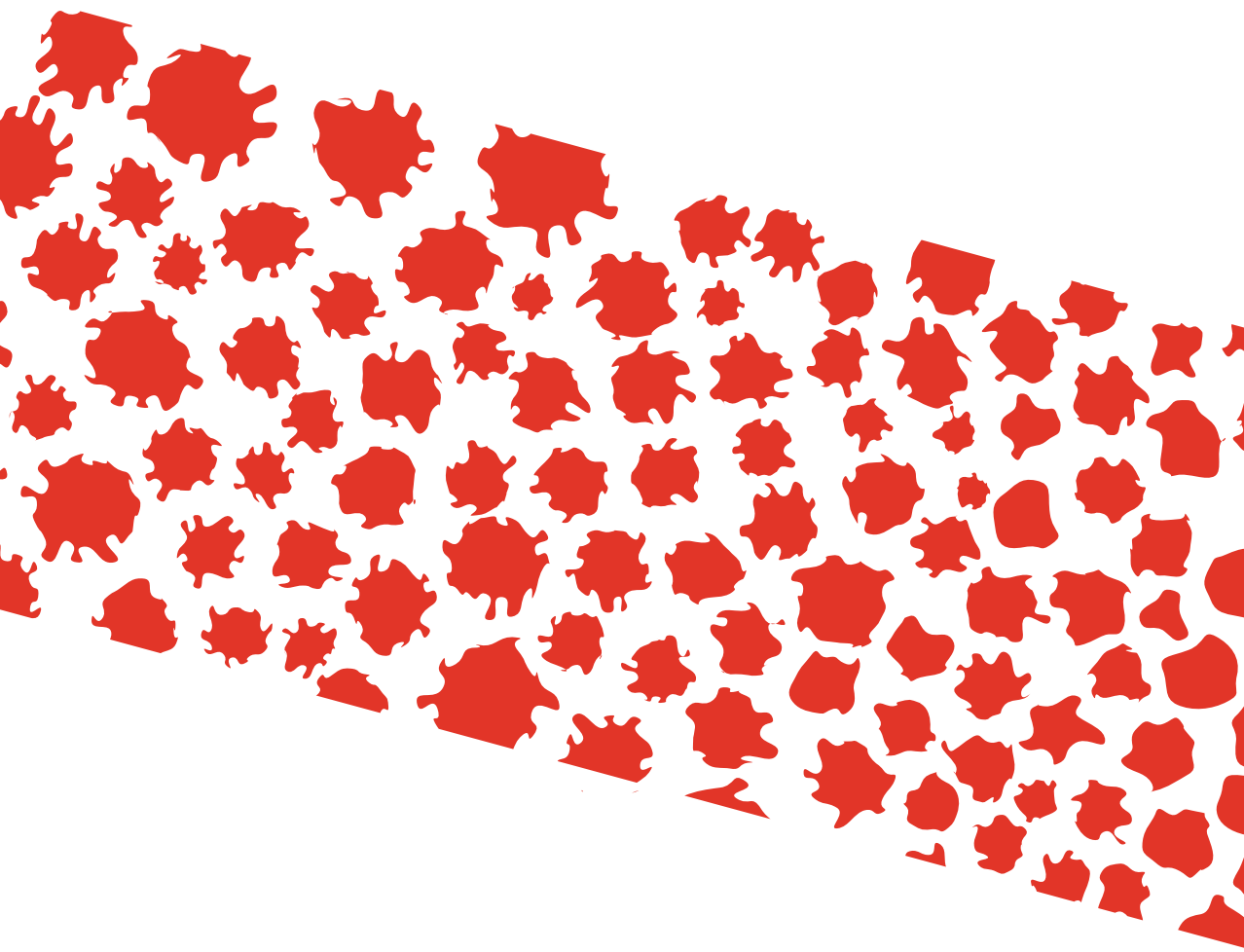
The 2013-2014 measles outbreak in the Netherlands:

New evidence on measles epidemiology and control



Tom Woudenberg

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Colofon

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De mazelenuitbraak van 2013-2014 in Nederland:

Nieuwe bevindingen ten aanzien van de epidemiologie en bestrijding van mazelen.

(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag

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Chapter 1

General introduction

Introduction

Measles is an infectious disease caused by measles virus. Measles virus is one of the most contagious pathogens known [4]. Symptoms generally consist of cough, fever, conjunctivitis, coryza, and a rash that appears 2-4 days after the first symptoms. In most cases, patients recover within 7 – 10 days after onset of disease. Common complications are otitis media (4% of reported cases), and pneumonia (6%) [5]. Measles encephalitis occurs in about 1-4 per 1000-2000 reported cases. Complication rates are higher in those below five and above 20 years of age [7]. Case fatality is approximately 0.05% in high-income countries to 5% in some African countries [8]. It is estimated that approximately 365 people, mostly children in developing countries, die every day due to measles, worldwide [9].

Measles incidence and outbreaks in the Netherlands

Between 2000 and 2017, the global annual incidence of reported cases of measles declined by 83%, from 145 to 25 cases per million population [10]. This success is due to the effort to increase the measles vaccination coverage. Globally, the coverage with the first dose of measles-containing vaccine (MCV1) showed a 13% increase to 85% in 2017. Approximately 90 – 95 % of children when vaccinated around 12 months of age, develop protective antibodies after one dose of MCV. A second dose is recommended by World Health Organisation (WHO) to protect children who failed to respond to the first dose [11]. The majority (>80%) of non-responders develop protective antibodies when revaccinated [11].

In the Netherlands, a single-dose measles vaccination programme was introduced in 1976 in the national immunisation programme for all infants at age 14 months. Since 1987, a two-dose programme using measles-mumps-rubella (MMR) vaccine is offered at ages 14 months and nine years. The birth cohort 1983-1985 was offered a catch-up programme at the age of four years (see Figure 1).

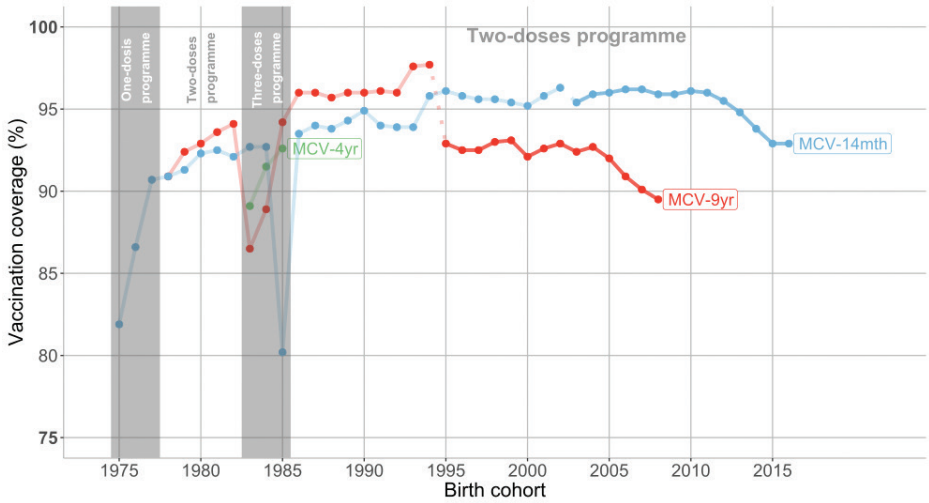

Figure 1. The vaccination coverage per vaccine and birth cohort in The Netherlands. Introduction of monovalent measles vaccination started in year 1976, vaccinating the birth cohort of 1975. The vaccination programme started with a one-dose programme, temporarily comprised of three doses, and currently comprises two vaccinations. Vaccination coverage from cohort 2000 for MCV at age 14 months and cohort 1995 for MCV at age 9 years, is reported based on the new information system Præventis, hence the interrupted lines. The vaccination status is measured at the individual age, and the vaccination coverage for MCV – 9yrs is the coverage for two doses rather one dose since 1995. Note that the y-axis starts at vaccination coverage of 75%. The data depicted is based on the annual vaccination report published by National Institute for Public Health and the Environment (RIVM) and is publicly available [12].

Since the early 90s, vaccination coverage for the first MMR dose was above 95% until birth cohort 2010. Subsequently, the vaccination coverage of both the MMR-1 and the combined coverage of two doses decreased (Figure 1). The last estimates of the MMR vaccination coverage for the first dose were around 93% (Figure 1) [12]. Coverage of two doses at the age of 10 was around 90% [12]. The coverage of the MMR-1 seems to stabilize around 93%. Vaccination coverage is, however, not homogenously distributed over The Netherlands (Figure 2). A region stretching from the south-west to the northeast in The Netherlands is characterized by low vaccination coverage. In some municipalities, vaccination coverage is even below 70% [12]. The low vaccination coverage is predominantly caused by individuals in the orthodox Protestant community who refrain from vaccination.

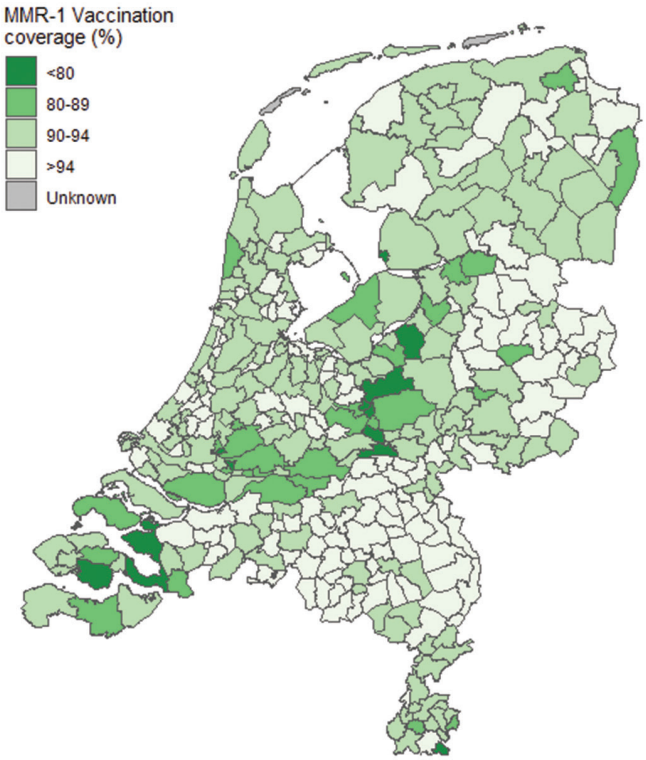

Figure 2. MMR-1 vaccination coverage at the age of 2 years for birth cohort 2016, by municipality in The Netherlands. The data depicted is based on the annual vaccination report published by National Institute for Public Health and the Environment (RIVM) and is publicly available [12].

The orthodox Protestant community comprises around 1% of the total population in the Netherlands [13]. Vaccine coverage in these communities is around 60% on average, but varies widely between churches, with coverage ranging from less than 30% among members of the most orthodox churches to vaccination rates comparable to the rest of the Netherlands in the least traditional churches [14]. In general, orthodox Protestants form close-knit communities. The majority (around 75%), live geographically clustered in the region known as the Bible belt. This region stretches from the south-west to the north-east of the country as can be seen in Figure 2 [12]. Children in these communities often attend orthodox Protestant primary and secondary schools.

Since the introduction of measles vaccination in the national immunisation programme, the occurrence of measles is notifiable by law. The yearly incidence

is visualized in Figure 3. The yearly incidence is low, except for some years with high measles transmission, mostly caused by outbreaks in the orthodox Protestant community [5,15]. Years with high incidences above 100 per million individuals were observed in 1976, 1977, 1988, and 1999 [5,15]. Since the outbreak of 1999/2000 [5], the incidence has remained below 5 per million population, except for 2008 [16] and 2013. A global incidence below 5 per million is one of the milestones towards global eradication of measles by WHO [17]. Despite the outbreaks, the number of measles deaths has remained limited in the Netherlands [18]. During the outbreak of 1999/2000, 3 persons died due to measles virus infection [5]. In addition to the mortality of measles during an outbreak, measles also leads to death cases that occur later after an outbreak [1]. These concern cases of subacute sclerosing panencephalitis (SSPE), a description of such a case can be found in Box 1.

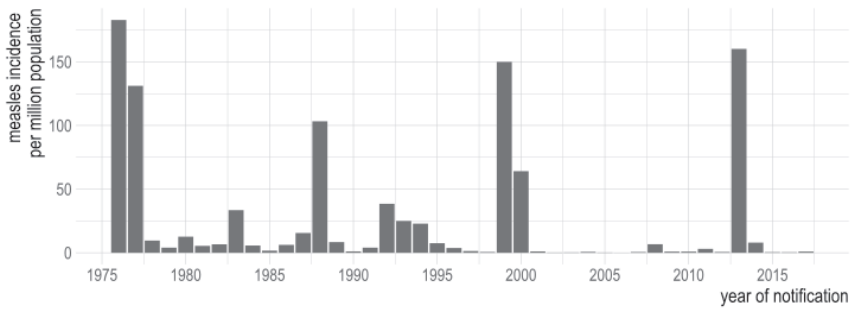

Figure 3. Measles incidence from 1976 until 2017 in The Netherlands. Data on measles cases is publicly available (https://www.volksgezondheidenzorg.info/onderwerp/ziekten-het-rijksvaccinatieprogramma/cijfers-context/mazelen#node-aantal-meldingen-van-mazelen), as well as population numbers (https://opendata.cbs.nl/statline/#/CBS/nl/).

Diagnosis of measles

The WHO clinical case definition for measles is a person with fever and maculopapular rash and cough, coryza or conjunctivitis. A typical case of measles is easily recognized during outbreaks. The diagnosis is, however, challenging when measles is rare and to clinicians who have not seen measles before [19]. Especially when measles cases present before onset of the rash. Laboratory confirmation is often by serology or by isolating measles virus [20]. The detection of measles-specific IgM in a specimen of serum or oral fluid is deemed to result from acute infection. Alternatively, acute infection can be confirmed with a four-times or higher increase in measles-virus-specific IgG antibody concentrations comparing acute and convalescent sera. The presence of IgG antibodies to measles virus in a single

serum specimen is evidence of previous infection or immunisation, which cannot be distinguished serologically. Measles can also be diagnosed by isolating measles virus in cell culture from respiratory secretions, nasopharyngeal and conjunctival swabs, blood, or urine by reverse transcriptase polymerase chain reaction (RT-PCR).

Box 1. A description of a case of subacute sclerosing panencephalitis in The Netherlands, 2013

A 17-year-old, previously healthy adolescent was taken by his parents to the Emergency Department [1]. Since a few weeks, he had been less talkative, behaving withdrawn and slow in thinking. He moved slowly and clumsily. He was not able to get dressed by himself. He experienced recurrent shudders occurring every minute, almost causing him to fall over. He was diagnosed with subacute sclerosing panencephalitis (SSPE). He was treated with carbamazepine as well as inosiplex and intramuscular interferon-beta 1a. After a relatively stable three months, a rapidly progressive deterioration occurred – within a week the patient became incontinent and comatose. He was admitted and treated with benzodiazepines for a short period of time, after which symptoms diminished. A few days after discharge, however, he died at home.

SSPE disease shows a relentless progression; only 5% of individuals with SSPE undergo spontaneous remission, with the remaining 95% dying within five years of diagnosis [2].

SSPE is a disease caused by measles virus and is vaccine-preventable. SSPE manifests itself after, on average, six years after the primary measles virus infection [2]. It is a very rare measles complication. Overall, 4 to 11 cases of SSPE are expected among every 100 000 cases of measles [3]. Incidence is, however, higher among children who contract measles virus aged less than five years (18/100,000). From 1976 until 1986, 77 cases of SSPE were registered in The Netherlands. More than 80% of these cases had experienced measles below the age of five [6].

During the measles outbreak of 1999-2000 in The Netherlands, the patient described above had experienced measles at the age of four. He was not vaccinated.

Antibody levels and protection against measles

Measles vaccines induce immune responses similar to natural measles virus infection [11]. Antibodies first appear between 12 and 15 days after vaccination and typically peak at 21 to 28 days. IgM antibodies appear transiently in blood, IgA antibodies are predominant in mucosal secretions, and IgG antibodies persist in blood for years. Vaccine-induced immunity, however, induces lower measles antibody concentrations than natural induced immunity [21,22]. While natural infection has been shown to provide life-long immunity [23], 2 – 10% of individuals with vaccine-induced immunity may not develop life-long protection [24-26]. Breakthrough infections can be either a primary vaccine failure or a secondary vaccine failure. Primary vaccine failure is the failure to respond to the vaccine and occurs in 5% of one-dose recipients when exposed to measles [11]. Secondary vaccine failure is defined as susceptibility due to a weak immune response or waning immunity after seroconversion. Most measles virus infections in twice-vaccinated individuals are considered secondary vaccine failures [27,28]. The

relevance of vaccine failures for measles control depends on the severity and infectiousness of measles in vaccinated individuals. The occurrence of measles cases in vaccinated individuals should therefore be monitored, with a particular focus on the severity and infectiousness of measles patients despite vaccination.

Seroprevalence studies and correlates of protection

Antibodies are presumed protective when the levels are above 0.12 IU/ml, the so-called correlate of protection. The correlate of protection has only been assessed by two studies. In the US [29], blood samples happened to be available before an outbreak of measles. Based on the clinical diagnosis of measles cases and prior antibody concentrations, the correlate of protection was determined to be 0.12 IU/ml [29,30]. This estimate was only confirmed once by a study conducted in Senegal [30]. Given the scarcity of estimates on the correlate of protection against measles, new studies need to be undertaken.

Seroprevalence studies are an essential guide in the evaluation of national immunisation programmes. Results of seroprevalence studies across all age groups and regions show the immunity of a population against a particular pathogen such as measles, and allow to identify risk groups [31]. In The Netherlands, the results of a large seroprevalence study in 8,000 individuals aged 0-80 years in 2005/6 showed that 96% of the general population was considered protected. Immunity levels above 95% are considered to provide herd protection against measles [32]. When immunity levels are high enough, transmission chains of infection will be disrupted and stop the spread of viral infection [33].

The seroprevalence study also predicted a future outbreak among orthodox Protestants; more than 50% of the orthodox Protestants below ten years of age were found susceptible to a measles virus infection. The seroprevalence study also highlighted the gap of immunity in infants from 6 to 14 months of age, less than 5% of infants aged 6 -13 months had protective levels of antibodies). While the majority of infants is susceptible at the age of 6 months after the decline of maternal antibodies [34,35], the first MMR is only given at 14 months of age. Other vulnerable groups might be specific age cohorts like the birth cohort 1972 – 1990 where seroprevalence, as measured by EIA, was found < 95%) [36]. However, there are uncertainties around the seroprevalence estimates measured by EIA, which can result in an underestimation of the protective seroprevalence.

The PRNT is regarded as the gold standard for measurement of neutralizing antibodies (standardized against a reference serum, currently the WHO 3rd international standard; NIBSC 97/648) [37,38]. PRNT measures antibodies that neutralize the virus. However, enzyme-linked immunosorbent assays (EIA) are the most widely used tests to measure measles-specific antibodies because results can be obtained more straightforward and cheaper. EIAs, however, have repeatedly been shown to display suboptimal sensitivity for detection of measles antibodies in cohorts with vaccine-acquired immunity [38-40]. Whereas PRNT measures neutralizing measles-specific antibodies, EIAs measure antibodies directed to a broader spectrum of measles virus proteins. Despite this suboptimal sensitivity, EIAs have been the preferred type of assay to study large number of samples in population-based seroprevalence studies [41], including in The Netherlands [36]. The suboptimal sensitivity could lead to overestimating the percentage of susceptibles in seroprevalence studies where the majority of individuals have vaccine-induced measles immunity [42,43]. Given that the majority of birth cohort 1972 – 1990 has vaccine-induced immunity, the susceptibility to measles for this cohort might be overestimated and should ideally be re-assessed with PRNT.

