Epidemiol. Infect. (2003), **131**, 703–709. © 2003 Cambridge University Press
DOI: 10.1017/S0950268803008641 Printed in the United Kingdom

Antibodies against mumps in The Netherlands as assessed by indirect ELISA and virus neutralization assay

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(*Accepted 22 February 2003*)

SUMMARY

To obtain insight into mumps immunity 9 years after introduction of vaccination in The Netherlands, antibodies were measured in a national sample ($n=8298$) and in clustered religious groups with low vaccine acceptance ($n=254$). All sera were tested by indirect ELISA, and agreement with neutralization assay was assessed in a subsample ($n=623$). Overall seroprevalence in the adult age groups in the national sample was 96·2% (95% confidence interval 95·4–97·0%). Seroprevalence was somewhat lower in the vaccinated age groups, but still sufficient to maintain herd immunity. After the first dose of vaccine, an increase up to age three years to 93·2% (89·8–96·6%) and a subsequent decline in prevalence to 88·9% (81·7–96·0%) at age 7 years was seen. Seroprevalence in those eligible for two vaccinations was 94·4% (91·3–97·4%). In the religious groups, seroprevalence was generally lower in the age group 1–4 years (30% (18–95%)) than in the national sample, but similar in the older age groups. Seroprevalence as estimated by neutralization assay was only slightly lower for all age groups $\geqslant 1$ year. Therefore, the indirect ELISA is a reliable method for measuring mumps virus-specific antibodies in population-based studies. However, to allow for inter-laboratory comparison, international unitage should be developed.

INTRODUCTION

In most cases, mumps is a relatively benign infection which is subclinical in approximately a third of all children, and 40–50% show non-specific or upper respiratory symptoms only. Before the introduction of mass vaccination mumps infection was responsible for considerable morbidity: deafness caused by mumps was one of the leading causes of acquired sensorineural deafness, and symptomatic meningitis occurs in 5% of mumps patients [1–3]. In The Netherlands, mumps vaccination (Jeryl Lynn strain) for children aged 14 months and 9 years was included in the national immunization programme in 1987 [4]. Before introduction of vaccination, 300–800 mumps cases were hospitalized annually, mostly for meningitis. After introduction, the number of hospitalizations decreased rapidly to less than 10 cases annually [5].

To study the effect of mass vaccination on immunity levels against mumps in the vaccinated, and unvaccinated Dutch population, we measured antibodies in 8298 sera from a national sample, and in 254 sera from orthodox reformed communities. Individuals from these religious communities are clustered geographically and socially within The Netherlands, and

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usually decline vaccination. All sera were tested in an indirect ELISA, a widely used method. Neutralizing antibodies are considered the most reliable indicators of immunity against infection, but a virus neutralization assay (NT) is very labour-intensive to perform. We explored the agreement between NT and ELISA in a subgroup of 623 sera.

METHODS

Study population

Between October 1995 and December 1996, we established a serum bank for the evaluation of the national immunization programme. The study design has been described in detail elsewhere [6]. In short, a sample of 40 municipalities was drawn proportional to the number of inhabitants. In each municipality an age-specific sample (0, 1–4, 5–9, …, 75–79 years) of 380 persons was drawn. To have access to orthodox reformed individuals, a similar sample was drawn in eight municipalities with low vaccine coverage. We requested all individuals to come to special clinics to give a blood sample, to fill out a questionnaire, and to show vaccination certificates. The responses were 55·0 and 52·5%. Testing for mumps antibodies was possible for 8298 sera of participants from the national sample, and for 254 sera of participants from the low vaccine coverage sample that had reported in the questionnaire to adhere to an orthodox reformed religion.

Indirect ELISA

Collected serum samples were stored at $-86\,^{\circ}C$ in Micronic blocks containing 96 cups of 500 μl until testing. IgG antibody concentrations against wild-type mumps virus (strain Enders) were measured by indirect ELISA in all sera, as described before [7]. Ninety-six wells microtitre plates were coated with 2 μg/ml purified antigen. No international reference serum exists for mumps. The internal reference serum (1000 RIVM units (RU)/ml) was added to each plate in a twofold dilution series, and the test sera and two control sera in a 1 in 100 dilution. The antibody concentration was determined by the four-parameter fit method in Kineticalc (KC4) with a Bio Tek plate reader (EL312d). The results of each plate were accepted if the reference serum revealed the original amount in the linear part of the curve $\pm 10\%$, and the two control sera were within their predefined 95% CI. The mean of two independent measurements was defined. The lower detection limit was 5 RU/ml. Sera with titres <45 RU/ml were considered mumps virus antibody negative, which has been determined using a panel of sera from 1-year-old children [8].

