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# Secondary measles vaccine failures identified by measurement of IgG avidity: high occurrence among teenagers vaccinated at a young age

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## SUMMARY

Failure to seroconvert (primary vaccine failure) is believed to be the principal reason (approx. $> 95\%$) why some vaccinees remain susceptible to measles and is often attributed to the persistence of maternal antibodies in children vaccinated at a young age. Avidity testing is able to separate primary from secondary vaccine failures (waning and/or incomplete immunity), but has not been utilized in measles epidemiology. Low-avidity (LA) and high-avidity (HA) virus-specific IgG antibodies indicate primary and secondary failure, respectively. Measles vaccine failures ($n = 142$; mean age 10·1 years, range 2–22 years) from an outbreak in 1988–9 in Finland were tested for measles–virus IgG avidity using a protein denaturating EIA. Severity of measles was recorded in 89 failures and 169 non-vaccinees (mean age 16·2 years, range 2–22 years). The patients with HA antibodies ($n = 28$) tended to have clinically mild measles and rapid IgG response. Among failures vaccinated at $< 12$, 12–15 and $> 15$ months of age with single doses of Schwarz-strain vaccine in the 1970s, 50 (95% CI 1–99), 36 (CI 16–56) and 25% (CI 8–42) had HA antibodies, respectively. When a single measles, mumps and rubella (MMR) vaccine had been given after 1982 at 15 months of age, only 7% (CI 0–14) showed HA antibodies. Omitting re-vaccinees and those vaccinated at $< 15$ months, Schwarz-strain recipients had 3·6 (CI 1·1–11·5) higher occurrence of HA responses compared to MMR recipients. Apart from one municipality, where even re-vaccinees had high risk of primary infection, 89% (CI 69 to $\sim 100$) of the infected re-vaccinees had an HA response. Secondary measles-vaccine failures are more common than was more previously thought, particularly among individuals vaccinated in early life, long ago, and among re-vaccinees. Waning immunity – even among individuals vaccinated after 15 months of age, without the boosting effect of natural infections should be considered a relevant possibility in future planning of vaccination against measles.

## INTRODUCTION

Exposure to the measles virus is steadily decreasing worldwide due to mass vaccination. Over a billion people live in areas where natural boosters are becoming increasingly rare, and hundreds of millions

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are protected solely by immunity induced by attenuated vaccines[1, 2]. However, the determinants of quality and duration of vaccine-induced immunity are not fully understood [3, 4]. Low vaccination age is known to adversely affect measles-vaccine efficacy, mainly via maternal antibodies [3]. Vaccine failures are largely attributed to the lack of a primary antibody response [3, 5], although evidence suggests that children primed for measles at an early age do gain immunological memory [5].

As the serological status preceding measles is usually unknown, it is difficult by conventional means to establish the occurrence of secondary vaccine failures (waning and/or incomplete immunity) and related factors [3]. In a study of students who donated blood just before infection, low antibody levels increased the risk of measles [6]. There is a description of four health care workers who contracted measles despite prior successful vaccination(s) [7]. Vaccination success rate has been determined in only one study, in which the antibody status of 188 vaccinees inoculated at 12 months was established soon after the first or second immunization [8]. In a 10-year follow-up, 6·9% of the vaccinees developed clinical measles, which was laboratory confirmed in only one case [8]. Case reports of secondary vaccine failures have been published, including one of a Chinese patient who seroconverted after vaccination at 8 months [3].

The avidity (functional affinity) of IgG antibodies has long been known to distinguish primary from secondary immune responses against many antigens. Notwithstanding protein-specific differences in antigen preparations and variation in assay calibration details [9, 10], virus-specific high-avidity (HA) antibodies generally correspond to pre-existing B-cell memory, whereas low-avidity (LA) IgG is a sign of the primary immune response [11]. The avidity of measles virus IgG has been shown to mature for several months after primary infection and remain high thereafter [12].

Avidity measurement has been used to assess the success of measles vaccination [13] and potentially offers a way of assessing the type of vaccine failure without knowledge of prior antibody status [12–15]. The technique was used to identify five measles patients with secondary vaccine failure [14, 15].