Timing of the first MMR vaccination

The timing of the first dose of measles vaccination, as suggested by WHO, depends on the measles transmission in a country [9]. In countries with high ongoing transmission, WHO advises administering the MCV1 at nine months of age due to the high risk of measles virus infection among infants. In countries with low levels of measles transmission, the MCV-1 is advised to be administered at 12 months of age. Vaccinating infants of 9-11 months results in lower vaccine effectiveness (84.0%) compared with vaccinations given at 12 months and older (92.5%) [44,45]. The reduced effectiveness is due to the immaturity of the immune system as well as the inhibitory effect of neutralizing maternal antibodies on developing an adequate immune response [46]. The decision for a specific age in the national vaccination program is thus largely dependent on the balance between reduced vaccine effectiveness at an earlier age and the risk of a measles virus infection prior to vaccination. As the majority of infants lost detectable antibody levels at six months [34,35] and the highest age-specific incidence is observed in infants below one year of age in Europe [47], vaccination campaigns offered to infants before nine months old has the potential to prevent cases of measles. Studies from high-income countries that assessed the vaccine effectiveness or the tolerability of MMR vaccination to infants below nine months are, however, lacking.

The 2014/2014 outbreak in the Netherlands and research of the thesis

Based on results from the seroprevalence study [36] and mathematical modelling [48], The Netherlands were at a high risk of a large measles outbreak. This outbreak started in May 2013. Two unvaccinated children attending an orthodox Protestant school were reported to have measles. The large epidemic that followed comprised 2700 reported cases. The epidemic peaked before the summer school holidays. The decrease in reported cases during the summer of 2013 could have been caused by a decreased reporting of measles but also by a decreased contact rate among susceptible schoolchildren. The epidemic ended in March 2014. In response to the outbreak, a national outbreak management team (OMT) gathered on 17 June 2013 and advised to implement several control measures including an early MMR offered to infants between 6-14 months of age. The rationale behind this control measure was the gap in immunity between 6 and 14 months old infants. Also, complication and hospitalisation rates are highest among infants. To evaluate the control measure as well as to assess whether a similar control measure should be implemented during future outbreaks, several studies were conducted to assess the uptake, adverse events and vaccine effectiveness. These studies would also contribute valuable information given the lack of vaccine effectiveness estimates among infants vaccinated at six months of age, as well as tolerability estimates.

Foreseeing a new outbreak among orthodox Protestants, we planned several research studies in order to

1. describe the epidemiology of the epidemic

2. estimate the underreporting of measles in The Netherlands during an epidemic in the Bible belt

3. determine the severity and infectiousness of vaccinated cases during the epidemic.

4. assess the uptake, the effectiveness and the side effects of an early MMR-vaccination administered to infants between 6 and 14 months.

5. assess the serological correlates of protection using a neutralisation assay among once vaccinated children.

6. estimate the seroprevalence in The Netherlands using the plaque reduction neutralisation assay.

7. quantify the disease burden of measles in monetary units in The Netherlands

8. estimate the reduction of measles transmission during annual school holidays

The studies that followed are discussed in this thesis. This **Chapter** concerned the introduction. In **Chapter 2**, we start with a detailed outbreak report of the measles epidemic 2013/2014. This outbreak report describes the course of the epidemic in relation to previous epidemics. The description of the outbreak is based on reported measles cases. As reporting of cases is subject to underreporting, we assessed the actual burden of measles in **Chapter 3** by estimating the completeness of reporting. In **Chapter 4**, we assess the severity and infectiousness of vaccinated cases observed during the epidemic. In response to the epidemic, infants of 6-14 months of age in high-risk areas were offered an early MMR. In **Chapter 5**, we assessed the uptake and its determinants of this intervention. In **Chapter 6,** we assessed the tolerability of this early MMR, and in **Chapter 7**, we assessed the effectiveness of this intervention, i.e. were vaccinated infants less likely to contract measles compared with unvaccinated infants during the outbreak? Protection against measles is determined by the level of antibodies against measles. In **Chapter 8**, we assessed what level of antibodies was needed to be protected against measles and subclinical measles among once vaccinated children of 4 to 8 years old during the epidemic. In **Chapter 9,** we assessed the seroprevalence of individuals born between 1972 and 1990 using the PRNT. This group was considered at risk for measles because of the relatively low seroprevalence. As the seroprevalence was measured with an EIA, while the PRNT is considered the golden standard in assessing the protective antibodies against measles, we re-assessed the seroprevalence of this birth cohort with the PRNT. Next to disease burden, we also estimated the economic burden associated with the measles epidemic in The Netherlands in **Chapter 10**. Last, in **Chapter 11**, we assessed what had caused the decrease in reported cases during the annual summer holidays. In **Chapter 12**, we re-assess the chapters using a different structure, namely by describing what was known before the study, our findings, and the added value. We end with general recommendations based on the work presented in this thesis.

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Chapter 2

Large measles epidemic in the Netherlands, May 2013 to March 2014: changing epidemiology

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Abstract

Since the early 1990s, the Netherlands has experienced several large measles epidemics, in 1992-94, 1999-2000, and in 2013-14. These outbreaks mainly affected the orthodox Protestants, a geographically clustered population with an overall lower measles-mumps-rubella first dose (MMR-1) vaccination coverage (60%) than the rest of the country (>95%). In the 2013-14 epidemic described here, which occurred between 27 May 2013 and 12 March 2014, 2700 cases were reported. Several control measures were implemented including MMR vaccination for 6-14-month-olds and recommendations to reduce the risk in health care workers. The vast majority of reported cases was unvaccinated (94%, n=2539), mostly based on religious grounds (84%, n=2135). The median age in the current epidemic was 10 years, 4 years higher than the previous epidemic in 1999-2000. A likely explanation is that the inter-epidemic interval before the 2013-2014 epidemic was longer than the interval before the 1999-2000 epidemic. The size of the unvaccinated orthodox Protestant community is insufficient to allow endemic transmission of measles in the Netherlands. However, large epidemics are expected in the future, which is likely to interfere with measles elimination in the Netherlands and elsewhere.

Introduction

Measles is a highly contagious infectious disease caused by measles virus. It can lead to serious illness, life-long complications and death [1]. Measles vaccination programmes have contributed to a steep decline in the number of infections and deaths, but in 2014 measles still caused an estimated 114,900 deaths worldwide, mostly in low income countries [2]. Case fatality is reported to be up to 6% in developing countries and is especially high in infants and young children [3].

In the Netherlands, a single-dose measles vaccination programme was introduced in the national immunisation programme (NIP) in 1976 for all infants at 14 months of age. Since 1987, a two-dose programme using measles-mumps-rubella (MMR) vaccine is offered at 14 months and nine years of age. Vaccine coverage of the first dose of MMR vaccination has been above 95% for 20 years [4]. Coverage for two doses at the age of 10 is around 93% during the past years. Introduction of measles vaccination in the Dutch NIP resulted in a large decrease in the number of reported cases [5]. However, epidemics still occur due to socio-geographically clustered individuals who refrain from vaccination. A large measles epidemic occurred in 1999-2000 with 3292 reported cases, of whom most were unvaccinated (94%) and orthodox Protestant (83%) [6]. Between 2001 and 2012 the incidence of measles was lower than the 5 cases per million which was set as a target by the WHO in 2010 [7], except for 2008 when the incidence was 6.7 per million caused by an outbreak among anthroposophic individuals [8].

The orthodox Protestant population comprises around 1.3% of the total population in the Netherlands [9]. The vaccine coverage in these communities is around 60% with a wide range from less than 30% among members of the most orthodox churches to vaccination rates comparable to the rest of the Netherlands in the least traditional churches [10]. In general, orthodox Protestants form close-knit communities. The majority of them, about 75%, live geographically clustered in the so-called “Bible belt”. In this region, stretching from the southwest to the northeast of the country, twenty-nine municipalities have MMR vaccination coverage less than 90% [11]. Children in these communities often attend orthodox Protestant primary and secondary schools. Some of these schools are known to have an MMR-1 and diphtheria-tetanus-pertussis vaccination coverage below 15% [12]. A serological survey carried out in 2006-07 confirmed the high risk of a large measles epidemic in these communities [13]. The seroprevalence was especially low in children 1-4 years of age (36%) and 5-9 years of age (63%).

The most recent epidemic started in May 2013 when two unvaccinated children attending an orthodox Protestant school were reported to have measles [14]. In response to this, a national outbreak management team (OMT) advised on 17 June 2013 an early MMR vaccination for infants aged 6-14 months of age living in municipalities with MMR-1 vaccination coverage < 90% [15]. These infants are too young to have been vaccinated in the regular schedule, but lost their maternal antibodies against measles [16] and are at highest risk for complications [17]. Parents of eligible infants were personally invited for this extra ('MMR-0' for 6-11 month-olds) or early ('MMR-1' for 12-14 month-olds) MMR vaccination. This intervention was implemented between July 2013 and February 2014. In total, 5,800 infants (57%) received an early MMR vaccination before 14 months of age.

Furthermore, the OMT advised to communicate via the media that children up to 19 years of age are entitled to receive a free catch-up MMR vaccination. This was also communicated through a newspaper and family magazines commonly used by orthodox Protestants, even though previous research showed low acceptance of catch-up vaccination among orthodox Protestants [18].

The OMT also advised to assess the immune status of healthcare workers (HCW) and to provide additional MMR vaccination when required [15]. HCW born before 1965 or vaccinated twice were considered protected, others were advised to complete their MMR vaccination schedule. All academic and community hospitals were approached by regular mail with the explicit request to bring the advice to the attention of the infection control committee.

Here we describe the epidemiology of the 2013-2014 measles epidemic in the Netherlands and compare it with the previous epidemic in 1999-2000.

Methods

Notification of measles

Measles is a mandatory notifiable disease in the Netherlands. Physicians and laboratories are required to report cases to Municipal Health Services (MHS). Directors of schools and day care centres are required to report clusters of rash in their institutions to MHS. For every reported case, a MHS physician or nurse is requested to complete a standardised questionnaire. The questionnaire covers, among others, demographic characteristics, disease onset dates, hospitalisation,

possible source, presence of complications, probable place of infection of infection, vaccination status, and reasons for non-vaccination. A possible source of infection is defined as contact with another reported case in 7 – 21 days before the start of the rash. Reasons for non-vaccination are pre-specified in the questionnaire and cases can be categorized in one of the following risk groups: orthodox Protestants, individuals with an anthroposophical attitude, individuals with a critical attitude towards vaccination, unknown or none of the pre-specified risk groups. The National Institute of Public Health and the Environment (RIVM) maintains an electronic web-based register for notifications by the MHS.

Case definition

Clinical measles is defined as fever and a maculopapular rash accompanied by at least one of the following three symptoms: cough, coryza, and conjunctivitis. Cases of measles are defined as clinical measles in a person with laboratory confirmed measles virus infection and/or an epidemiological link to a laboratory confirmed case. A case is epidemiologically linked if the individual had contact with a laboratory confirmed case in the three weeks before onset of disease. Laboratory confirmation is based on positive measles-specific IgM serology and/or detection of measles virus RNA by polymerase chain reaction (PCR) in a throat swab, oral fluid or urine specimen [19]. Individuals presenting with severe illness were advised to be rapidly diagnosed, which was mostly done by testing for measles-specific IgM. In other cases, the use of less invasive sampling of oral fluid was recommended, which comprised 60% of the specimens forwarded to the national laboratory for PCR testing, the remainder were throat swabs or urine specimens. The majority of PCR positive specimens were selected for genotyping using primers amplifying the N-terminal 450-nucleotide fragment of the measles nucleocapsid gene, according to WHO-approved sequencing methods for genotyping as previously described [20]. In case of successful and complete sequencing results, genotypes were generated and representative sequences were reported to the WHO/MEANS database.

From mid-July 2013 onwards, MHS located in the Bible belt were advised by the RIVM to limit the use of laboratory diagnostics of measles to cases with complications, vaccinated cases, cases in newly affected schools/villages/risk groups, and cases that were reported by general practitioners (GPs) without an epidemiological link.

We included in our analyses all cases reported between 27 May 2013 and 12 March 2014, respectively the first and the last case with a confirmed infection with the

predominant outbreak strain. Imported cases and cases with a genotype or strain other than the outbreak strain were excluded. Cases that were epidemiologically linked to excluded cases were also excluded.

Population data was retrieved from Statistics Netherlands. Vaccine coverages per municipality and postal code area were available from the national vaccination register. Proportions were compared using the Chi-squared test or the Fisher's exact test. The age distributions of both epidemics were compared using the Kolmogorov-Smirnov test. To test differences in medians we used the Mood's median test. All analyses were performed using R software version 3.1.0. Maps were created with ArcGIS version 10.2.2.

Results

Outbreak description

Overall, 2766 measles cases were reported between 27 May 2013 and 12 March 2014. Molecular typing of the outbreak strain showed a genotype D8 measles virus (strain MVs/Alblasserdam.NLD/22.13, WHO/MEANS Id 50730, Genbank Id KM066606), with a sequence indistinguishable from the strain that was first identified in the UK in 2012 (MVs/Taunton.GBR/27.12, WHO/MEANS Id 23447, Genbank Id JX984461). Two percent (n = 66) of the cases were excluded based on a different genotype (n = 11) or were imported (n = 25). Epidemiologically linked to these different genotypes and importations were 20 and 10 cases, respectively. Of the 11 different genotypes found, 10 were genotype B3 and one genotype H1. We included the remaining 2,700 cases in our analyses.

The first two cases were reported on 27 May 2013 in two unvaccinated children attending the same orthodox Protestant primary school. These children had not travelled abroad and the source of the measles infection was unknown. The epidemic peaked in the second week of July 2013 with 180 reported cases, with a subsequent rapid decline during school holidays in July and August 2013 (Figure 1). Coinciding with the new school year, from September 2013 onwards, reported cases increased until another peak of 122 cases occurred in the third week of October. Subsequently, the number of cases per week declined. The last case was reported on 12 March 2014.

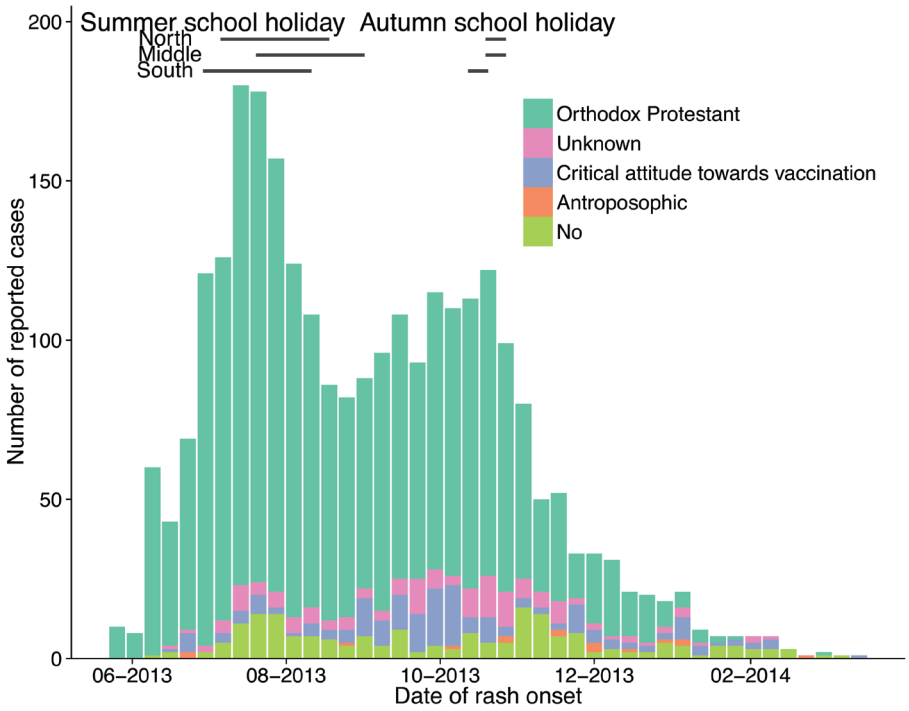

Figure 1: Reported measles cases by risk group and week of onset of rash[a] in the Netherlands, reported between 27 May 2013 and 12 March 2014 (n=2700). a) If the first day of onset of rash was not available, the date of the onset of the prodromal phase + three days was used [17]. Horizontal lines indicate the timing of school holidays, which are different per region.

The vast majority of reported cases was unvaccinated (94%, n = 2539) (Table 1), mostly based on religious grounds (84% of unvaccinated cases, n = 2135). Others who refrained from vaccination were anthroposophic individuals (1%, n = 16), had a critical attitude towards vaccination (7% , n = 172) or had other reasons to refrain from vaccination (4%, n = 108). Of vaccinated cases (n = 141), 89% (n = 125) was vaccinated once, 11% was vaccinated twice (n = 15), and one individual was vaccinated thrice (0.1%) (Table 1). Sixty-eight percent (n = 85) of the 125 once-vaccinated cases was between 14 months and eight years of age and among those, 49% (n = 61) was between 4-8 years of age. The majority of twice-vaccinated cases was older than 18 years of age (87%, n = 13).

Table 1: Reported measles cases by vaccination status (n = 2700), hospitalisation (n = 2677, 23 unknown) and complications (n = 2581, 119 unknown) during a measles epidemic, the Netherlands, May 2013 – March 2014.

	No. (%)[a] reported cases by age group							
	0-13 months	**14 – 48 months**	**4 - 8 years**	**9 – 17 years**	**18 - 40 years**	**> 40 years**	**Total**	**p value[b]**
Vaccination status	n = 78	n = 260	n = 824	n = 1268	n = 226	n = 44	n = 2700	
None	75 (96)	236 (91)	760 (93)	1246 (99)	183 (81)	39 (89)	2539 (94)	
Once	3 (4)	24 (9)	61 (7)	16 (1)	20 (9)	1 (2)	125 (5)	
Twice or more	0 (0)	0 (0)	0 (0)	3 (0)	13 (6)	0 (0)	16 (1)	
Unknown	0 (0)	0 (0)	3 (0)	3 (0)	10 (4)	4 (9)	20 (1)	
Complication status	(n = 75)	(n = 247)	(n = 787)	(n = 1208)	(n = 221)	(n = 43)	(n = 2581)	
All complications	12 (16)	41 (17)	108 (14)	111 (9)	17 (8)	7 (16)	296 (11)	<0.01
Pneumonia[c]	8 (11)	23 (9)	54 (7)	61 (5)	12 (5)	3 (7)	161 (6)	0.07
Otitis Media[c]	4 (5)	16 (6)	48 (6)	41 (3)	4 (2)	0 (0)	113 (4)	<0.01
Encephalitis[c]	0 (0)	0 (0)	1 (0)	1 (0)	0 (0)	0 (0)	2 (0)	NS
Dehydration/ diarrhoea[c]	0 (0)	4 (2)	8 (1)	12 (1)	3 (1)	3 (7)	30 (1)	0.07
Other[cd]	0 (0)	0 (0)	3 (0)	2 (0)	1 (0)	1 (2)	7 (0)	NS
Hospitalisation status	(n = 77)	(n = 257)	(n = 819)	(n = 1254)	(n = 226)	(n = 44)	(n = 2677)	
Hospitalision	8 (10)	24 (9)	51 (6)	55 (4)	32 (14)	11 (25)	181 (7)	<0.01

a Proportion of the number of reported cases by age-group
b Fisher's exact test or $\chi 2$ test; NS, not significant
c Individual cases could have had multiple complications. For example: Five cases had both otitis media and pneumonia
d Other complications comprised bilateral striatal necrosis (n=1), hepatitis (n=1), keratitis (n=1), stomatitis (n=1), tonsillitis (n=2), and transverse myelitis (n=1). Respiratory infections other than pneumonia were not included in category other complications.

