for the investigation of rash diseases. In the mumps negative-group ($n = 113$) only people aged below 20 years were included. It is expected that the majority of these individuals had never been exposed to mumps, because only very little mumps activity had been reported in the Netherlands since the introduction of vaccination in the Netherlands (1987); until the first major rise in 2007. The mumps positive and vaccinated-control group ($n = 17$) was chosen from mumps virus RT-PCR-positive [11] individuals that contracted the infection between 2007 and 2009 and were investigated at the National Laboratory for Infectious Diseases. Samples obtained very shortly after onset of disease (within four days) were discarded because of insufficient IgG seroconversion. Details on age and gender of the final control groups are given in Table 1.

2.2. School study population

The study population consists of 1175 individuals recruited from eight primary schools which had notified mumps cases during an ongoing outbreak of mumps in The Netherlands. Children attending one of the eight affected schools were assumed to have been exposed to mumps by infected classmates, family and friends. Exposure took place in 2008, the peak of the outbreak. The vaccine coverage in these schools ranged from 40% to 93% and individual vaccination statuses were confirmed using the Dutch national vaccine register (Praeventis). Individuals with unknown vaccination history were excluded from later calculations. Informed consent to participate in the epidemiological and laboratory study was given by all study participants. The details and epidemiological findings of this study will be published elsewhere.

2.3. Sample definition and analysis of mumps IgG antibodies

Oral fluid was collected with an ORACOL (Malvern Medical Developments, Worcester, UK) sponge device. The oral fluid was directly extracted from the sponge by centrifugation and stored at −80 °C. Before use, the samples were diluted 1:4 with 0.1% BSA in PBS. The commercial IgG capture EIA from Microimmune (London, UK) was used to determine the IgG levels in oral fluid samples of the study and control groups. Samples were analyzed as described by the manufacturer, with the exception that all samples on every microtiter plate were normalized in order to obtain three negative controls (xNC) that were equivalent to the optical density (OD) of 0.1. This way, it was possible to use constant cutoff values compared to the method recommended by the manufacturer. All samples were analyzed once and equivocal test results (between xNC and xNCx1.4) were considered negative.

2.4. Statistical methods

The groups were compared using a Chi^2- or Mann–Whitney-test. *P*-values <0.05 were considered statistically significant. Differences in the OD values between different groups were analyzed using an unpaired Kruskal–Wallis test. The cutoff was defined using a ROC curve with the aim to achieve >95% specificity. The area under the curve (AUC) was estimated with its 95% confidence interval. The AUC is a measure of the overall performance of the test across all cutoff-points, with values close to 1 being the most accurate and a test with an AUC of 0.5 is a non-discriminating test [12,13]. Sensitivity, specificity and attack rates were calculated for our control groups, with a 95% confidence interval. Hypothetical disease prevalence's in our control group were used to assess the changes in PPV and NPV. PPV and NPV were calculated as

follows: PPV = (sensitivity × prevalence)/(sensitivity × prevalence + (1 − specificity) × (1 − prevalence)); NPV = (specificity × (1 − prevalence))/((specificity) × (1 − prevalence) + (1 − sensitivity) × prevalence) [14]. All statistical calculations were performed using STATA (IC 10 for Macintosh); figures were produced using GraphPad Prism (Version 5 for OS X).

3. Results

3.1. Sample inclusion for cutoff determination

To identify which samples were suitable for the cutoff calculation we determined how the interval between first clinical symptoms and moment of sampling, as well as the number of vaccinations prior to infection influences the mumps IgG antibody levels using the Microimmune test on oral fluid. Optical density values in samples taken earlier than four days after the first symptoms proved to be significantly lower ($p < 0.01$, Mann–Whitney test) than for samples obtained more than four days after onset of symptoms. Hence, data for early samples were excluded. Second, we studied IgG levels in the uninfected control group and analyzed whether the history of vaccination had any influence on the levels of mumps specific IgG. When samples of the vaccinated, mumps PCR-negative group ($n = 113$) were stratified according to their number of vaccinations, no significant difference in the mean OD levels was observed (t-test, $p > 0.05$; mean OD values of 1 × MMR group ($n = 98$) 0.14 (CI95%: 0.12–0.18), mean OD value 2 × MMR group ($n = 15$) 0.15 (CI95%: 0.09–0.22)). Also in the mumps infected groups the difference between one and two doses of vaccine did not significantly differ (t-test, $p > 0.05$; mean: OD(1 × MMR) = 0.46 (CI95%: 0–0.97), OD(2 × MMR) = 0.93 (CI95%: 0.012–1.7)). Hence, we did not stratify by number of vaccinations in the following.