In this study we used IgG avidity to analyse a large series of vaccine failures, in order to separate secondary failures with HA from primary failures with LA IgG antibodies; we also compared the severity of measles among the vaccinees and non-vaccinees. We were particularly interested in discovering whether low vaccination age, or time since last vaccination, were associated with secondary measles vaccine failures.

## METHODS

### Setting and vaccination

The study was performed in Finland, a Nordic country with 5·1 million people, and approved by the ethical committee at the Department of Public Health of the University of Helsinki. In Finland, vaccinations are voluntary and free of charge. All children born between 1973 and 1981 were assigned to receive, at 12 months of age or above, a single dose of attenuated Schwarz-strain measles vaccine (Rimevax$^{R}$, SmithKline Biologicals, Rixensart, Belgium). Vaccination coverage was approx. 70% [16–18]. In the 1970s the vaccine was also administered to some 12-month-old children, and occasionally to children born before 1973.

Since 1982, the trivalent measles, mumps and rubella (MMR) vaccine (M-M-R$^{R}$ II, Merck & Co, West Point, PA, distributed in Finland as Virivac$^{R}$, SBL Vaccine Ab, Stockholm, Sweden) containing the more attenuated Enders–Edmonston strain of measles virus has been used exclusively, and administered first at 14–18 months and again at 6 years of age [16–18]. Some children born between 1978 and 1981 who had passed the first MMR vaccination age without being immunised in 1983 were vaccinated for the first time with MMR when they next visited health centres in 1983–6 [17]. Thus, 170000 children received three measles vaccinations [19]: (i) before 1982 the Schwarz-strain vaccine usually at 12 months or older, (ii) the first MMR vaccine exceptionally between 2 and 5 years of age, and (iii) the second MMR vaccine at 6 years of age. MMR vaccination coverage exceeded 97% after successful interventions [17].

### Measles surveillance and outbreak in 1988–9

Since 1987 serological confirmation of measles has been required for all suspected cases and serological diagnosis at National Public Health Institute has been free of charge for health centres.

Measles became rare [16, 18] as the comprehensive national MMR vaccination programme progressed. Only pockets of susceptible non-vaccinated individuals, mostly born in the early 1970s, remained in rural sparsely populated areas (Table 1, Fig. 1)

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Table 1. *Characteristics of study patients*

| Characteristics | Vaccines | | Non-vaccinees ($n = 169$) |
|---|---|---|---|
| | High avidity ($n = 28$)* | Low avidity ($n = 101$)‡ | |
| Age (years) | | | |
| Mean | 13·3 | 9·0 | 16·2 |
| Median | 14·1 | 6·2 | 16·2 |
| S.D. | 3·9 | 5·0 | 3·2 |
| Range | 2·0–22·0 | 2·0–20·9 | 2·0–22·0 |
| Male (%) | 6 (35) | 37 (58) | 79 (47) |
| Urban (%) | 4 (24) | 17 (27) | 45 (27) |
| Number of siblings | | | |
| Mean | 2·3 | 2·5 | 1·9 |
| Median | 2·0 | 2·0 | 2·0 |
| S.D. | 1·5 | 1·5 | 1·5 |
| Range | 0–6 | 0–9 | 0–9 |
| Within household infected (%) | 2 (12) | 11 (18) | 9 (5) |

* 17 returned questionnaire.
† 64 returned questionnaire.

[18–20]. In the 1988–9 outbreak area, measles had already become rare soon after 1975.

During the outbreak 1748 cases of measles were confirmed: 1297 of them by serological criteria at the National Public Health Institute and the remainder in the virology laboratories of the five universities.

The subjects of this study fell ill during a 273-day-period from 26 September 1988 to 27 June 1989 (Fig. 2). Notifications peaked in February. The mean time between disease onset and serum sample collection was 6·8 days (median 5·0, 95th percentile 24·5 days), and the mean time between paired samples was 15·9 days (median 14·0, 95th percentile 25 days).

## Vaccine failures

There were 153 cases of measles among vaccinated individuals [19]. Vaccination status was established based on records in official vaccination cards at the time of measles notification. Serum samples from 142 measles virus IgM positive vaccine failure cases were available for analysis.