The epidemic mainly affected low vaccination coverage areas. Nearly half (49%) of reported cases occurred in the 29 municipalities with vaccination coverage below 90% (range 59.6 – 89.8). In total, 41% of 408 municipalities (n = 169) reported at least one case. Within municipalities, there was a considerable heterogeneity in vaccination coverage and incidence by postal code area (Figure 2A and B). The incidence of reported cases by postal code area increased with a lower MMR-1 vaccination coverage (Figure 2C; Spearman's correlation coefficient: -0.42).

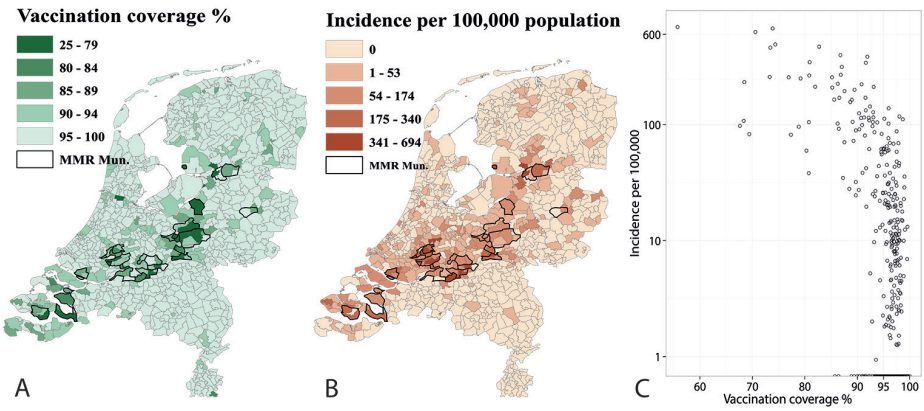

Figure 2: A) MMR-1 vaccination coverage[a] combined for birth cohorts 2011/2010/2009 at the age of two years by three digit postal code. B) Measles incidence from reported cases from May 2013 until March 2014 (n=2689, 11 missing data for location) by three digit postal code in the Netherlands. C) Scatterplot (log-scale) of three digit postal code areas' vaccination coverage and reported measles incidence.a) Data on vaccination coverage per postal code area were obtained from the national vaccination register. MMR Mun. are those 29 municipalities where the early MMR vaccination campaign was conducted. In the municipality in the far southeast of the Netherlands, Vaals, a considerable number of the infants receive their vaccinations in Germany and are therefore not registered in the Dutch vaccination register

The median age of reported cases was 10 years (range 0-68 years). Most reported cases were between four and 17 years of age (n = 2092, 77.4%) (Table 1). Three percent of the cases (n = 78) were under 14 months of age. Of these 78, three were vaccinated once prior to onset of disease. Six cases were below six months of age (0.2%). Highest incidence rates were found in 4-8 year-olds and 9-12 year-olds (88.8 and 87.9 cases per 100.000, respectively) (Figure 3). Males and females were equally affected (1355 out of 2684 cases with known sex were female (50.5%)).

Laboratory results

About a third (n=888, 32.9%) of reported cases were laboratory confirmed; all other cases were reported based on an epidemiological link. Most laboratory confirmed

measles cases (84%, n = 749) were confirmed using PCR testing of oral fluid or urine specimens. Another 13% (n = 116 cases) were confirmed by detection of measles-specific IgM antibodies in serum. In 2% of the cases (n = 16), both IgM and PCR test results were reported. For one percent of the cases (n = 7), the diagnostic test was not reported. Out of the 749 PCR confirmed cases, 73% (n = 548) were sent to the national laboratory for sequencing. In 7% (n = 39) the sequence could not be identified, 93% (n = 509) was sequenced the D8 measles virus (MVs/Alblasserdam.NLD/22.13).

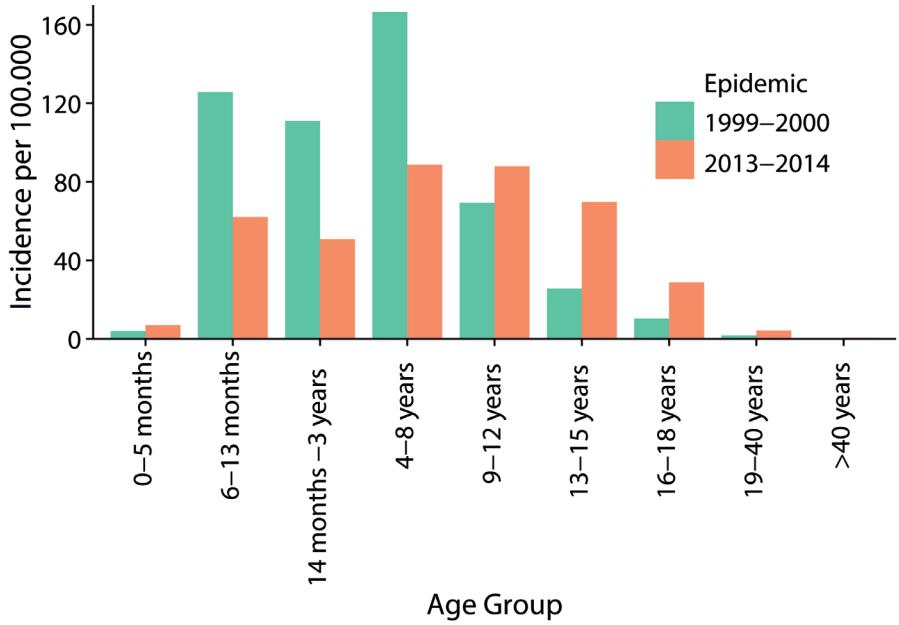

Figure 3: Incidence of reported cases by age group[a] for the 1999-2000 epidemic (n=3170) and the 2013-2014 epidemic (n=2700), the Netherlands. a) Age was calculated by subtracting the date of birth from the date of onset of rash. For individuals older than two years, only the year of birth was known. These cases were assumed to be born on June 30 of the reported year of birth. For cases younger than two years, the month of birth was known but the day of birth was lacking, these cases were assumed to be born on the 15th day of the reported month of birth.

Complications and hospitalisation

For 11% of the cases (n = 296) one or more complications were notified (Table 1). The occurrence of complications was unknown for 4% of the cases (n = 119). More than half of the cases with complications had pneumonia (54%) and about a third otitis media (38%). The risk of complications was highest below four and above 40 years of age (both 16%). Otitis media was especially prevalent among 14-month- to 3-year-olds (6%). Pneumonia occurred most frequently among cases younger than

four years of age (10%). Two reported cases were hospitalised with encephalitis: a 17-year-old girl and an 8-year-old boy. The girl had severe underlying medical conditions and died due to encephalitis and pneumonia.

Overall, seven percent of the cases (n = 181) was hospitalised, most commonly for pneumonia (48%, n = 86) or dehydration/diarrhoea (15%, n = 27). For one percent (n = 23) hospitalisation was unknown. Seven cases required intensive care admission for pneumonia (n = 5), encephalitis (n = 1) or both (n = 1). The median duration of stay in the hospital due to measles was four days (interquartile range 3 – 5 days). Adults with measles were at higher risk to be hospitalised than children (Table 1).

Health Care Workers

In total, 19 HCW were reported to have acquired measles at work. Two of these were born before 1965 and were unvaccinated. Eight of the HCW with measles were born between 1965 and 1975, of whom only one was vaccinated (one dose). Of the four HCW born in 1975, 1976 and 1977 (these cohorts were offered only one vaccination in their childhood), three were vaccinated once and one was unvaccinated. Five HCW were born after 1978, of whom two were unvaccinated and three were vaccinated at least twice. Most infected HCW were working at a general practice (eight HCW) and three HCW acquired measles while working in a hospital. There were no reports of infected HCW transmitting measles to patients or other HCW, nor reports from patients infected in the hospital.

Comparison with the 1999-2000 epidemic

The 2013-2014 epidemic was comparable with the 1999-2000 epidemic in that it took place in the same low vaccination coverage areas and affected mostly the unvaccinated orthodox Protestant population. The age distribution of the epidemics, however, differed markedly (Figure 3). First, the median age in the 1999-2000 epidemic was six years [*6*], compared with 10 years in the recent epidemic (p-value < 0.01).

Second, the incidence by age group of the two epidemics differed ($p < 0.01$). Older age groups (nine years and older) had a higher incidence in 2013-2014 than in 1999-2000, while the incidence in age groups below nine years of age were halved in 2013-2014 compared with 1999-2000. Among infants aged 6-13 months of age, who were offered an early MMR vaccination in 2013-2014 but not in 1999-2000, the incidence in 2013-2014 was 62.1 per 100.000. This is significantly lower than the incidence of 126 per 100.000 reported in this age group in 1999-2000 ($p < 0.05$).

In contrast, the incidence in infants below six months was higher in 2013-2014 than in the 1999-2000 epidemic (6.9 and 3.9 per 100,000, respectively) (p = 0.529).

Discussion

Despite an MMR-1 vaccination coverage above 95% for the last 20 years in the Netherlands, a large measles epidemic of 2700 reported cases, including cases with severe illness and one death, occurred among the socio-geographically clustered orthodox Protestant communities with low vaccination coverage. The total costs of the epidemic were recently estimated at €3.9 million [21].

In comparison with the previous epidemic in this group in 1999-2000, older age groups were more affected. There was a striking decline in reported cases during the summer holidays, which could be due to reduced transmission of measles and/or reduced reporting. The change of guidelines communicated by the RIVM to the MHS in mid-July to reduce the workload may also have had influence on reporting.

The vast majority of reported cases was among unvaccinated orthodox Protestant individuals. The number of cases in other risk groups remained relatively low, which suggests limited contact with orthodox Protestants and more protection by herd immunity. Of the 141 vaccinated cases, most were among once vaccinated children between four and eight years of age. Advancing the second MMR dose from 9-year-olds to 4-year-olds can reduce the susceptibility in this age group [22].

A limitation of our study is that it was based on reported cases only. Following the 1999-2000 epidemic it was estimated that only 7% of all individuals with measles were reported [23]. Another study carried out a survey after the epidemic and found 164 measles cases, among those cases only 9% (n = 15) was reported during the 1999-2000 epidemic [24]. We found similar completeness of reporting of measles infections in this measles epidemic using also capture-recapture methods (T. Woudenberg, data not shown). Based on this, the estimated number of individuals with measles infection in the 2013-2014 epidemic is approximately 30,000. The use of non-invasive samples such as saliva and urine for measles diagnosis contributed to a higher proportion of infections that was laboratory confirmed or could be epidemiologically linked to a confirmed infection, and hence to a more complete reporting.

Eleven percent of all reported cases had one or more complications. Similar to other epidemics [6, 25-27], complications and hospitalisations were more likely to occur in young children and adults [17]. Cases with complications and/or hospitalisations were probably more likely to be reported than cases without complications, thus the true rate of complications and hospitalisations among all measles infections during this epidemic is likely to be lower than the 11% and 7% we found in reported cases, respectively.

A rare complication of measles, subacute sclerosing panencephalitis (SSPE), occurs months to years after measles infection. Recently, a case of SSPE was reported in a Dutch 17 year old who died four months after diagnosis [28]. He had acquired measles in the Netherlands during the epidemic of 1999-2000 at the age of four years. SSPE is a very rare fatal complication of measles, estimates of SSPE incidence are approximately 0.4 – 1.1 cases of SSPE per 10.000 cases of measles [29]. Assuming 30,000 individuals acquired measles virus infection in the 2013-2014 epidemic, up to three cases of SSPE can be expected in the next two decades.

High measles vaccination coverage among HCW has been associated with decreased health-care-associated measles virus infections among patients and personnel [30]. During this measles epidemic 16 out of 19 HCW with measles were incompletely vaccinated although they were eligible to complete their MMR vaccination schedule according to the advice of the OMT. An assessment of the barriers to the implementation of the recommendations is ongoing.

Compared with the previous epidemic in orthodox Protestants, we found a higher median age in the 2013-2014 epidemic and higher incidence rates in age groups above eight years of age. This is likely due to the longer inter-epidemic interval before the 2013-2014 epidemic compared with the interval before the 1999-2000 epidemic [31]. The epidemic preceding the 1999-2000 epidemic was in 1992-1994, whereas the epidemic preceding the 2013-2014 epidemic was in 1999-2000. As a result, the population susceptible, consisting of individuals born after the previous epidemic, consisted of a wider age range in 2013 than in 1999.

The cause of the lower incidence in children below nine years in the 2013-2014 epidemic compared with the 1999-2000 epidemic may be due to an increase in the vaccination coverage among children below nine years old in orthodox Protestant communities. Evidence for this was found in the serological surveys performed in 2006-2007 and 1995-1996, in which a higher proportion of diphtheria protection

was found in the most recent survey [32]. Second, vaccination-uptake among orthodox Protestants seems to be increasing between subsequent generations, as found in 2013 by assessing vaccination status of orthodox Protestants in the age of 18-40 years, their parents and their children (W.L.M. Ruijs, data not shown). An increasing vaccination coverage within these communities may also explain the longer inter-epidemic period [31] and, at least partly, the higher median age. The distribution of cases comprises a smaller proportion of young cases compared to the previous epidemic.

The lower incidence among infants 6-13 months of age could reflect the administration of early MMR vaccination. However, results are difficult to interpret given that the incidence was also relatively low in the adjacent older age groups. The incidence in infants below six months was higher in 2013-2014 than in the 1999-2000 epidemic. This is likely to be related to the lower level of maternal antibodies in children born to vaccinated mothers compared with children born to unvaccinated mothers [16]. In 1976, measles vaccination was started in the Netherlands. Therefore, in 2000, the proportion of infants born to vaccinated mothers was probably lower than in 2013.

The source of the first measles cases from this outbreak is unknown. According to the MEANS database, the Taunton sequence was first identified in Wales, United Kingdom, in the second half of 2012, and subsequently in many other cities in the UK throughout 2012 and the first half of 2013. At the time when the first Dutch case was identified with the Taunton sequence in May 2013, about 900 identical sequences had been reported to MEANS, not only from UK but several other countries within the European region (e.g. Italy, France, Ireland, Austria, Russian Federation). Therefore, a particular source country is hard to identify [33, 34]. The epidemic in the Netherlands, however, was indicated as the origin of outbreaks in Belgium [35], Canada [36, 37] and onwards in the US [38]. The likely spread to Belgium led to an outbreak in a day care centre with 33 reported cases. In Canada an outbreak took place in Alberta with 43 reported cases and another in British Columbia with 444 reported cases. Social ties exist between orthodox Protestants in the Netherlands and Canada and the spread of infections such as poliomyelitis, measles, mumps, and rubella to Canada has been reported before [39].

Improved vaccination coverage among orthodox Protestants is essential to prevent future outbreaks. It is therefore one of the prioritized interventions in the national measles elimination plan of the Netherlands [40]. Since orthodox

Protestants base their vaccination decisions largely on religious arguments [41], specific information materials were developed focusing on religious arguments for and against vaccination. These brochures aim to facilitate decision making about vaccination among orthodox Protestants and were distributed during the epidemic [42]. An evaluation of their acceptability and impact is currently ongoing.

Apart from this intervention, the vaccination coverage seems to increase in the orthodox Protestant community. An improvement of the vaccination coverage will be reflected in a different epidemiology of future epidemics. As seen in the current epidemic, where a longer inter-epidemic period resulted in older age groups affected in comparison to the previous epidemic.

The number of individuals refraining from vaccination is insufficient to sustain endemic measles transmission in the Netherlands. Nevertheless, this situation poses a risk to public health in the Netherlands and contributes to the worldwide spread of measles, thus forming an impediment to the elimination of measles in Europe and elsewhere.

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Chapter 3

The tip of the iceberg: incompleteness of measles reporting during a large outbreak in The Netherlands in 2013–2014.

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Abstract

Measles is a notifiable disease, but not everyone infected seeks care, nor is every consultation reported. We estimated the completeness of reporting during a measles outbreak in The Netherlands in 2013–2014. Children below 15 years of age in a low vaccination coverage community (n = 3422) received a questionnaire to identify measles cases. Cases found in the survey were matched with the register of notifiable diseases to estimate the completeness of reporting. Second, completeness of reporting was assessed by comparing the number of susceptible individuals prior to the outbreak with the number of reported cases in the surveyed community and on a national level. We found 307 (15%) self-identified measles cases among 2077 returned questionnaires (61%), of which 27 could be matched to a case reported to the national register; completeness of reporting was 8.8%. Based on the number of susceptible individuals and number of reported cases in the surveyed community and on national level, the completeness of reporting was estimated to be 9.1% and 8.6%, respectively. Estimating the completeness of reporting gave almost identical estimates, which lends support to the credibility and validity of both approaches. The size of the 2013–2014 outbreak approximated 31400 measles infections.

Introduction

Measles virus is highly contagious. Measles disease is characterized by fever, cough, coryza, conjunctivitis, and a maculopapular rash lasting 3–5 days [1,2]. It also causes an immunosuppression that can last up to 2 years [3]. This immunosuppression leaves patients with measles susceptible to other pathogens [2], particularly in the respiratory tract. Pneumonia, caused by other pathogens or measles virus itself, is the most common fatal complication, occurring in 56–86% of measles-related deaths [4].

Worldwide, increased measles vaccination coverage caused a decline in the number of reported measles cases from 853 479 in 2000 to 254 928 in 2015 [5]. These numbers of reported cases are, however, incomplete: not every infected individual seeks care and not every consultation leads to a reported case [6].

The notification of only a fraction of measles cases may suffice to monitor transmission of measles and identify outbreaks [7], but it will result in biased estimates for risk of infection and risk of developing severe disease or death upon infection. Incomplete reporting will also result in an under- estimate of the true number of infections, which is an essential indicator in the context of measles elimination. If reporting is associated with certain characteristics of cases, underreporting may result in biased estimates of these.

So far, two approaches have been used to assess completeness of measles reporting. A first approach uses community-based surveys to identify measles cases, and then assess how many of them are reported to a register. This survey approach has been used as early as 1926 in the USA [8]. Since then a few other community-based surveys have been published worldwide, reporting that notified cases could range from 3% up to 64% of total infections [6,9]. These surveys, however, all originate from the 1900s [8,10-13] and lack laboratory confirmation of cases. The other approach used to assess the completeness of measles reporting involves comparing the number of cases reported with the number of people projected as susceptible and assuming that almost all are infected [14]. This approach resulted in an estimated completeness of reporting ranging from 7% for a measles outbreak in 1999–2000 in The Netherlands [15], up to 63% for endemic measles in England and Wales in 1946–1979 [14].

We used both approaches to assess completeness of reporting during the most recent measles outbreak in The Netherlands, which took place between May 2013 and March 2014, spread mainly among orthodox Protestant school-aged children [16], and consisted of 2700 cases reported to the national register of notifiable diseases. Orthodox Protestants form a socially and geographically clustered minority group in the Netherlands of about 250.000 individuals among whom vaccination coverage is approximately 60% [17]. In addition to measles outbreaks, this group has seen outbreaks of polio (last in 1992), rubella (last in 2004), and mumps (2008). Here, we used a community-based survey including laboratory testing of self-reported cases. In addition, we calculated the number of susceptible individuals in the community and nationally, and compared this with the number of reported cases to the national register.

Methods

National register of notifiable diseases

Measles is a notifiable disease in The Netherlands. Physicians and laboratories are required to report cases to the national electronic web-based register for notifiable diseases (Osiris) through local Municipal Health Services (MHS). Directors of schools and day-care centres are required to report clusters of children with rash in their institutions to MHS. Cases of measles are defined as clinical measles in a person with either laboratory-confirmed measles or epidemiologically linked to a laboratory confirmed case. Criteria for clinical measles are fever and a maculopapular rash accompanied by at least one of the following symptoms: cough, running nose, and red eyes.