3.2. Definition IgG cutoff values for vaccinated individuals

The receiver operator characteristics (ROC) curve was applied to compute different cutoff values (Fig. 1A) and their respective sensitivity and specificity in the control groups (Fig. 1B). Analysis of the ROC curve showed an AUC of 0.77 (95%CI: 0.63–0.91) for the use of the EIA in vaccinated individuals. The cutoff value that according to the ROC curve leads to the most balanced specificity and sensitivity was OD > 0.25, which resulted in a specificity and sensitivity of 82.3% (95%CI: 74.0–88.8%) and 58.8% (95%CI: 32.9–81.6%), respectively. To retrospectively distinguish infected from uninfected persons in a population where infection will be rare, we aimed at a minimum of 95% specificity [15]. This way the attack rate (AR) in the vaccinated groups will not be overestimated especially since a lower specificity would have only resulted in a slightly higher sensitivity. The cutoff that fulfilled this predefined condition for our study question was OD > 0.40 (Fig. 1B). At OD > 0.40 the test identifies mumps infections in vaccinated individuals with a specificity of 95.6% (95%CI: 90.0–98.0%) and a sensitivity of 47.1% (95%CI: 23.0–72.2%). We assumed different prevalence's for mumps infections among vaccinated persons (0.1%, 1%, 10%, 20%) to assess the positive and negative predictive values (PPV/NPV) of our test in different prevalence settings. To assess the PPV and NPV of the test in our control group ($n = 130$), the results obtained from the most balanced (OD > 0.25) and the chosen (OD > 0.40) cutoff were compared. This illustrates further how the respective sensitivity and specificity influences the predictive values of the test in different prevalence settings (Table 2). With increasing prevalence of mumps infections among vaccinated, the PPV of the assay ranges from 1.1% to 72.8% using the OD > 0.40 cutoff. At the same time the probability that a negative assay result is truly negative ranges from 99.9% to 87.8%.

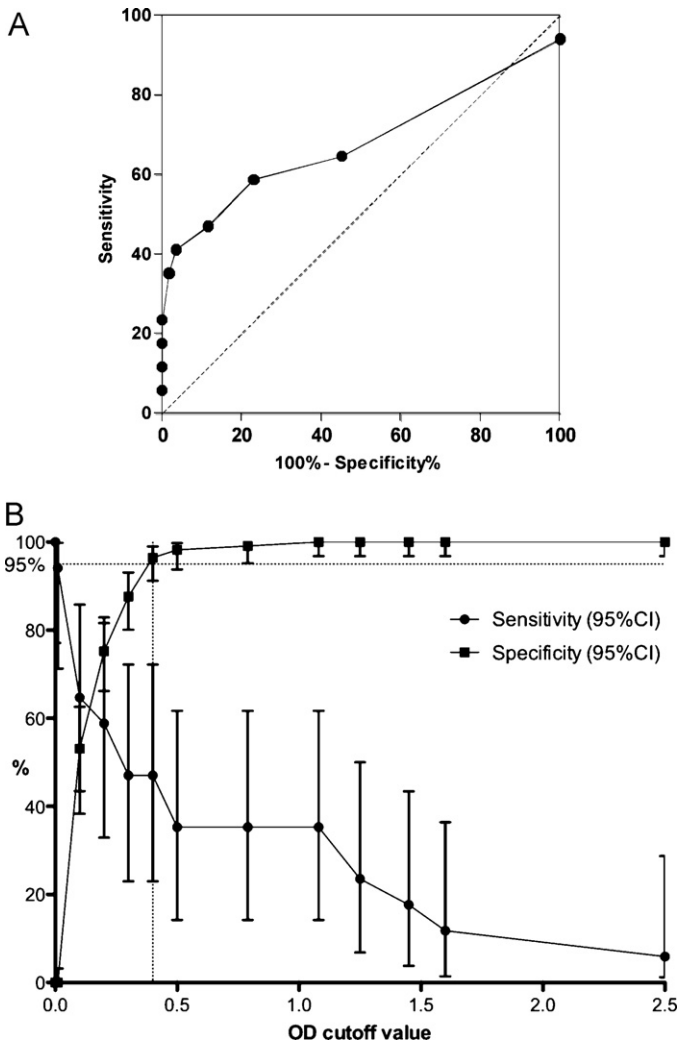

Fig. 1. Assay parameters to detect mumps infection in vaccinated individuals. (A) The ROC curve was used to assess the specific parameters for each cutoff and the overall AUC. (B) Sensitivity and specificity values and their respective 95%CI, are shown depending on the applied cutoff. The proposed new cutoff (OD > 0.40) is indicated with vertical dotted lines. In addition, 95% is marked with a horizontal dotted line.

Table 2
Hypothetical positive (PPV) and negative (NPV) predictive values for two different cutoffs, assuming different prevalences of mumps infection in our control population.

Prevalence (%)	Optical density > 0.25		Optical density > 0.40	
	PPV (hypothetical)	NPV (hypothetical)	PPV (hypothetical)	NPV (hypothetical)
0.1	0.3	99.9	1.1	99.9
1	3.2	99.5	9.8	99.4
10	27.0	94.7	54.3	94.2
20	45.4	88.9	72.8	87.8

Table 3
Attack rates among the school study population determined by the oral fluid EIA compared to epidemiological data.