A total of 113 vaccine failure patients were identified by fourfold or higher rises in measles virus antibody titre using the haemagglutination inhibition technique [21]. In 18 vaccine failure cases a diagnostic rise was not observed ($n = 18$), and only 1 sample was available for 11 vaccine failure patients; in these 29 cases the serological diagnosis was based on detection of measles virus specific IgM by enzyme immunoassay (EIA) (Enzygnost IgM/EIA Behringwerke, Marburg, Germany).

The mean age of the vaccine-failure cases was 10·1 years (range 2–22 years) (Table 1).

## Non-vaccinated cases

In 1995, 226 subjects were systematically sampled from the 1089 2–22-year-old non-vaccinated measles cases notified to the surveillance registry of the Finnish National Public Health Institute.

## Avidity measurement

All avidity measurements were carried out on coded samples. The avidity of IgG for measles virus was measured by a protein-denaturing EIA where the antibodies were first allowed to bind to the virus antigen, followed by elution with or without 6 M urea.

In preliminary experiments we compared two commercial measles-virus IgG EIAs (Enzygnost$^{R}$, Behringwerke, Marburg, and the measles-virus IgG EIA of Human Gesellschaft Taunusstein, Germany). As the two assays gave essentially similar results, the latter was chosen for regular use, while samples of special interest were examined with both EIAs.

We also compared two avidity-assay variants. In the technically simpler approach the serum samples were studied in single (fixed) working dilutions [11],

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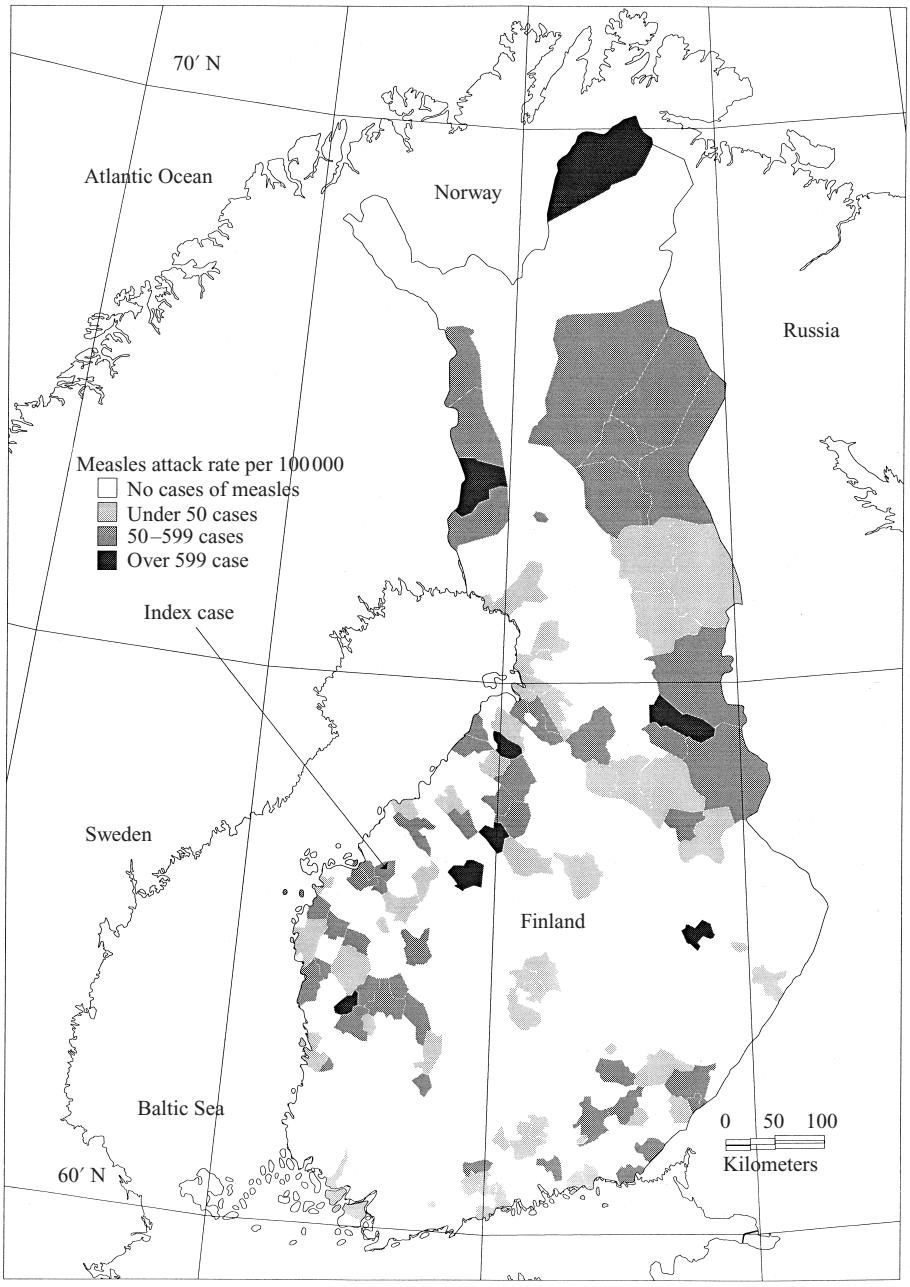