Virus neutralization assay

Mumps virus is closely related to some other paramyxoviruses, such as parainfluenzaviruses. Antibodies to some of these viruses may be detected by indirect ELISA, while they may not be protective against mumps infection. A virus neutralization assay is very specific, but is very labour- and time-intensive to perform. To explore the amount of cross-reactivity in the indirect ELISA, sera from eight random serum blocks from the national sample were tested for neutralizing IgG antibody against the same strain mumps virus (Enders) as in ELISA, essentially as described earlier [9]. Sufficient serum was available for 623 participants. Sera in twofold dilution series were mixed with 100 CCID50 virus and kept for 1 h at 37 °C in 96 well microplates. Vero cell suspensions (5×10^5/ml) were added to each well, incubated, and read after 6 days. Results were expressed in RU/ml as on each plate the internal reference (770 RU/ml) and a control serum were measured. The cut-off for seronegativity in the ELISA (45 RU/ml) corresponded to a titre in between the dilutions with 32 and 64 RU/ml in the NT. Therefore, sera with titres $\leqslant 32$ RU/ml were considered mumps virus antibody negative, and titres $\geqslant 64$ RU/ml positive.

Data analyses

Geometric mean titres were estimated using logarithmic transformation. To correct for age stratification in the sampling procedure, seroprevalences and (geometric) means of mumps antibodies according to indirect ELISA results within each municipality were weighted by the proportion of inhabitants per age groups in this municipality. As the municipalities in the national sample had been sampled proportional to their population size, the weighted prevalences and means were averaged over the 40 municipalities to obtain national estimates. To obtain estimates for the low vaccine coverage sample, weighted prevalences and means were averaged weighting by the population size of these eight municipalities. Differences were tested with the Wilcoxon rank sum test. The effect of differential non-response on the overall seroprevalence

https://doi.org/10.1017/S0950268803008641 Published online by Cambridge University Press

Table 1. *Age-specific geometric mean titre and seroprevalence with 95% confidence intervals (CI) in the national sample as estimated by indirect ELISA*

Age (years)	Number of mumps vaccinations	Number of persons	Seroprevalence ⩾45 RU/ml (%)	95% CI	GMT* (RU/ml)	95% CI
0	0	620	21·5	(17·9–25·8)	24	(22–26)
1–19	1–2	2417	91·0	(90·0–92·0)	143	(137–149)
2–7	1	905	90·2	(88·2–92·2)	127	(117–138)
8–9	1–2	227	96·2	(93·8–98·5)	154	(137–174)
10–12	2	351	94·4	(91·3–97·4)	136	(125–148)
13–18	1	617	94·9	(93·0–96·8)	172	(159–186)
20–79	0	5261	96·2	(95·4–97·0)	180	(171–189)

* GMT, geometric mean titre.

estimate was within one standard error of this seroprevalence estimate in both samples, and therefore was ignored.

As the mumps virus neutralization assay was employed for sera collected in 14 municipalities from the national sample only, crude prevalences and geometric mean titres were calculated. Differences in crude seroprevalences between NT and ELISA within groups were statistically tested pair-wise with the McNemar test. Differences with a P-value $<0·05$ were considered statistically significant.

RESULTS

Indirect ELISA

National sample

Mumps vaccination was introduced 9 years before sampling for this study was performed. As a result of a catch-up campaign however, persons up to the age of 18 years at the time of sampling would have been included in the mumps vaccination scheme (Table 1). Persons aged 2–7 years have been offered one dose of MMR vaccine at 14 months. Persons aged 8 and 9 years have been offered the first dose at 14 months, and some had already received the second dose which is scheduled at 9 years: of those participants who had brought vaccination certificates to the clinic, 15 and 53%, respectively, had already had their second dose. Persons aged 10–12 years have been offered two vaccinations (at 4 years and 9 years), and persons aged 13–18 years have been offered one dose at 9 years only.

In the national sample 94·1% (93·4–94·7%) had a titre ⩾45 RU/ml. Seroprevalence in infants declined from approximately 80% in the first 3 months to below 10% after the age of 6 months. Seroprevalence was lower in the age group 2–7 years than in the non-vaccinated cohorts, and GMTs were lower in the age groups under 13 years (Table 1 and Fig. 1).