Community-based survey

The study population consisted of all children born between 2000 and 2013 and living in the municipality of Rhenen (Figure 1A). These children were surveyed through a questionnaire in the third trimester of 2014. The survey was limited to this age group because it comprised most (76%) of the reported cases [16] and further, children born during this range of years could not have been infected in the previous outbreak in 1999–2000 [18]. The survey took place in the municipality of Rhenen [12], which had 19 116 inhabitants on January 1, 2014 (http://statline.cbs.nl). Rhenen is located in a region characterized by low vaccination coverage (known as the Bible belt), with a measles-mumps-rubella (MMR) vaccination coverage of 80% for the first dose at 14 months of age [19]. This is substantially lower than

the national vaccination coverage, which is approximately 95% in The Netherlands (Figure 1A).

In cooperation with the municipality of Rhenen and the MHS, we obtained the children's names and their addresses, where we mailed the questionnaires. Parents were asked to fill in the questionnaire on behalf of their children. The questionnaire could be returned either by regular mail or online. After 3 weeks a reminder was sent. The questionnaire ascertained date of birth, school, any history of measles infection and vaccination status. A measles virus infections was defined as having a red rash on the skin and fever possible accompanied with red watery eyes, coughing or running nose. The school was of interest because The Netherlands has schools of various denominations, including those with an orthodox Protestant denomination. Because there is a relationship between religion and vaccination behavior, information about school attendance of respondents gives an indication which groups in terms of religion and correspondingly vaccination behaviour will participate in our study.

Parents who reported that their child had a history of measles were requested to answer additional questions about the date of onset (month of first day of illness), general practitioner (GP) consultation, hospitalization, and complications, e.g., otitis media, pneumonia, encephalitis, diarrhea, other, or none. Parents who reported measles symptoms for their child were asked whether they were willing to donate a saliva sample from their child to test its immune status against measles.

Completeness of reporting and determinants

We matched the cases found in the questionnaire survey and those found in the national register by name, address, and date of birth at the MHS. We divided the matched cases by the total number of cases found in the questionnaire survey to estimate the completeness of reporting. We calculated a binomial proportion confidence interval using the Wilson score method.

We also assessed determinants of reporting: GP consultation and hospitalization, and variables such as birth cohort, date of onset, and sex that could provide insight as to the actual epidemiology of reported measles cases compared with unreported measles. The study participants were categorized by year of birth in three groups: 2000 – 2004, 2005 – 2008, and 2009 – 2013. Date of onset was dichotomized into groups of equal size. To discover whether reported cases were different from unreported cases, we used either Pearson's chi-squared test or Fisher's exact test

to compare proportions between groups. For all the analyses, we used R (version 3.2.0).

Laboratory testing

Parents who were willing to have their child's saliva tested received a measles saliva test kit and an additional questionnaire. They were instructed to collect a saliva sample of their child by gently rubbing a swab (a small sponge on a stick) in the subgingival area for about 1 minute. The sponge absorbs approximately 0.5 ml of crevicular fluid during this period. The swab was then sealed in a tube and transported at ambient temperature by posting the reply-paid envelope to the laboratory at the National Institute for Public Health and the Environment.

Saliva specimens were tested for measles-specific IgG antibodies using a measles-specific IgG capture enzyme immunoassay (EIA) developed by Microimmune Ltd. This assay has been reported to show good concordance with serum IgG results in detecting measles-specific IgG antibodies in both vaccinated populations [20,21] as well as in largely unvaccinated populations [22]. This assay does not distinguish between measles-specific IgG antibodies from a natural infection and those from a measles vaccination.

The additional questionnaire sent along with the measles saliva test kit consisted of one additional question assessing whether the participant was a first or subsequent measles case in the household.

Reconstruction of susceptible school-aged children in the community

Our second approach to assess completeness of reporting was by estimating the number of susceptible individuals in the most affected group prior to the outbreak, assuming that almost all will be infected, and to compare this number with the number of reported cases from this group. Those most affected during the outbreak in the municipality of Rhenen were from a group of unvaccinated orthodox Protestant school-aged children. From the questionnaire survey, we derived the number of susceptible school-aged children in orthodox Protestant schools based on the number of children and vaccination coverage of these schools. Subsequently, the number of susceptible school-aged children in orthodox Protestant schools was compared with the number of school-aged children reported to the national register from the municipality of Rhenen.

Reconstruction of susceptible school-aged children in The Netherlands

We conducted a similar assessment at the national level. Those most affected during the outbreak in The Netherlands were from a group of unvaccinated orthodox Protestant school-aged children. The number of orthodox Protestant school-aged children in The Netherlands can be estimated using data about the number of children by age per school with orthodox Protestant denomination, which are publicly available in The Netherlands (https://duo.nl/open_onderwijsdata/databestanden/).

The number of susceptible orthodox Protestant school-aged children can then be estimated using the vaccination coverage, which was approximately 60% among orthodox Protestants [23]. Because the previous outbreak among orthodox Protestants occurred 14 years earlier in 1999–2000 [18], unvaccinated orthodox Protestant children born after 1999 were assumed to be susceptible prior to the outbreak of 2013. Exposure to measles during the outbreak of 2013 is highly likely for unvaccinated orthodox Protestant children given the infectiousness of measles and high transmission within these schools with low vaccine uptake [16]. We can therefore make a comparison between the number of susceptible school-aged orthodox Protestant children registered in schools and those reported to the national register of notifiable diseases.

Having both the estimate from Rhenen and that of the entire country, we could assess whether the completeness of reporting estimate could be generalized to the national population.

Ethical considerations

Data concerning names, addresses, and dates of birth of children with measles cases reported to the national register were only available at the local municipal health service. After the matching, respondent names and addresses were erased from the data. Measles surveillance data obtained at the national registry are anonymized. Ethical approval was given by a medical ethics review committee (METC Noord Holland, M014-030).

Results

National register of notifiable diseases

In the national register of notifiable diseases we found 2700 measles cases for The Netherlands reported during 27 May 2013 and 12 March 2014, of which 39 measles cases were reported from the municipality of Rhenen. Of these, 35 were born between 2000 and 2013 (the age range of the questionnaire survey). Of these, 30 were orthodox Protestant. A total number of 1312 reported cases in The Netherlands were born between 2000 and 2013 and were orthodox protestant.

Community-based survey

In total, 3422 questionnaires were sent to all parents of all children born between 2000 and 2013 in the municipality of Rhenen, of which we received 2077 responses (response rate 60.7%). Of those 2077 respondents, 1067 were boys (51%) and the median age was 7 years (IQR 3 – 10). The responders did not differ from the non-responders in terms of sex ($p = 0.61$) and age ($p = 0.48$).

Overall, 307 respondents were reported to have had measles during the course of the outbreak of 2013–2014 (Figure 1B). Of the 307 outbreak-related cases, 171 patients were boys (56%) (Table 1). Nearly all cases were unvaccinated (n = 296, 96%), 2% (n = 6) were vaccinated once and 1% (n = 2) were vaccinated twice. The majority of cases (n = 236, 77%) did not consult a GP. In five cases hospitalization was reported. Almost a quarter of the cases (n = 69, 23%) reported at least one complication. Diarrhea (44 cases, 14% of all cases) was reported most commonly, followed by otitis media (n = 21, 7%), pneumonia (n = 12, 4%), and dehydration (n = 1, 0%). We found no record of measles related deaths in Rhenen.

Completeness of reporting and determinants

Of the 307 measles cases found in the survey 27 were reported to the national register (Figure 1B). Thus, the completeness of reporting was 8.8% (95%CI 6.0%–12.4%) (Figure 1E).

In Table 1, estimates of completeness of reporting are stratified by case characteristics. Cases of measles in children born in 2000–2008 were about three times more likely to be reported than cases in children born in 2009–2013 ($p = 0.11$). Cases with complications were six times more frequently reported than cases without complications ($p < 0.01$). Cases occurring before July 2013 were also more likely to be reported (14.7%) than those occurring in July 2013 or later (5.2%) ($p < 0.01$). Cases in children whose parents sought health care were more likely to be reported ($p < 0.01$).

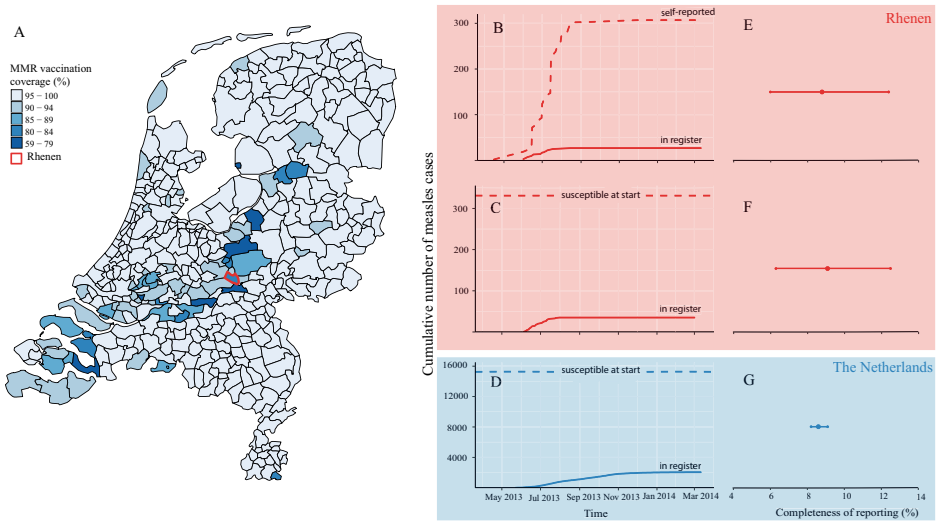

Figure 1. Completeness of measles notification in The Netherlands, 2013-2014. (A) First dose of measles-mumps-rubella vaccination coverage by municipality, The Netherlands, 2013 and the location of the municipality of Rhenen in the center of The Netherlands. (B) Cumulative number of self-reported cases from the community-based survey among birth cohort 2000-2013 in Rhenen (dashed line) and the cumulative number of self-reported cases matched to the national register of notifiable diseases (solid line). (C) Estimated number of susceptible orthodox Protestant children aged 4-12 years at the start of the epidemic in Rhenen, The Netherlands (dashed line) and the cumulative number of cases notified in the national register of notifiable diseases of 4-12 year-olds orthodox Protestants from Rhenen (solid line). (D)Estimated number of susceptible orthodox Protestant children aged 4-12 years in The Netherlands at the start of the epidemic (dashed line) and the cumulative number of orthodox Protestant cases aged 4-12 years notified to the national register of notifiable diseases in The Netherlands (solid line). (E) Completeness of measles notification, with 95% confidence interval in Rhenen as estimated with the community-based survey. (F) Completeness of measles notification with 95% confidence interval in Rhenen as estimated with the reconstruction of the number of susceptible children aged 4 – 12 years. (G) Completeness of measles notification with 95% confidence interval in The Netherlands as estimated with the reconstruction of the number of susceptible children aged 4 – 12 years.

Laboratory testing

Of the 307 measles cases identified in the survey whose parents were invited to submit saliva samples, we received samples of 126 children. Among these, four sent insufficient material to be tested. Five out of the 122 samples with sufficient levels of saliva were vaccinated and their samples tested positive. Of the remaining 117 saliva samples from unvaccinated children, all but one were positive for measles antibodies (n = 116, 99%). Thus, the positive predictive value of self-reported measles in unvaccinated individuals with a completed test was 99%. Those whose saliva was sampled were comparable in terms of sex (p = 0.3),

age (p = 0.9), complications (p = 0.7), and GP consultation (p = 0.3) with those who opted out from laboratory testing.

Table 1. Completeness of reporting stratified by case characteristics for cases in Rhenen, The Netherlands, 2013

	Cases in community survey in Rhenen (%)	Cases reported to the national register (%)	p value*	Completeness of reporting (%)
Total	307	27		8·8
Sex			0·67	
Male	171 (56)	14 (52)		8·2
Female	136 (44)	13 (48)		9·6
Complications†			<0·01	
Yes	69 (23)	17 (63)		24·6
No	237 (77)	10 (37)		4·2
Year of birth			0·11	
2000–2004	101 (33)	11 (41)		10·9
2005–2008	119 (39)	13 (48)		10·9
2009–2013	87 (28)	3 (11)		3·4
Date of reporting			<0·01	
Before 1 July 2013	115 (37)	17 (63)		14·7
1 July 2013 and later	192 (63)	10 (37)		5·2
MMR vaccination status‡			0·57	
None	296 (97)	26 (96)		8·8
Once	6 (2)	1 (4)		16·7
Twice	2 (1)	0 (0)		NA
Unknown	3 (0)	0 (0)		NA
GP consultation			<0·01	
Yes, in person	48 (16)	11 (41)		22·9
Yes, by telephone	23 (7)	5 (19)		21·7
No	236 (77)	11 (41)		4·7
Hospital admission			<0·01	
Yes	5 (2)	3 (11)		60·0
No	302 (98)	24 (89)		7·9

* The p value estimated by Chi square or Fisher's exact test indicates whether reported cases were different from unreported cases. † One missing for complications. ‡ Two missing for vaccination status.

On the basis of information from the additional questionnaire (n = 126), which was sent along with the saliva test kit, we found that young children born in 2009–2013

and children with complications were more likely to be a subsequent case in the household (Table 2).

Table 2: Case characteristics from children self-reported to have had measles and send in a saliva sample to test (n = 118)* , stratified by order of infection in the household.

	First (n = 41)	Subsequent (n = 77)	p value†
Year of birth			p < 0·05
2000–2004	12 (29)	25 (32)	
2005–2008	23 (56)	22 (29)	
2009–2013	6 (15)	30 (39)	
GP consultation			p = 0·25
Yes	13 (32)	17 (22)	
No	28 (68)	60 (78)	
Complication status			p = 0·03
Yes	5 (12)	23 (30)	
No	36 (88)	54 (70)	

* Eight out of the 126 from whom we received a sample of saliva and an additional questionnaire lacked information as to whether it was the first or subsequent case in the household. †p value estimated either by Chi square or Fisher's exact test indicates whether primary cases were different from secondary cases.

Representativeness of survey respondents

With regard to the survey, 1304 out of 2077 respondents were enrolled in an elementary school. Of those, 1231 children attended one of the eight elementary schools in Rhenen (Table 3). The majority of the measles cases found in the survey were reported from two schools with an orthodox Protestant denomination. For each school we compared the total number of children enrolled in school year 2013–2014 with the number of respondents. The percentage of respondents was similar among the different schools with different denominations, supporting that our study sample was representative of the community in terms of vaccination uptake.

Reconstruction of susceptible school-aged children in the community

In Rhenen, 588 children were enrolled in two orthodox Protestant schools, of who 482 were residents of Rhenen. The vaccination coverage among the children who were Rhenen residents in these schools was 22% and 46% (Table 3). Therefore, 331 children were estimated to be susceptible before the outbreak (Figure 1C). Thirty cases of orthodox Protestant school children from Rhenen, aged 4–12 years old, were reported to the national register. This means that the percentage

of susceptible children in the orthodox Protestant schools in Rhenen that were reported during the outbreak was 9.1% (95% CI 6.3% - 12.5%) (Figure 1F).

Table 3. Distribution of measles cases and respondents among elementary schools with different denominations with different vaccination coverage in Rhenen, The Netherlands, 2013

School	Children, residing in Rhenen, attending school in 2013–2014	Respondents	Response (%)	Cases among respondents	Vaccination coverage among responders (%)
A	218	155	71	0	97
B	266	163	61	5	93
C	143	104	73	0	91
D	282	195	69	1	97
E	187	135	72	66	46
F	295	190	64	141	22
G	284	178	66	2	96
H	198	111	56	0	92
Total	1883	1231	66	215	78

Reconstruction of susceptible school-aged children in The Netherlands

In orthodox Protestant elementary schools in The Netherlands, 38 131 children were registered in 2014. With a vaccination coverage of 60%, 15 252 children can be assumed to be susceptible prior to the outbreak (Figure 1D). During the outbreak, 1312 orthodox Protestant cases of children with measles 4–12 years of age were reported to the national register. If all the children who were assumed to be susceptible became infected in this outbreak, then only 8.6% (95% CI 8.2% - 9.1%) of these children were reported to the national register (Figure 1G).

Estimating the number of measles infections in the 2013–2014 measles outbreak in the community

The estimated reporting rate for measles of 9.1% as found in the calculation of the susceptible population for the municipality of Rhenen and the reported number of 35 measles cases for this community, suggest that during the outbreak approximately 384 (95%CI 280 - 555) individuals were infected with measles virus in the community of Rhenen.

Estimating the number of measles infections in the 2013–2014 measles epidemic in The Netherlands

The estimated reporting rate for measles of 8.6% for The Netherlands and the reported number of 2700 cases [16], suggest that the epidemic encompassed approximately 31 388 (95% CI 29 670 – 32 926) measles virus infections.

Discussion

During a large measles outbreak among predominantly orthodox Protestants in The Netherlands, only 8.8% of the measles cases in Rhenen were reported. Thus, for every reported case to the national register, there were approximately 10 other unreported cases. The congruity in the estimates between the community-based approach (8.8%) and the nationwide reconstruction method (8.6%) lends support to the credibility of these values.

Previous estimates of completeness of reporting in The Netherlands date back almost 15 years. A community-based survey found that 15 out of the 164 measles cases found (9%) were reported to the national register [12]. A reconstruction method estimated that 7% of the infections were reported to the national register during a previous measles epidemic in 1999–2000 [15]. These two estimates resemble our estimates from this study, despite a transition from paper-based to internet-based reporting. This suggests that the reporting rate is not much affected by the reporting system.

A major benefit of our community-based survey is that it allowed us to investigate the factors that affect the completeness of reporting. We found that having a complication and being infected early in the outbreak increased the likelihood of a given case being reported. While complications tend to be more common among children younger than 4 years of age [2], cases in children less than 4 years old were not as likely to be reported than those in older children. This observation might result from parents becoming accustomed to measles due to a first case in the household, most likely a school-aged child, and are then less inclined to seek health care for a subsequent case in the household.

Main factor of the incompleteness of reporting is for the most part due to the large number of measles cases who did not seek health care. Measles is a familiar disease in the orthodox Protestant community and after the first cases were diagnosed within a school or community, more infections were expected. Most infected individuals refrained from seeking health care, unless they were among the first

to be infected, and unless there were complications. The infected individuals that did seek health care could have exceeded the capacity for reporting as the measles cases were highly clustered in space in time.

Our survey had a high response rate. This response rate was high among respondents from schools with an orthodox Protestant denomination as well as in schools with other denominations. The equally high response rate among respondents from schools with an orthodox Protestant denomination is reassuring in estimating the completeness of reporting, as this group was the most affected during the outbreak of 2013–2014 [16]. Another strength of our study relative to previous studies is that we offered laboratory testing to a subset of the self-reported measles cases. The positive predictive value of cases that had a laboratory test result was almost 100%, which decreases the possibility of misclassification bias due to self-reporting of measles cases in the survey.

A limitation of the community-based survey is the restriction to one location (municipality of Rhenen) and one time period (the course of the outbreak in Rhenen took place mainly in June and July 2013). The close resemblance between the estimates from the community-based survey (8.8%) and the estimates from the reconstruction method for the study population (9.1%) strengthens our confidence that the estimate does not depend on the estimation approach. Furthermore, the close resemblance between the estimates for the reconstruction method for the study population (9.1%) and the national population (8.6%) suggests that the findings can be generalized beyond the single community. The outcomes of these various estimation approaches, when taken together, suggest that the completeness of measles reporting during the outbreak in The Netherlands was very close to 9%.