**Fig. 1.** Geographical distribution of the 1988–9 measles epidemic in Finland. The municipality where a measles vaccine failure outbreak including re-vaccinees was observed, is the most south-western municipality where measles attack rate exceeded 599/100000 (black).

whereas in the technically more demanding approach the sera were studied in serial dilutions [22].

As with other microbes [23], the latter approach proved better able than the former to distinguish between the acute phase and past immunity (data not shown). Consequently, in the present work the sera were serially diluted in fourfold steps for end-point titres using optical density (OD) = 0·2 as the cut-off. In one dilution series of each sample, the antigen-bound antibodies were washed with phosphate-buffered saline (PBS) containing 6 M urea and 0·05% Tween-20 (PBS), and in the other series, with PBST alone. Avidity was expressed as the percentage ratio of the two end-point titres:

[titre (urea wash)/titre (PBS wash)] × 100.

For definition of the cut-off levels of avidity, we tested the acute-phase sera of 13 non-vaccinees with

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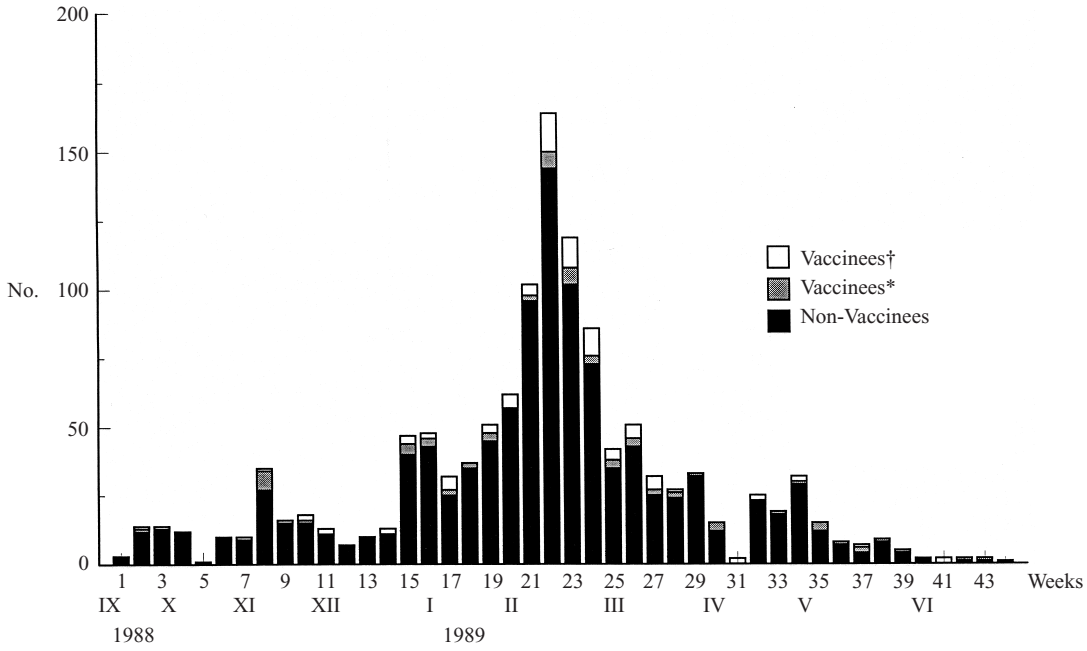