Vaccination status	Attack rates (%)	
	OD > 0.40 (CI95%)	Clinical (CI95%)
Non-vaccinated (n = 318)	n.a.	49.3 (46.4–52.1)
Vaccinated (n = 857)	14.4 (12.0–16.9)	2.7 (1.7–4.0)

3.3. Application of the mumps saliva EIA in the Dutch school-study group

The mumps IgG EIA was used on oral fluid samples collected as part of a retrospective epidemiological study in schools where mumps disease had been diagnosed during a mumps outbreak in the low vaccination coverage area, from 2007 to 2009. The samples were tested using the cutoff of the manufacturer (OD > 0.14) for the non-vaccinated group and using the adjusted cutoff value (OD > 0.40) for all persons with a vaccination history, to identify possible mumps infections in the vaccinated group (Table 3). The difference between the clinical and laboratory confirmed AR of mumps among vaccinated individuals was substantial, with an AR(infection) of 14.4% when laboratory data were taken into consideration, compared to an AR(clinical) of 2.7% according to the self reported clinical cases. Of the 21 individuals that accounted for the 2.7% clinical AR among the vaccinated study population, 7 (33.3%, 95%CI: 15.9–55.1%) were positive in the oral fluid assay with the adjusted cutoff.

4. Discussion

The interpretation of mumps IgG antibody levels as an indicator for recent mumps infection in vaccinated individuals is complicated, because vaccine-induced antibodies are expected to persist long after the last vaccination. To validate whether the Microimmune mumps IgG EIA for use with oral fluid of vaccinated persons was able to discriminate between a vaccine- and infection-mediated IgG response, a ROC curve suggests a moderately accurate test to identify mumps infection among vaccinated individuals [12,13]. By using a cutoff value of OD > 0.40 it can be assured that for vaccinated individuals, the antibody level induced by the vaccine is accounted for. We predefined a 95% specificity to prevent an overestimation of infected individuals in studies in populations with high vaccine coverage [15]. All obtained assay parameters, particularly the sensitivity, are influenced by the sampling strategy. For instance, consistent titer rises were only observed when sampling took place at least four days after onset of disease, but this might possibly still be too early to detect titer rises in some individuals leading to a mis-qualification of the sample in the control groups. Furthermore, the selection of positive control patients was made on basis of detection of viral RNA by RT-PCR. Though this is a valid parameter to diagnose mumps virus infection, this may not necessarily result in significant mumps-specific IgG titer rises in all patients. Oral fluid collection was chosen for this study despite the described pitfalls [16] because of its ease of use, safety and acceptability in larger public health studies. Overall, the chosen cutoff of OD > 0.40 achieves a very good specificity and NPV with a sensitivity that resulted in an acceptable PPV in a population with 10–20% infection prevalence. Using a range of prevalence's illustrates the influence of the disease prevalence on the accuracy and usefulness of the presented assay. Even though the PPV and NPV are calculated for hypothetical prevalence's, we believe based on our results that they will apply in our school study population. Yet at 10–20%, the PPV reaches only between 50% and 70%, which emphasizes that the described assay cannot be used for robust diagnosis of individual patients but only in population based studies during an outbreak. Despite its pitfalls, our analysis gives an insight in the underestimation of asymptomatic infection rates in a vaccinated population and a way to analyze this retrospectively. Even if we assume that based on our PPV, about 40% of the EIA positive, vaccinated persons were not infected, the remaining AR in our study population is still between 7% and 10%. This suggests that for every one clinical vaccinated mumps patient; at least three asymptomatic cases can be expected. This AR estimation takes the low sensitivity and the approximated PPV in our population into account. The substantial number of asymptomatic cases may contribute to mumps spreading and cannot be easily detected with any other method due to the lack of clinical symptoms. Only convalescent samples taken from the entire community, during an ongoing outbreak might be able to definitely confirm asymptomatic infection. Unfortunately this is very difficult to do in a large study population due to ethical constrains. The assessment of asymptomatic cases however, might be of great importance when outbreaks and transmission of mumps are reported in vaccinated communities [2,9,17], in order to identify linked cases or potential clusters and to assess vaccine effectiveness. In conclusion, our results show that it is possible to use the Microimmune mumps IgG EIA with oral fluid to retrospectively identify asymptomatic mumps infections in vaccinated populations, if an adjusted cutoff value is used. Such an analysis can also be performed using blood samples or even finger prick blood, but this is a more invasive approach and may not be feasible in a primary school setting such as the presented one. Based on our study we estimate that during a mumps outbreak in primary schools with intermediate vaccine coverage, between 7% and 10% of MMR-vaccinated children had an asymptomatic infection. This numbers are thought to be still an underestimations of the real AR among vaccinated, due to the low sensitivity of the adapted cutoff and the possible sampling constraints, even after taking the low PPV into account. With this in mind we have to assume that the AR of mumps infections among vaccinated primary school children in an outbreak setting will be even higher. Our findings are of importance for assessing the role of asymptomatic shedders in the spread of infection during an outbreak. Additionally, vaccine effectiveness studies traditionally only take clinical cases into account but the effectiveness of a vaccine to protect from infection rather than disease is also an important parameter when it comes to herd immunity [18]. This could in turn lead to the reassessment of vaccination schedules and target vaccination coverage. In addition, the investigation of AR among vaccinated individuals can also be a tool to assess the virulence of different mumps genotypes that are currently causing outbreaks all over the world.

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