The 2700 reported measles cases in The Netherlands over the 2013-2014 epidemic represent just the tip of the iceberg of the true number of measles infections. Our findings show that the measles epidemic in The Netherlands in 2013–2014 consisted of approximately 30 000 to 33 000 individuals infected with measles virus. The completeness of reporting varies with case characteristics. Epidemiological analyses on severity using only reported cases should be viewed in light of this knowledge.

Further, this study shows that our assessment of the number of susceptible children prior to an outbreak closely approximated the estimate from a community-based

survey. An assessment of the susceptible population prior to an outbreak may therefore be sufficient to assess the true number of infections of a future outbreak in The Netherland but also in other high income countries that have an overall high but heterogeneous vaccination coverage with pockets of communities with lower coverage. The epidemiological pattern of having periodic and sometimes large outbreaks has been seen recently in for example Brazil [24], Canada [25], and in the USA [26]. Calculating the expected number of susceptible individuals in the groups at risk could help to estimate the completeness of reporting and to assess the true extent of the measles outbreak in those populations. An important condition, however, is to have accurate census data and data on vaccination uptake.

That reported cases represent only a very small proportion of the actual incidence emphasizes the difficulty in achieving measles elimination. Having accurate estimates of the number of measles virus infections allows us to calculate the risk of complications upon infection with measles virus, to measure the health burden of measles, and to assess the possibility of breaking the chain of transmission to eliminate measles.

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Chapter 4

Severity and infectiousness of measles vaccine failures in a large epidemic, the Netherlands, 2013-2014

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Submitted

Abstract

A large outbreak of measles in the Netherlands in 2013-2014 provided an opportunity to assess the effect of MMR vaccination on severity and infectiousness of measles.

Measles is notifiable in the Netherlands. We used information on vaccination, hospitalization, complications and most likely source(s) of infection from cases notified during the outbreak. When a case was indicated as a likely source for at least one other notified case, we defined it as infectious. We estimated the age-adjusted effect of vaccination on severity and infectiousness with logistic regression.

Of 2,676 notified cases, 2,539 (94.9%) were unvaccinated, 121 (4.5%) were once-vaccinated and 16 (0.6%) were at least twice-vaccinated, 328 (12.3%) cases were reported to have complications and 172 (6.4%) cases were hospitalized. Measles in twice-vaccinated cases led less often to complications and/or hospitalisation than measles in unvaccinated cases (0% and 14.5%, respectively, aOR 0.1 (95% CI 0-0.89), p=0.03). Of unvaccinated, once-vaccinated and twice-vaccinated cases, respectively 194 (7.6%), seven (5.1%) and 0 (0%) were infectious. These differences were not statistically significant (p>0.05).

Our findings suggest a protective effect of vaccination on occurrence of complications and/or hospitalisation as a result of measles and support the WHO recommendation of a two-dose MMR vaccination schedule.

Background

Measles is a highly contagious viral disease. The number of secondary cases from one patient in a fully susceptible population ranges between 12 -18 (1). Globally, measles remains one of the leading causes of death in young children, despite the availability of safe and effective vaccines against measles (2). Initial symptoms of measles, including high fever, cough, coryza and conjunctivitis, develop 10- 12 days after exposure. A few days later, a rash develops which usually spreads over the entire body. Complications of measles include pneumonia, otitis media, diarrhoea and encephalitis.

Measles virus infection in vaccinated individuals can be due to primary or secondary vaccine failure. Primary vaccine failure is the failure to respond to the vaccine and occurs in 5% of one-dose recipients (3). Secondary vaccine failure is defined as susceptibility due to waning immunity after seroconversion and depends mainly on the time since vaccination and the number of doses received (4). The relevance of vaccine failure for measles control depends on its frequency of occurrence and the severity and infectiousness of measles in vaccinated individuals. Measles transmission has been reported from twice-vaccinated cases (5). Limited information available suggests, however, that measles in fully vaccinated individuals is less infectious and presents with milder symptoms than measles in unvaccinated individuals (6) (7).

Vaccination against measles has been part of the Dutch national immunisation programme since 1976. Children are offered vaccination against measles, mumps and rubella (MMR) in a two-dose schedule, at 14 months and nine years of age. Despite high overall vaccination coverage, large measles outbreaks occurred in 1987/1988, 1999/2000 and 2013/2014, mostly affecting unvaccinated individuals of orthodox Protestant denomination (8, 9). This group of orthodox Protestant individuals live in a socio-geographically clustered area in the Netherlands, described as the 'bible belt'. About 40% of this group refuses vaccination because of religious reasons (10). In the 2013/2014 outbreak, 2,700 measles cases were notified predominantly among unvaccinated primary school-aged children of orthodox Protestant denomination. The circulating genotype was D8 (11).

This large outbreak provided an opportunity to assess the effect of vaccination on the occurrence of complications, hospitalization, and infectiousness of measles.

Methods

Measles is a notifiable disease in the Netherlands. We included all notified cases with day of rash onset between 23 May 2013 and 11 March 2014 in our analyses. A confirmed case was defined as any person not recently vaccinated and meeting the clinical and laboratory criteria for measles. The clinical criteria included fever, maculopapular-rash and at least one of the following: cough, coryza or conjunctivitis. The laboratory criteria included either detection of measles-specific IgM antibodies in blood specimens or specific detection of measles virus RNA by polymerase chain reaction (PCR) in throat swabs, oral fluid or urine specimens. A probable case was also notifiable and is defined as any person meeting the clinical criteria who has been in contact (< 3 weeks prior to the date of onset) with a confirmed case. Regional and national laboratories tested and genotyped all collected specimens.

Clinicians and laboratories reported cases to the Municipal Health Services (MHS). The MHS collected information on the cases by interviewing them or their physician using a standardized measles surveillance form. The MHS notified cases meeting the case definition criteria to the national surveillance database 'Osiris'.

The standardized surveillance form included a question on vaccination status, which was verified in the national vaccination register, by a vaccination card or by consulting the cases' GPs. Questions on the presence of complications and hospitalization were other items in the form. Encephalitis, pneumonia, otitis media were defined as complications in the form, next to an open text field for other complications. At the start of the outbreak, questions about the source of infection were added to the standardized surveillance form. MHSs were asked to indicate one or more likely sources for each notified case by recording the unique notification identifier of this/these source(s). A likely source of a case was defined as another notified confirmed or probable case with whom there was contact 7-21 days before the onset of rash and whereby the generation interval of the linked cases was between 9-14 days (12). RIVM separately collected information on the duration of hospitalization.

We considered two outcomes in our analyses: severity and infectiousness. We defined severity as the presence of at least one complication and/or hospitalization due to measles. Infectiousness was defined as a case being indicated as a likely source of infection to other cases.

Vaccination status was the independent variable of interest. We excluded cases with unknown vaccination status and those where the vaccination status was not verified by national vaccination register, by a vaccination card or by a GP. In the analyses of complications, we excluded cases for which no information was available on the occurrence of complications.

We used logistic regression to compare the frequency of complications and infectiousness between unvaccinated, once and at least twice MMR vaccinated cases. We used Firth logistic regression where there were zero cases in subgroup analyses (13). This produces finite parameter estimates by means of penalized maximum likelihood estimation. We adjusted for age group (⩽13 months, 14 months-8 years, 9-18 years, ⩾19 years) in all analyses. Associations with a p-value below 0.05 were considered statistically significant. We calculated the vaccine effectiveness (VE) for protection against complications/hospitalisation and infectiousness for one and two doses of MMR as VE=1-aOR. For two doses of MMR, we also estimated the total VE against measles and infectiousness and against measles and complications/hospitalisation as:

VE_{Total}=1-((1-VE) * (1-$VE_{I,C}$)), where VE is the VE against measles (which we assumed was 0.94) and $VE_{I,C}$ is the VE against infectiousness or complications (as estimated in our study), and both VEs are expressed as fractions rather than percentages (14) (15). We used STATA software version 14.0 and R for the analyses.

Results

In total, 2,700 measles cases were notified during the 2013/14 outbreak. Twenty-four cases were excluded from the analyses because of unknown vaccination status (n=20) or since their vaccination status was not verified by national vaccination register, by a vaccination card or by a GP (n=4). The median age of cases was 10 years (range 0-68) and 50% were female. Most cases (2,161, 81%) were orthodox Protestants (Table 1). Of 2,676 notified cases with a known vaccination status, 2,539 (94.9%) were unvaccinated, 121 (4.5%) were vaccinated once, 15 (0.6%) were vaccinated twice, and one case received three doses. The MHSs verified cases' vaccination status in the national vaccination register (67%), with the vaccination card (24%), or by a GP (9%).

Severity

Of 2,676 cases with a verified vaccination status, the occurrence of complications was known for 2,563 (96%). For 328 (13%) of these, complications were reported. Of cases with complications, 311 (95%) reported one complication and 17 (5%) two complications. In total 158 (6%) cases had pneumonia, 113 (4%) otitis media and two (0.1%) cases had encephalitis. Other complications were reported for 72 (3%) cases. These other complications were most often a respiratory infection or dehydration. fOR 317 (15%) of unvaccinated cases and 11 (10%) of vaccinated cases a complication was reported (Table 2). All complications, except otitis media, were more prevalent in the unvaccinated group (Table 2). One unvaccinated case with encephalitis and pneumonia died (case fatality ratio among unvaccinated cases 0.04%).

In total 172 (7%) cases were hospitalized. The median duration of hospital admission was four days, and it did not differ between unvaccinated and vaccinated hospitalised cases. Cases of orthodox Protestant denomination (6%) and other risk groups (2%) were less often hospitalized than cases that did not belong to a risk group (14%) (p <0.000) (adjusted for vaccination status).

We combined hospitalization and complications in the analyses of severity and MMR vaccination status. Of the 2,563 cases, 371 (14%) had complications and/or were hospitalized.

Of 2,428 unvaccinated cases, 353 (14.5%) had complications and/or were hospitalized and 18 (13.3%) of the 135 vaccinated cases had complications and/or were hospitalized [aOR 0.72 (95% CI 0.5-1.5), p 0.22]. Taking into account the number of doses of MMR, 18 (15.1%) of the 119 once-vaccinated cases and none (0%) of the 16 at least twice-vaccinated cases had complications and/or were hospitalized [aOR 0.87 (95% CI 0.5-1.4), p=0.60] and [aOR 0.12 (95% CI 0.0-0.89), p=0.03, VE=88%], respectively (Table 3). The estimated total VE against measles and against complications/hospitalisation, for two doses of MMR, was 99% (95% CI 11-100).

Infectiousness

A total of 709 cases (26%) indicated a source of infection. After correction for the contact period, as described in the methods, 376 cases could be linked to 201 likely sources. The mean number of cases linked to a likely source was 1.9, SD 1.35 (range 1-11).

Table 1: Characteristics of measles cases by MMR vaccination status, the Netherlands, May 2013- March 2014 (n=2676)

		Number of cases#	Unvaccinated n (%)#	Vaccinated: 1 dose n (%)#	Vaccinated: 2 doses n (%)#	Vaccinated: 3 doses n (%)#	p-value
n*		2,676	2,539 (94.9)	121 (4.5)	15 (0.6)	1 (0.04)	
Median age in years (range)		10 (0-68)	10 (0-68)	5 (0-41)	26 (12-35)	30	
Age group	Infant (⩽ 13 months)	78 (2.9)	75 (2.9)	3 (2.5)	0 (0)	0 (0)	
	Child (14 months-8 years)	1,081 (40.4)	996 (39.2)	85 (70.3)	0 (0)	0 (0)	
	Adolescent (9-18 years)	1,292 (48.3)	1,275 (50.2)	14 (11.6)	3 (20)	0 (0)	
	Adult (⩾ 19 years)	225 (8.4)	193 (7.6)	19 (15.7)	12 (80)	1 (100)	<0.000
Sex	Male	1,317 (49.2)	1,251 (49.3)	59 (48.8)	6 (40)	1 (100)	
	Female	1,343 (50.2)	1,272 (50.1)	62 (51.2)	9 (60)	0 (0)	
	Unknown	16 (0.6)	16 (0.6)	0 (0)	0 (0)	0 (0)	0.871
Case definition	Confirmed	871 (32.6)	771 (30.4)	87 (71.9)	12 (80)	1 (100)	
	Probable	1,805 (67.4)	1,768 (69.6)	34 (28.1)	3 (20)	0 (0)	<0.000
Risk group	None	191 (7.1)	108 (4.3)	71 (58.7)	11 (73.4)	1 (100)	
	Orthodox Protestant denomination	2,161 (80.8)	2,135 (84.1)	24 (19.8)	2 (13.3)	0 (0)	
	Anthroposophist	16 (0.6)	16 (0.6)	0 (0)	0 (0)	0 (0)	
	Critical attitude towards vaccination	177 (6.6)	172 (6.8)	5 (4.1)	0 (0)	0 (0)	
	Unknown	131 (4.9)	108 (4.2)	21 (17.4)	2 (13.3)	0 (0)	<0.000

Percentages displayed of column total
* Percentages displayed of row total

Table 2: Measles complications by MMR vaccination status, the Netherlands, May 2013-March 2014 (n=2563)

MMR doses received	Number of cases	Cases with pneumonia n (%)	Cases with otitis media n (%)	Cases with other complications n (%)	Cases with encephalitis n (%)	Hospitalized cases n (%)#
0	2,428	153 (6.3)	107 (4.4)	71 (2.9)	2 (0.1)	163 (6.7)
1	119	5 (4.2)	6 (5)	1 (0.8)	0 (0)	9 (7.6)
2*	16	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

* One case received three MMR doses
# 127 of the unvaccinated hospitalized cases had (a) complication(s) and 2 of the once vaccinated hospitalized cases had (a) complication(s)

T**able 3:** Association between severity (any complication and/or hospitalization) and MMR vaccination status, the Netherlands, May 2013- March 2014.

MMR doses received	No. of measles cases	No. (%) of cases with complications	OR (95% CI)	p-value	VE %	aOR (95% CI)#	p-value#	aVE %#
0	2,428	353 (14.5)	ref	ref	ref	ref	ref	ref
≥1	135	18 (13.3)	0.90 (0.5-1.5)	0.69	10 (-50-50)	0.72 (0.5-1.5)	0.22	28 (-50-50)
1	*119*	*18 (15.1)*	*1.1 (0.6-1.7)*	*0.37*	*-9 (-74--38)*	*0.87* (0.5-1.4)	*0.60*	*13 (-43-49)*
2*	*16*	*0 (0)*	*0.18 (0-1.3)*	*0.11*	*82 (-32-100)*	*0.12 (0-0.89)*	*0.03*	*88 (11-100)*

Adjusted for age group (≤13 months, 14 months-8 years, 9-18 years, ≥19 years)
* One case received three MMR doses

Of 2,538 unvaccinated cases, 194 (8%) were reported as a likely source whilst of the 137 vaccinated cases seven (5%) were reported as a likely source [aOR 0.74 (95% CI 0.3-1.6), p=0.45]. All vaccinated likely sources had only one secondary case whilst unvaccinated likely sources had a mean of 1.9 secondary cases (p=0.02). Of the seven vaccinated likely sources 71% of their secondary cases were also vaccinated whilst of the 194 unvaccinated likely sources only 5% of their secondary cases were vaccinated.

Taking into account the number of doses of MMR, seven (6%) of the once-vaccinated cases and none (0%) of the 16 at least twice- vaccinated cases were indicated as a likely source [aOR 0.9 (95% CI 0.4-1.8), p=0.77] and [aOR 0.39 (95% CI 0-3), p=0.45, VE=61%] respectively (Table 4). The estimated total VE against measles and against infectiousness, for two doses of MMR, was 98% (95% CI -203-100).

Table 4: Association between infectiousness and MMR vaccination status, the Netherlands, May 2013-March 2014

MMR doses received	No. of measles cases	No. (%) of cases indicated as a likely source	OR (95% CI)	p-value	VE %	aOR (95% CI)#	p-value#	*aVE %#*
0	2,538	194 (7.6)	ref	ref	ref	ref	ref	ref
≥1	137	7 (5.1)	0.65 (0.3-1.4)	0.27	35 (-40-70)	0.74 (0.3-1.6)	0.45	26 (-60-70)
1	*121*	*7 (5.8)*	*0.79 (0.3-1.6)*	*0.53*	*21 (-57-66)*	*0.90 (0.38-1.80)*	*0.77*	*10 (-80-62)*
2*	*16*	*0 (0)*	*0.39 (0-2.7)*	*0.56*	*63 (-171-100)*	*0.39 (0-3.0)*	*0.45*	*61 (-203-100)*

Adjusted for age group (≤13 months, 14 months-8 years, 9-18 years, ≥19 years)
* One case received three MMR doses

Discussion

During a large measles outbreak in the Netherlands in 2013/14, we found that none of the at least twice-vaccinated measles cases had complications, was hospitalized nor was indicated as a likely source for other cases. Among measles cases, those who were vaccinated with two doses of MMR were less likely to develop complications and/or were hospitalized as a result of measles.

Our results are consistent with findings by others. Misra et al report a lower proportion of complications, such as pneumonia, ear infection and diarrhoea among at least once- vaccinated cases (16). In a study of Mitchell et al, unvaccinated cases were 2.8 times more likely to have more severe clinical outcomes, such as height and duration of fever, number of days needing medication (other than paracetamol) and days required in bed, compared to vaccinated cases (17). De Serres et al also found that twice-vaccinated cases had milder illness than those who were unvaccinated or once-vaccinated cases (18). This is in line with our results, where none of the at least twice-vaccinated cases reported complications. The once-vaccinated cases reported complications, but the proportion of the different complications was lower, albeit not significantly so, for the once-vaccinated cases compared with unvaccinated cases, except for otitis media. In one study, measles vaccination was found to be associated with lower mortality (19). The low number of deaths in our study did not allow an assessment of the effect of MMR vaccination on measles mortality among cases.

None of the at least twice-vaccinated cases were hospitalized in our study. De Serres et al also showed that twice-vaccinated cases had a significantly lower risk of hospitalization than those who were unvaccinated or once-vaccinated (18). Another study reported also lower hospital rates in once-vaccinated cases (20). In our study, there was no difference between the unvaccinated and once-vaccinated cases, but the reason for hospital admission seems less severe in the vaccinated cases (data not shown).

In our study, none of the at least twice-vaccinated cases were indicated as a likely source by other cases. A few case reports were published which document no transmission from vaccinated cases (6, 7, 21). One study described transmission from a twice-vaccinated individual with documented secondary vaccine failure (5). We found seven once-vaccinated cases who were a likely source to other cases. Of these vaccinated likely sources, three were hospitalized and one had pneumonia. Their relatively severe course of illness and infectiousness may indicate primary vaccine failure. Coleman et al suggested vaccinated cases are less infectious because of the relatively mild nature of their illness (22).

The relatively small proportion of vaccinated cases during this outbreak, compared with other outbreaks in Europe (23-27), limited the power of our analyses. Another limitation is that we could not distinguish the role of primary or secondary vaccine failure since we lacked information on the immune response and avidity levels (28) of vaccinated cases.

During this outbreak only 9% of measles cases were notified (29), consistent with the underreporting estimated in the previous outbreak (30, 31). The proportion of complications and hospitalizations among all infected individuals might be lower than the proportion among notified cases when taking the underreporting into account. Cases with complications and hospitalized cases will probably be notified, because of the severity of disease. In the recent underreporting study, the proportion of unreported cases in the vaccinated group was 88% and in the unvaccinated group 91%. Hence, we believe that underreporting of cases did not influence our results.

It is possible that cases developed complications after being notified, thus leading to underestimation of the frequency of complications. However, we do not believe there is a relation between the completeness of reporting complications and the vaccination status of cases.

For only a small percentage of the cases we identified a likely source (14%). In vaccinated cases, the source of infection was easier to identify for cases that do not belong to a risk group than in the group of orthodox Protestant denomination, because there were many orthodox Protestant cases.