**Fig. 2.** Weekly number of reported measles cases during the last epidemic season in Finland, 1988–9 (*Schwarz and †MMR failure-notification programmes).

primary infection and the same number of sera from non-symptomatic subjects with past immunity of natural origin. In the patients with primary infection the avidity values were 0–13% (mean 4·1%) and in the past-immunity controls, from 22–93% (mean 48·2%). Based on these data and those of others with measles virus [12, 13, 15], and on longstanding experience with similar avidity-EIAs for other microbial pathogens [9, 22, 23], we defined values 26–100% as the high-avidity range, and values 0–15% as the low-avidity range. Values 16–25% were considered borderline, or equivocal. One vaccine failure with high avidity antibodies was bled 62 days after onset of measles, and was subsequently categorized as equivocal.

In the LA, HA and EQ avidity classes the occurrences of measles virus IgM-positive patients showing at least fourfold increases of measles-IgG titres in paired samples were 92/100 (92%), 22/28 (79%) and 10/14 (71%), respectively.

Additional internal validity assurance was done as follows. First, as the specific IgG response should be more rapid in cases with immunity than in a primary response [24], measles virus antibody responses among the HA and LA responders and the non-vaccinated reference patients were measured by a sensitive plaque neutralization (NT) assay [25] at three time points after onset of symptoms: (i) 0–5, (ii) 6–10 and (iii) 11+ days. The respective geometric mean NT titres in the HA patients were 3600 ($n = 9$), 3000 ($n = 5$) and not assessed ($n = 0$); in the LA group 60 ($n = 42$), 250 ($n = 34$), and 1000 ($n = 2$); and among the non-vaccinees 493 ($n = 10$), 1042 ($n = 9$) and 1871 ($n = 11$), respectively. Secondly, the HA responders should have milder disease than the LA responders or the non-vaccinees, and they had. Thus, avidity testing seems to yield results compatible with current understanding of the natural history of secondary vaccine failures [3, 5, 7, 24, 26–29].

## Mail survey of vaccine failures and non-vaccinated measles cases

In 1995, 6 years after the outbreak, a mail survey was performed to establish the natural course of measles and living conditions. Severity of measles was established by asking what was the peak body temperature during the illness, and how long (in days) the convalescence took. Subjects were also asked whether they had a rash during the course of the illness. The subjects were not reminded about their vaccination status nor were told the purpose of the study. Non-responders were contacted five times, questionnaires returned by 89 vaccinees and 169 non-vaccinees. The survey was done prior to avidity testing. The vaccinated non-responders ($n = 53$) were older (mean age = 11·5 years; median = 13·6 years) and had more often (45 vs. 36%) received a single

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Table 2. *Number of measles vaccine failures presenting with high avidity, low avidity, or with an equivocal immunological response*

| Failure group* (vaccination age) | High avidity | Low avidity | Equivocal | % High avidity (95% CI) |
|---|---|---|---|---|
| 1 × MMR (> 15 months) | 4 | 48 | 5 | 7 (0–14) |
| 1 × Schwarz (> 15 months) | 6 | 15 | 3 | 25 (8–42) |
| 1 × Schwarz (12–15 months) | 8 | 9 | 5 | 36 (16–56) |
| 1 × Schwarz (< 12 months) | 2 | 2 | 0 | 50 (1–99) |
| Re-vaccinees (10 months–19 years) | 8† | 1† | 0 | 89 (69–100) |

* All failures from low efficacy municipality omitted.
† Five received first vaccination (Schwarz-strain) at < 15 months, and three (Schwarz-strain) at > 15 months of age; in the former group both the mean and median time between vaccinations was 5·8 years (range 5·0–6·6), and in the latter group of three vaccinees the mean, median and range were 8·9, 11·1 and 2·5–18·9, respectively.
‡ Received first Schwarz-strain at < 15 months of age.