Seroprevalence, but not GMT, was significantly ($P=0·02$) higher in the age group 10–12 years (eligible for two doses of vaccine) than in the age group 2–7 years (one dose). Seroprevalence in 2-year-olds was 88·1% (81·4–94·7%), 93·2% (89·8–96·6%) in 3-year-olds and subsequently decreased to 88·9% (81·7–96·0%) in 7-year-olds, and increased to 98·4% (96·2–100%) in 9-year-olds. GMT in these participants amounted to 142 (119–173) RU/ml in 2-year-olds, 157 (131–170) RU/ml in 3-year-olds and decreased to 112 (91–129) RU/ml in 7-year-olds, and was 153 (145–216) RU/ml in 9-year-olds. Similar seroprevalences and GMTs were observed in those with registered vaccination, reflecting the high vaccine uptake in the national sample.

In the indirect ELISA no gender-specific differences were observed in seroprevalence, but GMTs in the age groups over 10 years were consistently lower ($P<0·001$) for males (163 (155–172) RU/ml) than for females (192 (183–201) RU/ml).

Orthodox reformed participants

In comparison with the national sample, the seroprevalence and GMT as estimated by indirect ELISA in the 1- to 4-year-old orthodox reformed participants was significantly lower (30% (CI 18–95%) *vs.* 79% (CI 77–82%), and 111 (CI 102–120) *vs.* 39 (CI 28–54) RU/ml). For other age groups eligible for vaccination and in the adult cohort (20–79 years), seroprevalence was slightly, but not statistically significantly, higher

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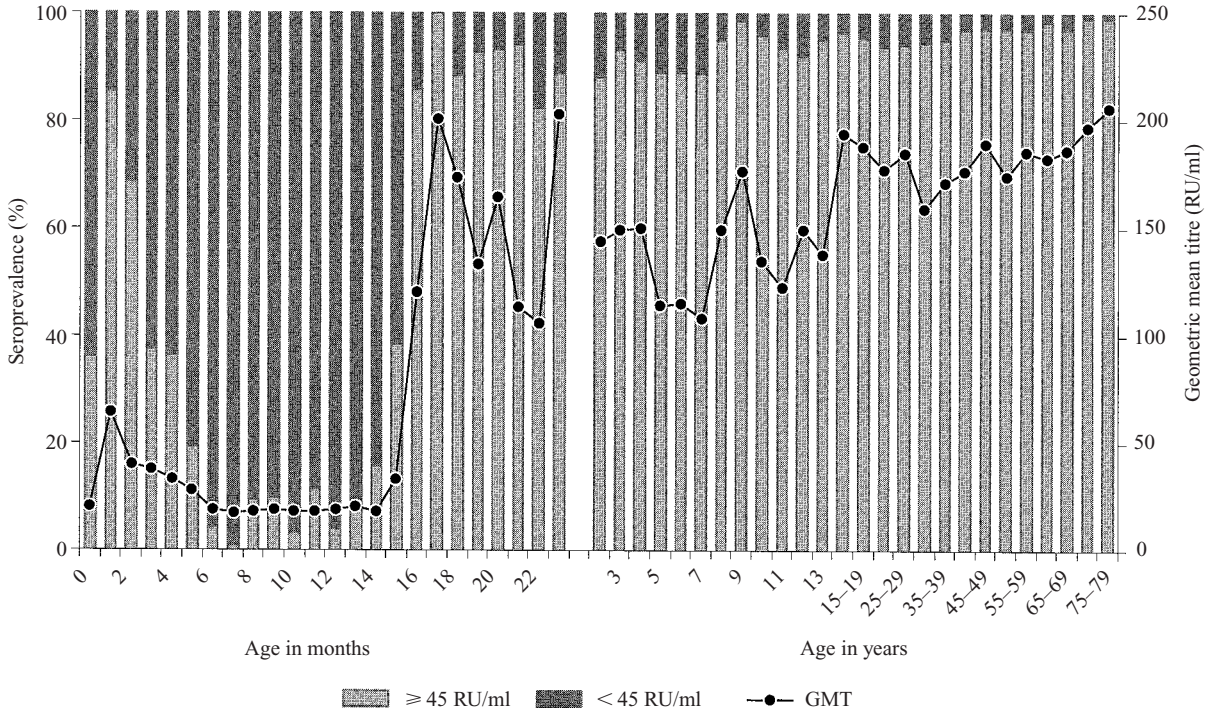

Fig. 1. Age-specific seroprevalence and geometric mean titre as estimated by indirect ELISA in the national sample. (Note that age is expressed in months for those 0–1 years, in years for those 2–14 years, in 5-year age groups for those 15–79 years).