Besides the results could be biased because vaccinated cases mainly have contact with vaccinated cases, and unvaccinated cases with unvaccinated. Therefore the calculated VEi can be overestimated. We tried to analyse this by assessing the vaccination status of the secondary cases of the likely sources. The results show that vaccinated cases indeed tend to cluster with vaccinated cases and unvaccinated with unvaccinated cases. As vaccinated cases have less chance to get measles infection, the probability of transmission of measles to vaccinated individuals is lower than the probability of transmission to unvaccinated individuals. This bias can lead to overestimation of the OR. We intended to carry out the analyses on transmission for the risk group of orthodox Protestant denomination only, because this (mainly unvaccinated) group tends to cluster. Unfortunately, there were no vaccinated likely sources in this group and therefore we could not assess the presence of this bias.

In conclusion, our findings suggest a protective effect of MMR vaccination on the occurrence of complications and/ or hospitalization. These are important findings for global measles control policies. None of the at least twice- vaccinated cases had complications, were hospitalized or were indicated as a likely source to other cases. Our study therefore supports the WHO recommendation of a two-dose MMR vaccination schedule (2). The severity and infectiousness of vaccinated measles cases are important indicators for measles surveillance and outbreak investigation. We recommend including these indicators in measles surveillance.

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Conflict of interest

None.

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Chapter 5

A novel measles outbreak control strategy in the Netherlands in 2013–2014 using a national electronic immunization register: A study of early MMR uptake and its determinants

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Vaccine, 2017

Abstract

Background

During a large measles outbreak in the Netherlands in 2013–2014, infants aged 6–14 months living in municipalities with low (<90%) measles-mumps-rubella (MMR) coverage were individually invited for an early MMR using the national electronic immunization register, Præventis. We estimated uptake of early MMR prior to and during the 2013-2014 outbreak and assessed determinants for early MMR vaccination.

Methods

We obtained vaccination records from Præventis, and defined early MMR as vaccination before 415 days (13 months) of age. A multi-level multivariable logistic regression model, restricted to infants with three diphtheria-pertussis-tetanus-polio (DPTP) vaccinations was used to examine the association between early MMR uptake and sex, parents' country of birth, socioeconomic status (SES; at postcode level) and voting proportions for the Reformed Political Party (SGP; at municipal level), used as a proxy for religious objections towards vaccination.

Results

In the 29 municipalities with low MMR coverage, uptake of early MMR was 0.5–2.2% prior to the outbreak. Between July 2013 and March 2014, 5,800 (57%) invited infants received an early MMR. Among infants with three DPTP, 70% received an early MMR. Only 1% of infants without prior DPTP received an early MMR. Lower early MMR uptake was associated with a higher SGP voter-ship (OR 0.89 per 5% increase, 95%CI 0.83–0.96), parents' with unknown country of birth (OR 0.66 95%CI 0.47–0.93) and compared with very high SES, high SES had significantly lower early MMR uptake (OR 0.66 95%CI 0.50–0.87).

Discussion

This is the first study describing use of Præventis during an outbreak and to assess determinants of early MMR uptake. More than half of invited infants obtained an early MMR. SES, parents' with unknown country of birth and religious objections towards vaccination were found to be associated with lower early MMR uptake. In future outbreaks, these determinants could be used to tailor intervention strategies.

Introduction

Measles is a highly contagious viral disease, that is transmitted via airborne or droplet exposure causing fever and rash, and can lead to serious and sometimes fatal complications, with young children and adults at increased risk of serious complications [1]. The Dutch National Immunization Programme (NIP) offers the first dose of measles, mumps and rubella (MMR-1) at 14 months and the second MMR (MMR-2) at nine years [2]. In addition, it recommends children aged between 6 and 14 months should be considered for an early MMR before travel to countries with endemic or epidemic measles transmission [3]. Most infants by 6 months of age lack detectable measles maternal antibodies [4], [5] and are therefore at increased risk of infection during an outbreak.

When infants receive an MMR between 6 and 12 months, it is referred to as an MMR-0 and subsequent MMR-1 and MMR-2 vaccinations are recommended according to the NIP schedule. Obtaining an MMR between 12 and 14 months is referred to as an early MMR-1 and it is recommended to obtain the MMR-2 according to the NIP schedule [2].

Vaccination coverage in the Netherlands is monitored via Præventis, the national immunization registration database [6]. Coverage of MMR-1 and MMR-2 in the Netherlands is around 95% and 92%, respectively [7].

In May 2013, a large measles outbreak was declared in the Netherlands [8], which led to 2,700 reported measles cases [9]. The outbreak ended in March 2014 and occurred primarily among unvaccinated members of orthodox Protestant communities [9]. In the Netherlands, there are an estimated 200,000 orthodox Protestants, who are socio-geographically clustered in the so-called 'Bible-belt'. Its boundaries are often defined by municipality voter-ship for the Reformed Political Party 'Staatkundig Gereformeerde Partij' (SGP) of 5% or greater [10]. This community adheres to a strict orthodox Protestant faith with religious objections towards vaccination, and an estimated 40% are unvaccinated [11].

In response to the measles outbreak, a multidisciplinary outbreak management team (OMT) consisting of physicians, infectious diseases experts from the National Institute for Public Health and the Environment (RIVM), local authorities and academic institutions was convened in June 2013. Vaccination coverage for MMR-1 is determined at 2 years of age. Therefore, the most up-to-date MMR-1 vaccine

coverage from Præventis was available for birth cohort 2010, which was low (<90%) in 30 municipalities, of which 29 were within the 'Bible-belt' [8] (Figure 1). The other municipality, Vaals, in the far south-east of the Netherlands had MMR-1 coverage of 84%, as some infants are vaccinated in Germany and are therefore not registered in Præventis. The OMT recommended early MMR vaccination for all infants aged 6-14 months in the 29 municipalities within the 'Bible-belt' with low MMR-1 coverage. Measles catch-up vaccination was also offered to all unvaccinated individuals aged 14 months to 19 years, irrespective of vaccination coverage in their municipality [8]. These control measures were announced by RIVM and Dutch politicians in June 2013. Advertisements were also placed in an orthodox Protestant newspaper ('Reformatorisch dagblad').

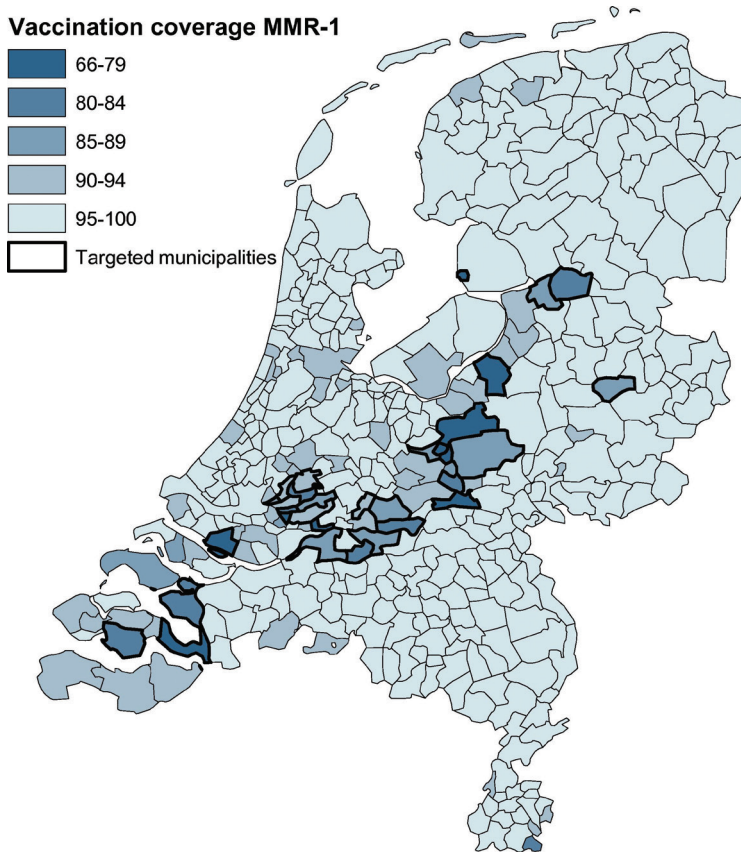

Figure 1. Vaccination coverage for MMR-1 among birth cohort 2010. The black outline indicates the 29 municipalities targeted for early MMR with low MMR-1 coveragea.aThirty municipalities had MMR-1 vaccination coverage <90%, of which, 29 are within the 'Bible belt'. The other municipality, Vaals, in the far south-east of the Netherlands had MMR-1 coverage of 84%, as some infants are vaccinated in Germany and are therefore not registered in Præventis

Using demographic and vaccination data obtained from Præventis, the objectives of this study were to estimate the uptake of early MMR in the 29 municipalities with low MMR coverage within the 'Bible-belt' prior to the early MMR campaign, and assess early MMR uptake during the 2013–2014 measles outbreak. Finally, we aimed to assess determinants for early MMR uptake.

Material and methods

Study population

We assessed vaccination records for birth cohorts 1995–2013 registered as active (living) in Præventis in the 29 municipalities with low MMR coverage. Firstly, we estimated the baseline uptake of early MMR prior to the outbreak among birth cohorts from 1995 to 2011. Secondly, we assessed uptake of early MMR among eligible infants invited for an early MMR during the 2013–2014 measles outbreak.

During the outbreak, a search within Præventis, beginning 13 July 2013, was used to identify infants eligible for early MMR aged between 171 days (6 months) and 399 days (13 months) without a prior invitation nor MMR vaccination. Subsequently, a weekly search within Præventis was conducted to capture infants turning 6 months of age living in one of the 29 municipalities with low MMR coverage or who had moved into one of the 29 municipalities after the 13 July 2013.

Data collection

We gathered data at different levels of aggregation as detailed in Table 1. We obtained individual records for MMR and diphtheria-pertussis-tetanus-polio (DPTP) vaccinations administered in the first six months of life (at 6-9 weeks, 3 months and 4 months) from Præventis.

The infants date of birth, area of residence (four-digit postcode and municipality), and parents' country of birth (a proxy for ethnic background) were available at individual level in Præventis. The date of invitation for early MMR was also available. Præventis does not record the reason an infant receives an MMR-0 e.g. for travel. However, during this outbreak, an indicator variable was added for eligible infants invited for an early MMR.

Table 1. Summary of data sources collected according to level of aggregation, details and year.

Data source	Details	Year
Individual level (Dutch national immunization register "Præventis")		
Date of birth	Month/day/year	2014
MMR vaccination status	MMR-0 or early MMR-1	2014
DPTP vaccination status	DPTP vaccinations in first six months of life (DPTP 1-3)	2014
Country of origin of parents	Country name	2014
Date of vaccination invitation	Month/day/year	2014
Postcode	Four-digit postcode	2014
Municipality	Municipality name	2014
Postal code level (Netherlands Institute for Social Research)		
Socioeconomic status (SES)	SES score ranging from −4 to +4; the lower the SES score, the higher the SES	2012
Municipality level (Statistics Netherlands)		
Election results for the Netherlands	Proportion of votes for the Reformed Political Party (SGP)	2012

MMR: measles, mumps rubella vaccine; DPTP: diphtheria, pertussis, tetanus, polio vaccine.

Group-level data on voting statistics for the SGP and socioeconomic status (SES) were collected. The proportion of votes for the SGP from the 2012 election is publicly available at municipality level from Statistics Netherlands (www.cbs.nl) and was used as a proxy for religious objections towards vaccination among orthodox Protestants. The "status score" calculated by the Netherlands Institute for Social Research (www.scp.nl) was used as a proxy for SES, and is available at four-digit postcode level. This score takes into account the average income per household in a given postcode area as well as the percentage of households with low income, without a paid job and with low education level [12]. We included 183 postcode areas for the 29 municipalities with low MMR coverage. For data that were not available at individual level (SES and religious objection to vaccination), all children in a given geographic area (e.g. postcode or municipality) were given the value of that area.

Data analysis

A cut-off of 415 days (13 months) was chosen for early MMR uptake to prevent interference with routine MMR, which is given, on average, at 426 days (14 months). MMR vaccination before 365 days (12 months) was recorded as an MMR-0 and an MMR between 365 and 415 days (12–14 months) was recorded as an early MMR-1.

In the descriptive analyses, we used Chi-square tests to compare categorical variables between infants who were invited for an early MMR and were vaccinated and those who were not. To assess associations of early MMR uptake and predictor variables, we restricted our analysis to infants with three DPTP vaccinations, as the proportion of our study population who did not have the NIP recommended number of DPTP vaccinations and who were vaccinated with an early MMR were too small.

We calculated odds ratios (ORs) and corresponding 95% confidence intervals (95%CIs) using multi-level multivariable logistic regression models. A multi-level model was used to take into account the hierarchical structure of the data; municipality was included as a varying intercept. All predictors were included in the multivariable model. Continuous variables were categorised in the model if a linear trend with early MMR uptake was observed. We categorised the parents' country of birth by ethnic background based on both parents. Ethnic backgrounds with smaller numbers were grouped into the category "other-other". Infants of whom the country of birth of at least one parent was unknown were categorised in the group "unknown". R statistical software (version 3.2.0) and package lme4 were used for all analyses [13]. ArcGIS was used to visualize vaccination coverage per municipality for MMR-1 for birth cohort 2010 in the 29 municipalities with low MMR coverage.

Results

Prior to the early MMR vaccination campaign in 2013–2014, background coverage of MMR-0 was 0.5–2.2% in the 29 municipalities with low MMR coverage (Figure 2). Between 13 July 2013 and 31 March 2014, 10,097 infants aged between 6 and 14 months living in 29 municipalities with low MMR coverage were invited for an early MMR. We found one duplicate in the database; therefore, our final study population was 10,096 infants (Figure 3).

In total, 5,800 (57%) infants living in the 29 municipalities with low MMR coverage received an early MMR before the age of 415 days (13 months) (Table 2). Among these infants, 5,006 (86%) received an MMR-0, and 794 (14%) received an early MMR-1. Twenty-five percent (n = 1,436) of infants had their early MMR administered before they received an invitation letter, and 78% (n = 4,531) received their early MMR vaccination within 14 days of invitation. The majority of infants (91%, n = 5,254) were vaccinated with an early MMR within a month of invitation.

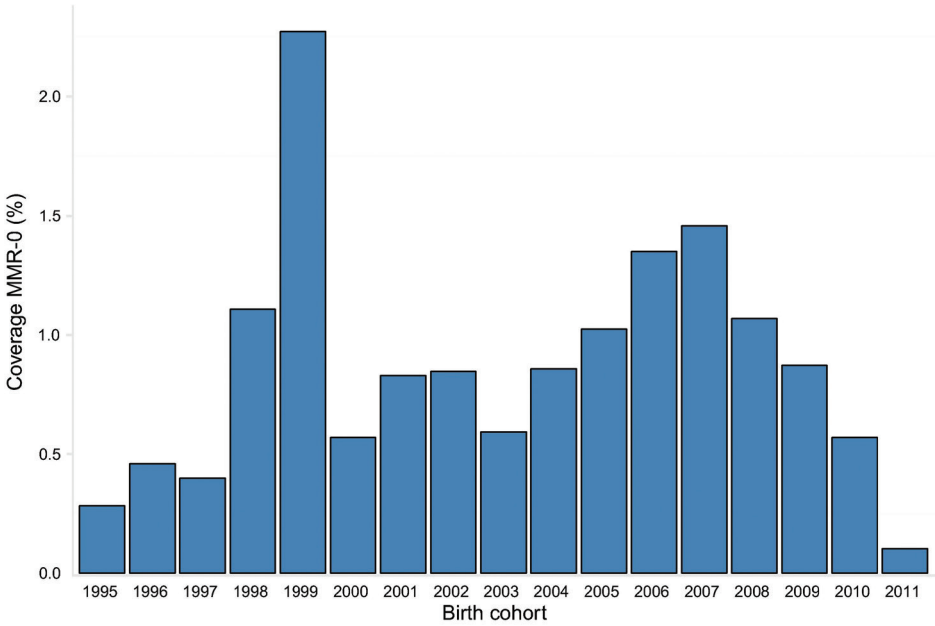

Figure 2. Background coverage of MMR-0 among infants from birth cohorts 1995/2011 in the 29 municipalities with low MMR-1 coverage prior to the 2013–2014 early MMR vaccination campaign (n = 180,145).

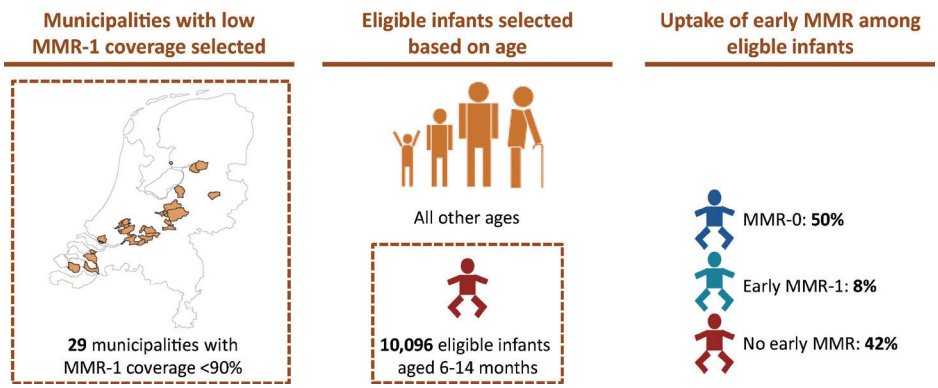

Figure 3. Selection within Præventis of 29 municipalities with low MMR-1 coverage and of eligible infants aged 6-14 months invited for an early MMR (dashed line) and uptake of early MMR, July 2013–March 2014 (n = 10,096).

Table 2. Early MMR uptake in the 29 municipalities targeted for the outbreak intervention, the Netherlands, July 2013–March 2014 (information available for 10,096 individuals and 183 postcode areas).

Determinants		**Invited**	**Vaccinated**	**Vaccine uptake**	**p - value**
		N	**n**	**(%)**	
Total		10096	5800	57	
Sex	Male	5194	2979	57	0.84
	Female	4902	2821	58	
Previous DPTP vaccination	Zero doses	1773	17	1	<0.001
	One dose	30	4	13	
	Two doses	59	27	46	
	Three doses	8234	5752	70	
Parents' country of birth	The Netherlands - The Netherlands	8960	5091	57	0.003
	The Netherlands - Turkey	56	34	61	
	The Netherlands - Morocco	44	28	64	
	The Netherlands - European Country	194	122	63	
	The Netherlands - Other	233	135	58	
	Morocco - Morocco	74	57	77	
	European - European	101	64	63	
	Turkey - Turkey	40	28	70	
	Other - Other	216	139	64	
	Unknown	178	102	57	
SGP voters	<12.5%	2390	1555	65	<0.001
	12.5%–19%	2493	1467	59	
	20%–25%	3573	2018	56	
	>25%	1479	671	45	

Table 2. Continued

Determinants		Invited	Vaccinated	Vaccine uptake	p - value
		N	n	(%)	
Socio ecomonic status (SES)	Very high SES	427	293	69	<0.001
	High SES	4602	2654	58	
	Low SES	4781	2686	56	
	Very low SES	125	78	62	

DPTP: diphtheria, pertussis, tetanus, polio vaccine; SGP: Reformed Political Party.