Table 3. *Self-reported disease severity of measles patients*

| | Vaccinees | | |
|---|---|---|---|
| Symptom | High avidity ($n = 17$) | Low avidity ($n = 64$) | Non-vaccinees ($n = 169$) |
| Fever °C | | | |
| Mean* | 38·6 | 39·3 | 39·7 |
| (95% CI) | (38·2–39·0) | (39·1–39·5) | (39·6–39·8) |
| Duration (days) | | | |
| Mean | 6·3 | 8·1 | 8·6 |
| SD | (5·1–7·5) | (6·9–9·3) | (8·1–9·1) |
| Rash (%) | 76 | 86 | 90 |
| (95% CI) | (56–96) | (77–94) | (85–95) |

* Mean fever between groups all different (Scheffé corrected $P < 0{\cdot}05$).

Schwarz-strain dose than those who returned the questionnaires (mean age = 9·6 years, median = 10·2 years). HA response was 50% more prevalent among the non-responders than among the responders.

### Statistical methods

The clinical and personal data among the vaccinated (LA and HA separated) and non-vaccinated patients were compared with the $\chi^2$ test and variance analysis. Multiple comparisons of continuous variables were corrected with Scheffé's conservative post hoc correction [30]. Statistical significance of time since last vaccination between different groups was assessed with the Mann–Whitney *U*-test [31]. The occurrence of HA responders was determined according to different levels of factors related to vaccine failure. Occurrence ratios (OR) and 95% confidence intervals (95% CI) were calculated [32]: 95% CI were also constructed for group means and proportions. Fisher's exact test was performed when appropriate [33]. Restriction was used to control for confounding.

## RESULTS

Among vaccine failure patients vaccinated in the 1970s with a Schwarz-strain virus at 12 months, between 12 and 15 months and at > 15 months, a high avidity (HA) response was present in 50% (2/4), 36% (8/22) and 25% (6/24), respectively (Table 2). Among those who had received a single MMR dose in the 1980s at > 15 months, an HA response was present in 7% (4/57).

Omitting re-vaccinees, those vaccinated at < 15 months and vaccine failure patients from one exceptional municipality, Schwarz-strain recipients had 3·6 (95% CI 1·1–11·5) higher occurrence of HA responses compared to MMR recipients.

Omitting the odd municipality, 89% (8/9) of the infected re-vaccinees had a HA response (Table 2). The first measles vaccination had been given before 15

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months of age in 63% (5/8) of the re-vaccinated HA responders.

The HA vaccine failure patients tended to have a longer interval since vaccination than the LA vaccine failure patients ($P < 0{\cdot}05$). The four single-dose MMR vaccine failure patients vaccinated at $> 15$ months and who presented with HA had had their vaccination within 1·2 years prior to measles infection. Mean, median and range of years since last vaccination of HA and LA responders were 8·7, 12·3, 0·5–13·9 vs. 6·5, 4·2, 0·7–14·3, respectively.

The re-vaccinated vaccine failure patients with HA had varying time periods (range 0·8 months–6 years) since last vaccination. In the exceptional municipality where even re-vaccinees had a high risk of measles and were as contagious as non-vaccinees, all 26 failures, including 8 re-vaccinees, showed LA responses ($P < 0{\cdot}002$).

The HA vaccine failure patients usually had a milder clinical measles than the LA responders and the non-vaccinees (Table 3). There was one thrice-vaccinated HA vaccine failure patient who had severe exanthematous disease with high fever ($> 39{\cdot}5$ °C) and pneumonia. This patient recovered slowly in 3 weeks. The proportions among HA responders, LA responders and non-vaccinees who graded their measles as 'mild flu-like disease' were 41% (7/17), 14% (9/64) and 4% (6/169), respectively ($P < 0{\cdot}001$). The contrast in clinical severity of measles between the vaccinated HA responders and the non-vaccinees was obvious when analyses were restricted to 14–16-year-old subjects only (data not shown). The LA responders' measles was of intermediate clinical severity, but when considering 14–16-year-old subjects only, the LA responders' disease was as severe as that of the non-vaccinees.