(⩾97%). Age-specific seroprevalence and GMT in the non-orthodox reformed participants in the sample of the municipalities with low vaccine coverage, was similar to that in the national sample (data not shown).

Virus neutralization assay

Of the 623 sera from the national sample that were tested both in the indirect ELISA and NT, 571 (92%) results were concordant, of which 566 were positive in both tests. Fifty-two results were discordant: 22 (4%) sera were positive (⩾45 RU/ml) by ELISA but negative (⩽32 RU/ml) by NT, while 30 (5%) sera were negative by ELISA but positive by NT. Assuming NT is the gold standard, the positive predictive value of indirect ELISA was 96%. Estimated seroprevalence was similar according to ELISA and NT, except for the infants under 1 year of age (Table 2). However, the difference in this age group is not significant and may be due to chance in this small sample.

DISCUSSION

Effects of mumps vaccination

Before the introduction of mumps vaccination in The Netherlands, seroprevalence of neutralizing antibodies as measured by plaque reduction neutralization assay in children aged 3 years was 25%, and increased from 50 to 90% in children aged 4–14 years [10]. For adult cohorts, seroprevalence was slightly over 95%. Seroprevalences as estimated by indirect ELISA and NT in the present study were similar for the unvaccinated adult cohorts while seroprevalences in the vaccinated cohorts under 15 years from the general population were higher than in the parallel age group in the pre-vaccine years. This implies that mass vaccination has led to an increase in mumps virus antibody positivity. However, only three birth cohorts have had two doses of MMR vaccine, and as a result of the catch-up campaign, they received their first vaccination at age 4 and some will have been naturally infected before then. Those aged 13–18 years have had their first vaccination at 9 years, and many of them probably were naturally infected before receiving vaccination. Thus, time since start of mass vaccination was too short to be able to study the developments in antibody levels after the second dose in cohorts with exclusive vaccine-induced immunity.

The second dose of vaccine is given to immunize children with primary vaccine failure, and those children that did not receive the first dose. Seroprevalence, but not GMT, was significantly higher in the twice-vaccinated 10–12-year-old group than in the once vaccinated 2- to 7-year-old group. This effect of

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Table 2. *Age-specific seroprevalence with 95% CI as estimated by indirect ELISA and virus neutralization assay in a subgroup of the national sample*

		ELISA		NT*	
Age	Number of persons	Seroprevalence ⩾45 RU/ml (%)	95% CI	Seroprevalence ⩾64 RU/ml (%)	95% CI
0	10	10	(0–29)	50	(19–81)
1–19	198	91·9	(88·1–95·7)	89·9	(85·7–94·1)
1–9	114	88·6	(82·8–94·4)	88·6	(82·8–94·4)
10–19	84	92·9	(92·5–100·0)	91·7	(85·8–97·6)
20–79	415	97·6	(96·1–99·1)	95·7	(93·7–97·6)

* NT, neutralization assay.

the second dose of MMR vaccine was also seen for measles and rubella. However, mumps seroprevalence in the partly once and partly twice vaccinated 8- and 9-year-olds was also higher than in the once vaccinated 2- to 7-year-old group. This effect of the second dose in this only partly twice vaccinated group was not seen for measles and rubella. This may be explained by the somewhat lower efficacy of the mumps component compared with the measles and rubella virus components in the MMR vaccine, as a second dose of a less efficacious vaccine will result in a greater reduction in susceptibility due to a larger proportion of primary vaccine failures.

Interpreting our cross-sectional data longitudinally, we observed an increase in prevalence and GMT, on average up to the age of 3 years, after the first dose of MMR. An unexplained increase in titres, despite low incidence of disease, has been recognized before, after Jeryl Lynn mumps vaccination [11], but not after measles and rubella vaccination. The subsequent decline in titres with time has been recognized for measles and rubella also, and implies a low level of virus circulation in the general population [1, 2].