Among those with three DPTP vaccinations (n = 8,234), the uptake of early MMR was 70% (Table 2). Eighteen percent (n = 1,773) of eligible infants had no prior DPTP vaccinations, of whom only 1% (n = 17) availed of an early MMR. These infants all had Dutch born parents and 65% (n = 11) lived in municipalities with SGP voter-ship greater than 20% (data not shown). At municipality level, as the proportion of SGP voter-ship increased, early MMR uptake decreased (Table 2). The uptake of early MMR was highest among infants with both parents' country of birth from either Morocco or Turkey, 77% and 70% respectively. However, these numbers were small. At the postcode level, the proportion of infants receiving an early MMR was highest among those classified as living in an area of very high SES (Table 2).

Among those with three DPTP vaccinations, 148 infants were missing their four-digit postcode, as they had moved out of one of the 29 municipalities with low MMR coverage, resulting in 8,086 infants eligible for the multi-level multivariable logistic regression analysis. Early MMR uptake did not differ by sex (OR 1.02 95%CI 0.93–1.12) (Table 3). Municipalities with a higher proportion of SGP voters (OR 0.89 per 5% increase, 95%CI 0.83–0.96) were associated with lower early MMR uptake. No marked difference in early MMR uptake was observed among infants with parents' country of birth known, while infants whose parents' country of birth was unknown were associated with lower early MMR uptake (OR 0.66 95%CI 0.47–0.93). Compared with very high SES areas, only areas with high SES had significantly lower early MMR uptake (OR 0.66 95%CI 0.50–0.87).

Table 3. Multi-level multivariable logistic regression analysis of factors associated with uptake of the early MMR among infants with three doses of DPTP in the 29 targeted municipalities, the Netherlands, July 2013–March 2014 (n = 8086).

Determinants		Adjusted OR	95% CI
Sex	Male	Reference	–
	Female	1.02	0.93–1.12
SGP voters	+5%	0.89	0.83–0.96
Parents' country of birth	The Netherlands - The Netherlands	Reference	–
	The Netherlands - Turkey	0.57	0.33–1.00
	The Netherlands - Morocco	0.74	0.39–1.42
	The Netherlands - European country	0.82	0.59–1.13
	The Netherlands - Other	0.87	0.64–1.19
	Morocco - Morocco	1.19	0.68–2.10
	European country - European country	0.84	0.53–1.32
	Turkey - Turkey	0.77	0.39–1.54
	Other - Other	0.80	0.59–1.09
	Unknown	0.66	0.47–0.93
Socio economic status	Very high SES	Reference	–
	High SES	0.66	0.50 – 0.87
	Low SES	0.80	0.61 – 1.06
	Very low SES	0.95	0.53 – 1.68

SGP: Reformed Political Party; OR: odds ratio.

Discussion

This is the first use of Præventis to perform a novel outbreak control strategy in response to a measles outbreak in the Netherlands. In addition, this is the first study describing the use of Præventis to assess early MMR uptake and its determinants. The existence of a national electronic immunization register such as Præventis allowed a targeted outbreak intervention, whereby infants were individually invited for an early MMR based on their risk (low MMR-1 coverage in their municipality and aged 6-14 months). We showed that in the 29 municipalities with low MMR coverage targeted for this outbreak intervention, SES, parents' with unknown country of birth and having religious objections towards vaccination were associated with low early MMR uptake.

The strengths of this study are largely due to the availability of individual level data from Præventis. For every infant born or registered in the Netherlands, their vaccination status is available in real-time. This significantly reduces the risk of bias in terms of the population studied and recall bias in relation to the collection of demographic and vaccination history. In addition, the accessibility of immunization histories in Præventis provided sufficient years of data to assess MMR-0 uptake prior to the 2013-2014 measles outbreak.

We found that MMR-0 uptake in the 29 municipalities prior to early MMR campaign was low, with two notable exceptions: birth cohorts 1998/1999 and 2006/2007. The first increase corresponds to the previous large measles outbreak in the Netherlands in 1999–2000 [14]. The second increase corresponds to the large measles outbreak in France in 2008 [15]. This was an interesting finding, as MMR-0 is outside the routine NIP schedule, and in contrast with the 2013–2014 measles outbreak, these infants did not receive an individual invitation for an early MMR based on national outbreak response recommendations. However, we were unable to decipher from the Præventis data if MMR-0 uptake among the 1998/1999 and 2006/2007 birth cohorts was due to outbreak response at local level or recommendations for travel to countries with endemic or epidemic transmission. Nonetheless, this indicates that a proportion of parents with young infants living in municipalities with orthodox Protestants are aware of the option of an early MMR and the risks of measles in infants.

Overall, 57% of the infants invited for an early MMR in 2013–2014 availed of the intervention, with 78% vaccinated with an early MMR within 14 days of invitation. We also observed that a quarter of infants were vaccinated with an early MMR before they received an invitation letter. This was possibly due to the announcement made by RIVM and Dutch politicians in June 2013 relating to the additional control measures for groups considered most at risk for measles [8], [16].

In the regression models, we found a difference between previous DPTP vaccination and early MMR uptake. According to the NIP schedule, by four months of age, infants in the Netherlands should have received three DPTP vaccinations, followed by a fourth at 11 months, a fifth at four years and a diphtheria-tetanus-polio (DTP) vaccine at nine years [7]. Overall, the uptake of DPTP in the Netherlands is high (95% of infants receive the full primary series by 12 months) [7]. However, parents who refuse to vaccinate their child with DPTP are likely to refuse other vaccines. This was evident in our study, as we found only 1% of infants without

any DPTP vaccinations were vaccinated with an early MMR. Uptake was also low for the small group with one or two DPTP doses. From a public health perspective, this is concerning, particularly in the event of future vaccine preventable disease outbreaks.

At municipality level, we found differences in uptake of early MMR with the proportion of SGP voting, which we used as a proxy for religious objections towards vaccination among orthodox Protestants. In our multi-level study, limited to infants with three DPTP vaccinations, we found infants living in municipalities with a high proportion of SGP voter-ship were negatively associated with early MMR uptake. This suggests that some orthodox Protestant parents accept DPTP vaccination, but are reluctant to accept early MMR. This may be due to parents' perceiving the risks of measles, mumps and rubella, differently from diphtheria, tetanus, pertussis and polio, which DPTP confers protection. Previous polio outbreaks in the 1970s and 1990s led to much discussion about vaccination acceptance among orthodox Protestants [17].

We found infants whose parents' country of birth was unknown, which accounted for <2% of the study population, were associated with lower early MMR uptake. In the Dutch municipal population registration, the "unknown" category is used if a parent is deceased or in the case of immigrants, from a country that no longer meets a standard definition for classification of a population e.g. Portuguese Mozambique [18]. Therefore, the unknown group may comprise of hard to reach populations with regard to healthcare access. Further engagement at local health authority levels could improve uptake of outbreak control measures, as they would have a better awareness of minority groups in their communities.

Compared with very high SES, infants living in areas with high SES were associated with lower early MMR uptake. In a previous study in the Netherlands by van Lier et al. [19], SES was also found to be associated with vaccine uptake. As we restricted our study to the 29 municipalities with low vaccination coverage, the effect of SES as a determinant could be indicative of residual confounding of orthodox Protestant families in these municipalities. The inclusion of SGP voting in the model is probably not sufficient to take into account the confounding effect of religion.

Given the historical precedence of low vaccine uptake among the orthodox Protestant communities [17], further vaccine preventable disease outbreaks are

likely. The use of Præventis to monitor areas with low vaccine coverage is hugely advantageous in preparation for and in the event of a vaccine preventable disease outbreak. Coupling vaccination data with other datasets enables identification of risk groups and associations of low vaccine uptake. In addition, having a national electronic immunization register, whereby data is regularly updated versus conducting routine vaccine coverage surveys, saves a great deal of time and is also an accurate and representative data source [6].

Some limitations of this study were the use of aggregated data with regard to SES and religion, which can make the interpretation of the results more difficult. As a result, associations at aggregated levels do not directly apply to individuals, but to a group of individuals within a given area, thus, assuming the group is homogeneous. Given the variations in SGP voter-ship, DPTP and early MMR uptake, it is apparent that this group was not completely homogenous. Furthermore, for SES and parents' country of birth, some data for the study population was missing. However, there were a sufficient number of observations remaining to observe statistically significant differences. Finally, we used available data that were not collected for the purpose of this study. Therefore, the list of presented determinants is not complete and it is possible that some were missed.

During the 1999–2000 measles outbreak, 6% of the reported measles cases (n = 196) were in infants below 14 months [14]. Older age groups were more affected in the 2013–2014 outbreak [9], while there was a 60% decrease in the number reported measles cases in infants below 14 months (n = 78) [14]. Further evaluations of immunological response to early vaccination are on-going and studies evaluating adherence to the NIP schedule following an early MMR during the measles outbreak are planned.

Conclusions

This is the first study describing the use of a national electronic immunization register to perform a targeted outbreak intervention and assess determinants of early MMR uptake in the Netherlands. It is encouraging that uptake of early MMR was achieved in more than half of the infants invited; firstly, considering early MMR is outside of the NIP schedule and secondly, considering low vaccination coverage in these municipalities. In future outbreaks, determinants of early MMR uptake found in our study could be used to tailor intervention strategies.

Acknowledgments

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Chapter 6

Vaccine effectiveness of early MMR vaccination among 6-14 month-old infants during an epidemic in the Netherlands: an observational cohort study

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Abstract:

Background

Routinely, the first measles-mumps-rubella (MMR) vaccine dose is given at 14 months in the Netherlands. However, during a measles epidemic in 2013-2014, MMR vaccination was also offered to 6-14 months-olds in municipalities with <90% MMR vaccination coverage. We studied the vaccine effectiveness (VE) of this early MMR vaccination.

Methods

Parents of all infants targeted for the early MMR were asked to participate. When parent(s) suspected measles, their infant's saliva was tested for measles-specific antibodies. VE against laboratory-confirmed and self-reported measles was estimated using Cox regression, whereby VE=1-hazard ratio.

Results

Three vaccinated and 10 unvaccinated laboratory confirmed cases occurred with a total observation time of 106,631 and 23,769 days, respectively. The unadjusted VE against laboratory confirmed measles was 94% (95%CI 79% to 98%). Adjusted for religion and sibling's vaccination status, the VE decreased to 71% (-72% to 95%). For self-reported measles the unadjusted and adjusted VE was 67% (40% to 82%) and 43% (-12% to 71%), respectively.

Conclusions

Infants vaccinated between 6 and 14 months of age had a lower risk of measles than unvaccinated infants. However, part of the effect was caused by herd immunity, since vaccinated infants were more likely to be surrounded by other vaccinated individuals.

Introduction

Measles is a highly contagious viral disease. It can lead to severe illness and even death with the greatest burden in the youngest children [1, 2]. Most acute measles deaths are due to secondary infections resulting from measles induced suppression of immune responses [3]. Measles vaccination programs have led to a large decline in global mortality, from an estimated 562,400 annual measles deaths in 2000 to 114,900 in 2014 [4].

Infants under the age of one year were at highest risk of measles in recent outbreaks in Europe [5] [6]. This is worrisome as the risk of measles-associated complications and case fatality rates are highest among infants [2, 7]. Passively acquired maternal antibodies protect infants in the first months against measles. However, infants of vaccinated women have significantly lower concentrations of maternal antibodies than infants of naturally immune women [8] and protection is on average 2-3 months shorter [8, 9]. At the age of 6 months, most infants lack detectable maternal antibodies (95% of infants of naturally immune women and 99% of infants of vaccinated women) [8].

WHO advises to administer the first dose of measles-mumps-rubella (MMR) vaccine in countries with endemic measles at the age of 9 months and in countries with low rates of measles transmission at the age of 12 months [10]. In the Netherlands, children are offered MMR vaccination at 14 months and at 9 years of age. Infants who have lost their protection from maternal antibodies are susceptible until their first vaccination. Administering vaccinations at an earlier age than 9 months may be beneficial when the risk of measles is high.

However, measles vaccination below 9 months of age has been associated with lower proportions of children who develop protective antibody levels after measles vaccination. The median proportion of children who seroconvert after measles vaccination at 8-9 months of age was 90% (IQR 82, 95) among 44 studies, while the median was 99% (IQR 93, 100) in infants vaccinated at 11-12 months in 21 studies [11]. However, the majority of these studies were conducted in developing countries. Seroconversion results by age may be different in industrialized countries. Reasons for this include lower levels of maternal antibodies since most mothers have vaccine induced immunity to measles only. In a study where infants were included without maternal antibodies, no differences were found in the seroconversion rates for infants vaccinated at 9 and 12 months of age [12].

In a systematic review of case-control and cohort studies, vaccine effectiveness (VE) against laboratory confirmed measles of a one-dose MCV (measles containing vaccine) at the age of 9-11 months was estimated to be 84%, while VE for infants vaccinated at the age of 12 months or older was 93% [13]. VE estimates for infants vaccinated <9 months of age are scarce. In a retrospective cohort study in Niger in 1995, a single dose of MCV below 9 months of age resulted in a VE of 87% (95%CI 81% to 91%) among children 6 to 59 months of age against self-reported clinical measles [14]. To date, no VE estimates have been reported against laboratory confirmed measles of infants vaccinated <9 months in observational studies. VE estimates against laboratory confirmed measles are more accurate because they discriminate measles from other diseases with rash and fever.

Here we investigated VE against self-reported and laboratory confirmed measles for infants who received an MMR vaccination between 6 and 14 months of age during a measles epidemic in The Netherlands. The epidemic started in May 2013, and lasted until March 2014 with 2700 reported cases [15]. Most cases were unvaccinated orthodox Protestant primary and secondary school aged children (Woudenberg et al, submitted). The epidemic peaked in July 2013, slowed down during the summer holiday, and progressed with a second, lower peak in October 2013. This study was possible because the Ministry of Health offered an MMR vaccination temporarily to all infants between 6 and 14 months of age living in municipalities with MMR vaccination coverage below 90%, and to infants in orthodox Protestant families living elsewhere.

Methods

Study procedures

We conducted a prospective observational cohort study during the measles epidemic in the Netherlands in 2013-2014. As part of the vaccination campaign, infants between 6 and 14 months of age living in municipalities with an MMR-1 vaccination coverage below 90% [16] were invited for an additional or an early MMR vaccination. Infants of 6-11 months of age were offered an extra vaccination (and would thus still be eligible for their second MMR vaccination at the age of 14 months), while 12-14-month-old infants were offered an early MMR vaccination as an alternative for the regular time point at 14 months of age. All infants are eligible for another dose of MMR scheduled at 9 years of age.

Approximately four weeks after the personal invitation for vaccination, all parents of infants targeted for the early MMR in the 29 municipalities received an invitation to enroll their infant(s) in the study. We could not invite parents of infants in orthodox Protestant families living outside of the 29 targeted municipalities to participate in the study, as religion is not registered in the vaccination registry of the Netherlands. Invitations to participate in the study were sent from week 35 of 2013 up to week 8 of 2014 [17]. Parents of invited infants were asked to register for the study by sending a reply form by regular mail indicating their e-mail address. Subsequently, they received a link to the online baseline questionnaire. Infants were followed until the end of the epidemic (14-03-2014). Along the follow-up period, parents were reminded monthly by e-mail to report suspected measles in their infant. When parents did so, they received a second questionnaire and a measles saliva sampling kit. The Central committee on Research Involving Human Subjects of the Netherlands approved the study.

Data collection

In the baseline questionnaire, vaccination status was asked as well as permission to check vaccination status in the national vaccination register. Parents were also asked whether their infant(s) had measles in the preceding 3 months. In the baseline questionnaire measles was defined as having fever (temperature > 38°C), exanthema, and at least one of the following symptoms: cough, runny nose, or sore eyes [18]. Other questions, among others, were about gender; day-care center attendance; vaccination status of the parent(s), and sibling(s); education level of the parent(s); religion; travel history; medication use; co-morbidities; breastfeeding, birth weight, and duration of pregnancy. The second questionnaire, which parents received when they reported to suspect their infant to have measles, consisted of questions to ascertain symptoms to diagnose self-reported measles.

Laboratory testing

When parents reported measles in their infant, they were sent a saliva sampling kit, consisting of a tube and a swab. Briefly, we applied an IgM capture enzyme immunoassay specifically designed for the detection of IgM antibodies in oral fluid specimens, according to procedures recommended by the manufacturer (MicroImmune, Hounslow, Middlesex, UK). The relative specificity and sensitivity of IgM antibody detection in oral fluid, as compared to serum, is near 100%, as reported by the manufacturer. An infant of whom the parents reported a suspected measles case and from whom the saliva sample tested IgM positive was regarded a laboratory-confirmed measles case. Laboratory testing was only offered to

suspected cases occurring after the baseline questionnaire was completed. Infants for whom it was indicated in the baseline questionnaire that they had had measles in the 3 months prior to filling out the baseline questionnaire were not offered saliva testing.

Outcomes

We estimated VE against laboratory confirmed measles and self-reported measles. For VE estimation against laboratory confirmed measles the observation time started at the date the baseline questionnaire was filled in and stopped at either the reported date of onset of disease, a second MMR vaccination or the end of the epidemic (14-03-2014), whichever came first. For self-reported measles the baseline questionnaire included a question about the occurrence of measles in the preceding 3 months. Therefore, we included this 3-month period in the observation time for outcome self-reported measles. The start of the observation time for outcome self-reported measles was therefore 3 months before the baseline questionnaire with a minimum at 6 months of age. The end of the observation time for self-reported measles was the date of onset of measles, a second MMR or the end of the epidemic, whichever came first.

Statistical analysis

Infants with missing address or no permission to check their vaccination status were excluded. We also excluded self-reported cases before the start of the observation time, cases reported 5 – 12 days after vaccination, and we excluded infants who enrolled after their 2nd MMR or enrolled after the epidemic.

VE was calculated as 1 minus the hazard ratio (HR) times 100 [19]. The HR is the ratio of the hazard rate of vaccinated infants versus the hazard rate of unvaccinated infants. Kaplan-Meier estimates were used to visualize empirical probabilities of laboratory confirmed and self-reported measles in vaccinated and unvaccinated infants. A Cox's proportional hazard model, which gives a HR as outcome, assessed the association between vaccination status and outcomes laboratory confirmed measles and self-reported measles. Due to the varying exposure to measles during an epidemic, we used calendar time as the time scale [20]. Vaccination status was included as a time-varying exposure variable; infants could contribute person-time to both the unvaccinated and vaccinated group. The vaccinated person time started 12 days after the MMR vaccination. Age was also included as a time varying variable, and was updated every quintile of the observation period.

The following covariates were considered a priori as potential confounders: age, breastfeeding, religion, sibling's vaccination status, day-care center attendance and travel history. To test which covariates we had to include in our model, we first performed bi-variable analyses. The covariate that gave the biggest relative change of the VE (with a minimum of 10%), was included in the model. Subsequently, we added the remaining covariates one by one to the model to check for another change of >10% in the VE. A final model was reached when none of the remaining covariates led to a >10% change. We tested the proportional hazards assumption by using scaled Schoenfeld residuals, where we considered the proportional hazards assumption to be valid with a P value > 0.05 for the variables in the final model. Data analysis was conducted using R (version 3.2.0). Cox proportional hazards regression model and Kaplan-Meier estimates were conducted by using the package "survival".

Vaccine

The vaccine administered during this vaccination campaign was the same as the live attenuated MMR vaccine used in the national immunization program (M-M-RVAXPRO; Sanofi Pasteur MSD). This vaccine contains at least $1x10^3$ 50% cell culture infectious dose of measles virus Enders' Edmonston strain [21].

Results

Between July 13, 2013 and March 1, 2014, 10,097 infants were invited for an early MMR vaccination in 29 municipalities (Figure 1). For 123 infants the address was not available or parents had indicated that they did not want to receive regular mail from the vaccination registry. We invited 9,974 infants to participate in the study, of whom 1,866 (19%) agreed and 1304 (13%) filled in the baseline questionnaire. In total 74 (6%) infants were excluded since parents did not give permission to check their infant's vaccination status, resulting in 1,230 (12%) eligible infants for analysis.