## DISCUSSION

A high occurrence of secondary vaccine failures was found among measles patients vaccinated over a decade ago and/or at an early age, especially among re-vaccinees. In one municipality, where even re-vaccinees had a high risk of measles [20], all patients presented with LA and probably lacked B-cell memory.

Secondary vaccine failures are probably more common than suggested by studies relying on specific IgM [26, 34]. Likewise, our results challenge a recent meta-analysis conclusion that in epidemic conditions nearly 0% of vaccine failures are of secondary type [35]. Nor were we surprised by the occurrence of nearly 50% secondary failures in a study [24] which identified secondary vaccine failures using an elaborate IgM/IgG ratio. However, neither vaccination ages nor intervals since last vaccination were disclosed in that study [24].

Vaccinees who were primed at too young an age were especially likely to have sub-neutralizing levels of humoral immunity against measles when they contracted it. This has been suggested by epidemiological reasoning, e.g. when West African children vaccinated at a very young age (even at 6 months) were subsequently infected, they tended to experience only mild measles and were also less contagious [5]. Very recently investigators have not only shown data supporting their previous findings but also have been able to show that sub-clinical infections occur and sustain epidemics among children who have been vaccinated early in life [36], thus further strengthening the potential importance of natural boosters in maintaining immunity. Before these results Danish investigators had shown in Greenland that a clinically not apparent epidemic among fully vaccinated individuals probably had explained their serological findings [37]. Thus, it seems quite possible that a single dose of measles-containing vaccine, on many occasions, does not necessarily yield life-long immunity without the boosting effect of natural infection [3].

Until very recently [36], there has been a lack of convincing evidence for waning immunity after measles vaccinations without the boosting effect of natural infection [3]. If waning immunity occurs, a higher occurrence of an HA response with increasing time since vaccination would be expected. This was the case in our study, with the notable exception that MMR vaccinees with an HA response were very recently inoculated. However, this phenomenon could result from selection bias or even differences in immunogenic properties of the Schwarz and MMR vaccines. However, it is widely believed that older vaccines were significantly less heat stable than those manufactured in the 1980s [3]. Thus, our findings not only raise concerns that immunity after measles vaccination might wane even among children vaccinated after 15 months of age in an affluent society, but also cast doubt on the common interpretation that old heat labile vaccines would have yielded a high primary vaccine failure rate [3].

Six very recently (re)vaccinated HA responders in this study and three re-vaccinees with known im-

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munity against measles described by others [7], all had mild measles soon after the (re)vaccination. Perhaps successful vaccination for full protection sometimes requires a longer period than assumed because of vaccine-induced immunosuppression [38].

Almost all the re-vaccinees except in the low-efficacy municipality were HA responders, probably since re-vaccination as a rule effectively corrects primary failures [39]. It is of concern that protection against measles is not 100% even after a successful series of vaccinations, but the risk of measles is considerably lower compared to that in single-dose recipients, and those who receive their first inoculation at $>15$ months might enjoy even smaller risk compared to those re-vaccinees who receive their first vaccination at 15 months [19].

In the single isolated rural community that experienced an explosive school-based outbreak, even the re-vaccinees had a high measles risk; all presented with LA and were contagious [20], which indicates that humoral immunity can remain absent even among re-vaccinees. However, vaccine failure patients in this low-efficacy municipality might have had some immunological memory [20], since T-cell responses against measles virus are better sustained and could explain certain peculiar characteristics of the outbreak in that community [38].

In conclusion, there is enough evidence for monitoring possible waning immunity in measles vaccine failure patients, particularly in areas where natural infection boosters are rare. As measurement of IgG avidity is increasingly being applied in vaccine research [13–15, 40, 41] and as our results were consistent, we believe that this method is a reliable and feasible tool for monitoring and studying the determinants of quality and duration of immunity after measles vaccinations.

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