Orthodox reformed

The religious communities that decline vaccination are clustered both geographically and socially. Within the orthodox reformed participants, 30% of 1- to 4-year-old children were mumps virus antibody positive. However, this is probably primarily due to vaccination and not to natural infection. Some orthodox reformed adherents do accept vaccination for their children, and those that participated in this study are more likely to accept vaccination than those that did not accept our invitation to give blood. Of the participants aged 1–19 years in this study, 44% reported to have been vaccinated according to the regular schedule versus 30% of those that did not give blood but did fill out a questionnaire. Thus, it seems probable that orthodox reformed children under the age of 5 who were mumps virus antibody positive acquired immunity chiefly through vaccination. Over 97% in the age groups 5 years and older were mumps virus antibody positive. Given the limited vaccine acceptance, immunity in these older age groups is expected to be acquired primarily through natural infection. Thus, in between introduction of vaccination and serum collection in our study, mumps infections must have occurred in these religious groups.

No indications for extensive circulation of mumps virus have been observed in The Netherlands since introduction of vaccination in 1987. Reported mumps incidence dropped below 1 per 100 000 inhabitants after introduction of vaccination until 1999, since when mumps has no longer been a notifiable disease. Furthermore, the number of mumps hospitalizations and laboratory confirmations has been at an all time low for a decade now (unpublished results). Our surveillance data do not seem to be sensitive enough to detect mumps outbreaks within this relatively small population, especially as many infections remain subclinical. Therefore, it is uncertain whether mumps infections still take place within these groups at the moment. If mumps is no longer endemic, orthodox reformed children may reach adult age without immunity. In the future, this may lead to an epidemic with many adolescent and adult cases, in which complication rates are increased [1, 2].

Need for international unitage

International standard sera enable comparison of inter-laboratory results by calibration of local units

https://doi.org/10.1017/S0950268803008641 Published online by Cambridge University Press

into international units. However, for mumps virus no international unitage nor cut-off for antibody positivity has been been achieved. In the European Sero-Epidemiology Network (ESEN), a working standard was used as a reference serum and country-specific, including Dutch, standardized test results were compared [12, 13]. In this paper, we have shown the unstandardized Dutch test results.

According to unstandardized results, ELISA mumps virus antibody positivity was higher than according to standardized results, most specifically in the vaccinated age groups [12, 13]. The fact that we employed a different ELISA than the reference laboratory could have resulted in poor comparability of antibody titres, possibly due to differences in the test antigen [12]. To enable comparison of mumps virus antibody test results, an international standard serum should be developed.

Comparison of indirect ELISA and NT results

We compared our indirect ELISA results with the gold standard for measurement of protective antibodies, the neutralization assay. Sensitivity of an NT is lower than that of an ELISA, but the detection limit of our NT is still below the cut-off we used for mumps virus antibody positivity. Since the same antigen and reference was used in both tests, we directly compared NT and ELISA antibody titres.

The NT seroprevalence results were similar or slightly lower for all age groups 1 year and older, both for vaccinated and unvaccinated cohorts (Table 2). Only for infants, the seroprevalence as estimated in NT seemed higher than the prevalence as estimated in ELISA, using a 1 in 100 serum dilution. Although numbers are too small to draw definite conclusions, it may indicate that maternal antibodies persist longer than assumed based on our ELISA. The number of sera tested both in ELISA and NT was too small to allow further stratification by age. Because of pairwise testing of NT and ELISA antibody positivity, small differences already lead to significant results. However, estimated ELISA and NT immunity levels were not really divergent, as indicated by overlapping 95% CI. Thus, the indirect ELISA we employed seems to be a reliable indicator of immunity in population-based studies.

Seroprevalence in the national sample was high enough to achieve herd immunity in the general Dutch population, both in the vaccinated and unvaccinated age groups. In contrast, immunity in the orthodox reformed age groups born after introduction of vaccination is expected to have become too low to prevent outbreaks. To follow developments in (herd) immunity in NL with increasing proportion of vaccinated persons, this immunosurveillance study should be repeated in a few years, in which the aim is predominantly on vaccinated cohorts. Such a study should also include orthodox reformed individuals to give insight into whether mumps outbreaks still occur in these unvaccinated groups, despite lack of evidence from disease and laboratory surveillance sources. The indirect ELISA is a reliable method for measuring mumps virus-specific antibodies in population-based studies. However, to allow for inter-laboratory comparison, international unitage and cut-off for mumps virus antibody positivity, preferably based on NT, should be developed.

ACKNOWLEDGEMENTS

Marina Conyn-van Spaendonck initiated this immunosurveillance study. We thank all participating public health services and municipalities, and the Pienter project team members for their help in the data collection. We thank Petra van der Kraak, Anja Schakelaar and Michiel van de Wetering for their useful contributions to the laboratory part of this study.

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