Characteristics of the cohort are presented in Table 1. The vaccinated and unvaccinated groups differed considerably. Vaccinated infants were on average 31 days older at enrollment. Unvaccinated infants were more likely to have an unvaccinated sibling or parent, and to go to a church with low vaccination coverage. Vaccinated and unvaccinated infants were similar regarding gender, parents' education, medication usage, co-morbidities and birth weight.

Table 1. Characteristics of early vaccinated and unvaccinated infants (n = 1230) during an outbreak of measles in the Netherlands, 2013-2014.

Characteristic	Vaccinated	Unvaccinated	p-value[a]
Infants	919	311	
Gender (male)	460 (50%)	157 (51%)	0.90
Age at enrollment days median (IQR)	273 (232,357)	242 (226,301)	< 0.001
Day care center attendance	613 (67%)	150 (48%)	< 0.001
Unvaccinated mother	147 (16%)	128 (41%)	< 0.001
Unvaccinated father	313 (34%)	166 (53%)	< 0.001
Unvaccinated sibling	5 (1%)	55 (18%)	< 0.001
Sibling with a measles infection	3 (0%)	24 (8%)	< 0.001
Religion[b]			< 0.001
high coverage	818 (89%)	192 (62%)	
intermediate coverage	99 (11%)	89 (29%)	
low coverage	2 (0%)	30 (10%)	
Education mother			0.35
Low	25 (3%)	6 (2%)	
Medium	427 (47%)	158 (51%)	
High	467 (51%)	147 (47%)	
Education father			0.46
Low	85 (9%)	28 (9%)	
Medium	492 (54%)	155 (50%)	
High	342 (37%)	128 (41%)	
Medication usage	65 (7%)	18 (6%)	0.43
Breastfeeding			< 0.001
No	237 (26%)	54 (17%)	
Breastfed	535 (58%)	164 (53%)	
Breastfeeding	147 (16%)	93 (30%)	
Holiday in a foreign country	376 (41%)	92 (30%)	< 0.001
Co-morbidities	63 (7%)	13 (4%)	0.09
Birth weight in grams median (IQR)	3565 (3215, 3910)	3590 (3280, 3878)	0.75
Duration pregnancy in weeks median (IQR)	40 (39, 41)	40 (39, 41)	0.05

[a] Differences between groups were tested with Chi-square for categorical variables and Kruskal-Wallis test for continuous variables.
[b] Religion is grouped according to the vaccination coverage in infants' community. High coverage is comparable to the general population in the Netherlands (around 95%). Medium coverage is categorized by communities with vaccination coverage ranging between 50% and 70%. Low coverage churches have vaccination coverages ranging from 10% to 30 %
IQR: Interquartile range

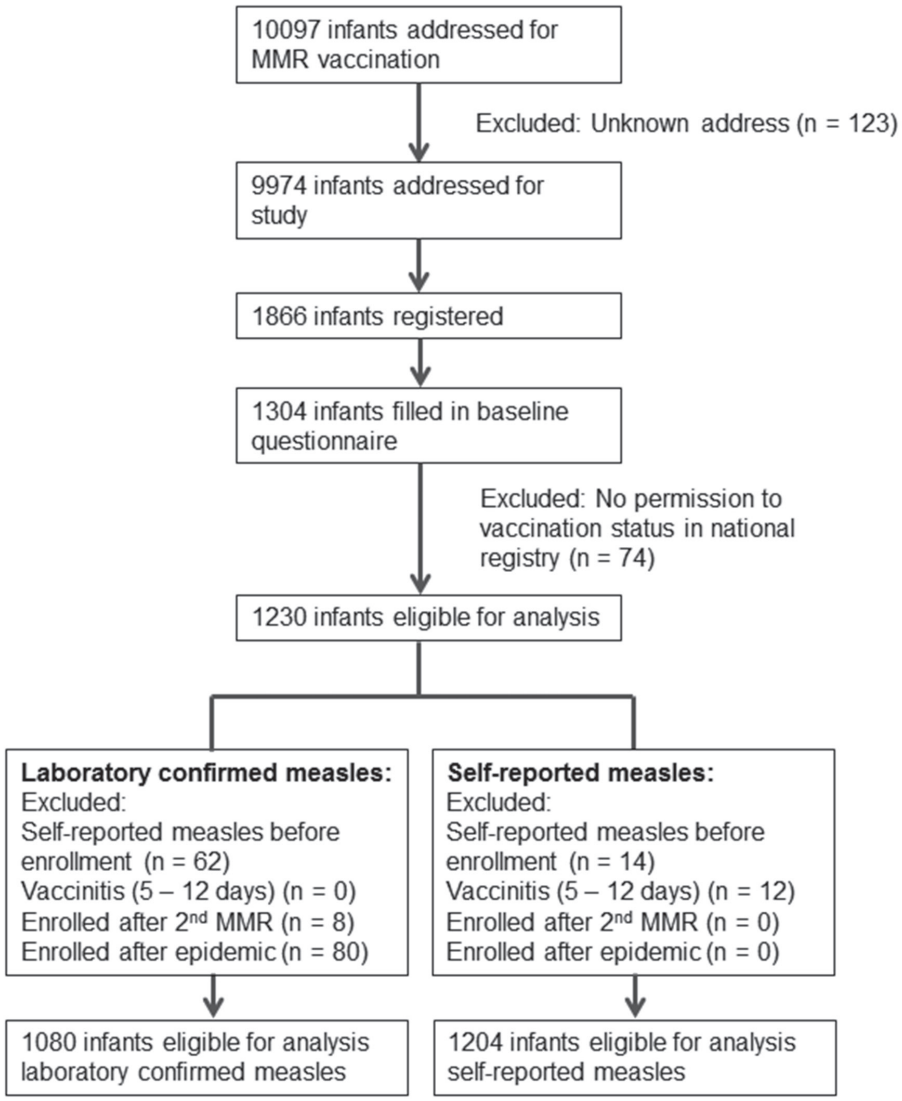

Figure 1. Flowchart study population. MMR: measles-mumps-rubella

In total, 1,080 infants were eligible for the analysis with outcome laboratory confirmed measles after the exclusion of infants with self-reported measles before the start of the observation time (n = 62), infants who enrolled after their second MMR dose (n = 8), and infants who enrolled after the measles epidemic (n = 80) (Figure 1). During the observation period, 3 vaccinated and 10 unvaccinated laboratory confirmed cases of measles were reported (Table 2). Two of the

vaccinated infants were vaccinated at 6 months of age and one at 8 months of age. Most cases occurred between September and November 2013 (Figure 2). Using Cox's proportional hazard model, we found an unadjusted HR of 0.06, which corresponds with a VE of 94% (95%CI 79% to 98%) (Table 2). When we adjusted for confounding (sibling's vaccination status and religion), VE decreased to 71% (95%CI -72% to 95%).

Table 2. VE estimates of MMR vaccination among infants 6-14 months of age against laboratory confirmed measles and self-reported measles using Cox's proportional hazard model.

	Laboratory confirmed measles		**Self-reported measles**	
	Vaccinated	**Unvaccinated**	**Vaccinated**	**Unvaccinated**
Cases	3	10	20	37
Observation time (days)	106,631	23,769	140,075	72,993
Unadjusted HR (95%CI)	0.06 (0.02 to 0.21)	Ref	0.33 (0.18 to 0.60)	Ref
Unadjusted VE (95%CI)	94% (79 to 98)		67% (40 to 82)	
Adjusted HR (95%CI)	0.292 (0.05 to 1.72)[a]	Ref	0.573 (0.29 to 1.12)[a]	Ref
Adjusted VE (95%CI)	71% (-72 to 95)		43% (-12 to 71)	

[a]: adjusted for sibling's vaccination status and religio
VE: vaccine effectiveness, MMR: measles mumps rubella, HR: hazard rate ratio, CI: Confidence interval

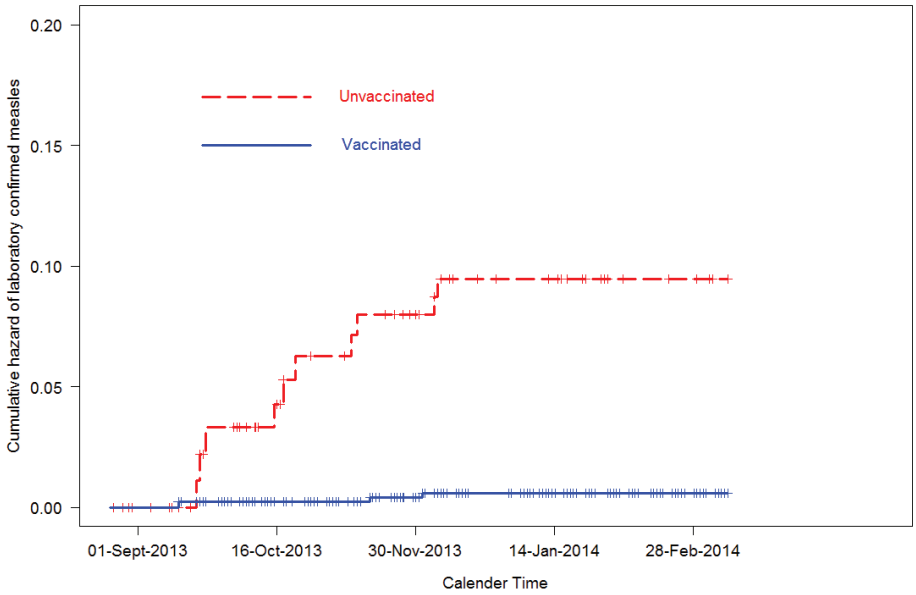

Figure 2. Kaplan-Meier curves for vaccinated and unvaccinated infants. Cumulative hazard of laboratory confirmed measles infection over time.

For the analysis with outcome self-reported measles, we excluded 12 cases who reported measles 5-12 days following after the early MMR vaccination and 14 cases who reported measles before the start of the observation time. In total, there were 20 vaccinated and 37 unvaccinated self-reported cases of measles (Table 2), which were reported throughout the observation time (Figure 3). The unadjusted VE for self-reported measles was 67% (95%CI 40% to 82%) and the VE adjusted for religion and sibling vaccination status was 43% (95%CI -12% to 71%) (Table 2).

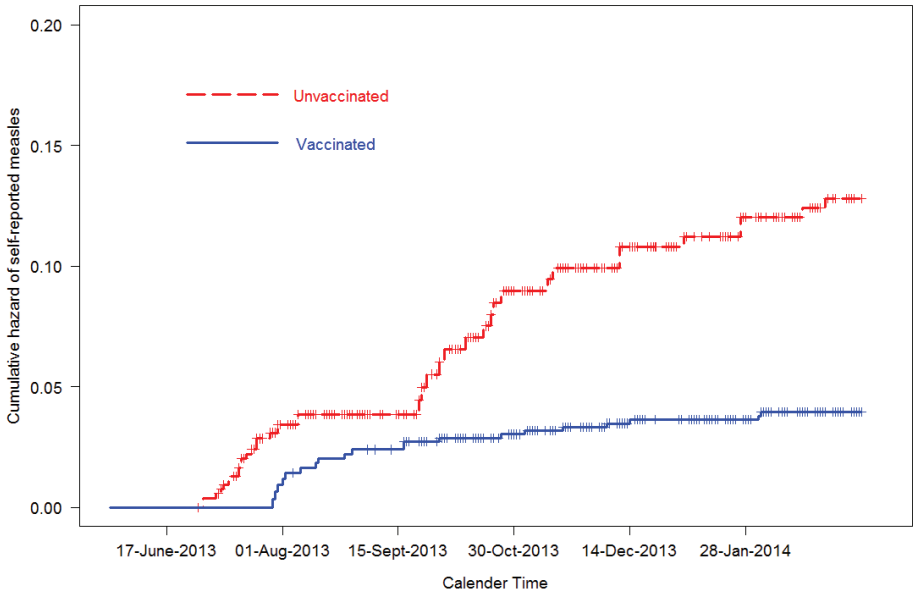

Figure 3. Kaplan-Meier curves for vaccinated and unvaccinated infants. Cumulative hazard of self-reported measles infection over time.

Discussion

We showed that infants vaccinated between 6 and 14 months of age had a reduced risk compared with unvaccinated infants of laboratory confirmed measles during an epidemic in the Netherlands, with an unadjusted VE estimate of 94%. This reduction cannot be solely attributed to the effectiveness of the vaccine. Vaccinated infants were probably to a lesser extent exposed to measles than unvaccinated infants, as the latter were more frequently a member of the orthodox Protestant community with a low vaccination coverage and had more often an unvaccinated sibling or parent. When we adjusted for these differences in exposure to measles, the VE against laboratory confirmed measles reduced to 71%. Due to low numbers, this estimate was no longer statistically significant.

Unadjusted and adjusted VE estimates against self-reported measles were 67% (95%CI 40% to 82%) and 43% (95%CI -12% to 71%) respectively. The lower VE estimates against self-reported measles compared with laboratory confirmed measles most likely reflect misdiagnosis. First, with an effective vaccine, the presence of cases misdiagnosed as measles results in a lower VE, as relatively

more of these cases are present in the vaccinated group [22]. Second, it could be that vaccinating parents may be more likely to erroneously interpret any rash appearance as measles, since they are probably less familiar with measles than parents who are opposed to vaccination. This could lead to a selective increase in false positive cases among vaccinated infants compared to unvaccinated infants and hence an underestimation of the VE. Furthermore, most laboratory confirmed cases occurred from September to October, which coincided with a peak of reported cases during the measles epidemic in the Netherlands [15], while self-reported cases in our study population occurred constantly over time. This also suggests that the misclassification of cases was more prevalent among vaccinating parents. Our estimate against outcome laboratory confirmed measles is therefore more accurate, as the laboratory test excludes most rash cases that are not caused by the measles virus.

Our adjusted point estimate against laboratory confirmed measles is consistent with the lower end of the IQR of the VE (84%, IQR 72%,95%) found in a systematic review [13]. This VE estimate was based on 44 MCV estimates using laboratory confirmation of cases and studies with a cohort or case-control design. However, this estimate was limited to infants vaccinated at 9-11 months of age while in our study infants of 6, 7 and 8 months old were also included.

A study more comparable in respect to age with our study was conducted during an outbreak in Canada [23]. Deserres et al. estimated the VE for infants 6 to 11 months of age (96%, 95%CI 72% to 99%) against clinical measles. Our adjusted estimate against laboratory confirmed measles borders the lower value of the CI, despite the inclusion of infants vaccinated between 12-14 months of age in our study. However, the Canadian study assumed comparable levels of exposure to measles between the vaccinated and unvaccinated infants, whereas we tried to include exposure to measles in our model through adjustment for surrogates of exposure to measles.

Our results indicate that exposure to measles as assessed through such proxies differed between vaccinated and unvaccinated infants and that it influenced the VE estimates. Adjustment of the VE with surrogates of measles exposure led to lower VE estimates for both self-reported as laboratory confirmed measles. This was in line with our expectations, given that the measles epidemic in the Netherlands largely took place among unvaccinated orthodox Protestant children [15], who live socio-geographically clustered [24]. Thus, we think that exposure to measles

is an important factor to take into account in the estimation of VE in observational studies. Especially, given that the parents' choice to vaccinate also depends on the choices of its social network [25] and in the event the networks of the parents' children overlap it creates clusters of unvaccinated children [26].

To our knowledge, only one randomized clinical trial has been conducted to estimate the measles efficacy of MCV in children vaccinated <9 months of age in an outbreak setting [27]. Because infants were randomly assigned to be vaccinated, different levels of exposure to measles can most likely be ruled out in this clinical trial. Martins et al. followed 1333 infants of 4.5 months of age, of whom 441 were vaccinated, for 5 months and found a VE of 94% (95%CI 74% to 98%) against laboratory confirmed measles. In comparison with our estimate this is substantially higher, all the more since infants were vaccinated at 4.5 months of age. It is, however, important to note that in this trial the Edmonston Zagreb vaccine was used, which has been reported to have a higher immunogenicity in infants than other vaccines [27, 28].

The main limitation of our study is that infants were not randomized to early MMR vaccination or not, but self-selected whether to vaccinate or not, and therefore we studied different groups in respect to exposure to measles. We have addressed this difference in exposure to measles by correcting for surrogates, but residual confounding cannot be excluded. Another limitation of our study is the low response rate and small number of cases. As a result, we did not have sufficient statistical power to find precise VE estimates, which may account for some of the variance between our VE estimates and previous estimates in literature. In addition, the small number of cases limited us to study differences in severity of disease between vaccinated and unvaccinated cases and to stratify the results by age at vaccination. [In a subgroup analysis of infants vaccinated below 9 months of age and unvaccinated infants enrolling before 9 months of age, we found an unadjusted VE against laboratory confirmed measles of 81% (95% CI, 7.6% to 96%).]

Since infants are at the greatest risk during recent outbreaks in Europe and as they are at the highest risk for complications too, it is important to protect them during outbreaks. Recently, a study by our group already concluded that MMR vaccine is safe to protect infants aged 6-14 months of age [29]. The trade-off, however, is a lower VE, leaving relatively more vaccinated infants susceptible. This lower VE can be largely voided by the additional measles vaccination recommended in the WHO schedule, given that the majority of children who failed to develop sufficient

antibodies after their first measles vaccination will develop protective antibody levels after their second measles vaccination [11].

However, a concern is that vaccinated infants who received their first MCV vaccination at 6 months of age - and despite subsequent secondary and tertiary doses - had lower levels of humoral responses at 7 – 10 years of age compared with those who received the first dose of MMR at 12 months [30]. This blunting could be associated with the interference of maternal antibodies and an immature immunity. That this effect may be of clinical relevance is suggested by first results of an outbreak investigation among students in Canada [31]. Here relatively more twice vaccinated cases were reported who received their first MMR dose at 12 months of age than twice vaccinated cases who received their first MMR at 15 months of age.

In conclusion, MMR vaccinated infants between 6-14 months of age were at lower risk of measles than unvaccinated infants. However, part of the effect was caused by herd immunity of the regular national immunization program in the Netherlands; vaccinated infants were more likely to be surrounded by vaccinated individuals and were therefore to a lesser extent exposed to measles. Our VE estimates adjusted for exposure to measles through the use of proxies suggest that the early MMR vaccination campaign in the Netherlands was effective, but precise estimates are lacking and further research on vaccine effectiveness at a young age is required. In the meantime, given the high disease burden in infants < 14 months of age and the early loss of maternal protection, early MMR vaccination is recommended when the risk of measles is high.

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Chapter 7

Tolerability of early measles-mumps-rubella vaccination in infants aged 6-14 months during a measles outbreak in the Netherlands in 2013-2014

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Abstract

Background

In 2013-2014, a measles outbreak spread through the Netherlands. To protect young infants, measles-mumps-rubella (MMR) vaccination was offered to 6-14-month-olds in municipalities with MMR1 coverage below 90%. We assessed tolerability of this early MMR.

Methods

After study-entry, parents of eligible infants (n=10,097) filled in a questionnaire. In case the infant received an early MMR (n=962), we asked information on adverse events (AEs). AE-frequencies were compared between 6-8-, 9-11- and 12-14-month-olds. Using multivariable logistic regression, we assessed the association between the risk of AEs and age at early MMR.

Results

Parents of 59 (6.1%) and 350 (36.4%) infants receiving early MMR reported local and systemic AEs, respectively. Parents of infants vaccinated at 6-8 months reported less frequently systemic AEs (32%) than parents of children vaccinated at 9-11 (45%) and 12-14 (43%) months (p=<0.001). For local AEs there were no differences (5%, 7% and 10%, respectively; p=0.08). Compared to vaccination at 6 months, all older infants, except 14-month-olds, showed an increased risk for any AE and for systemic AEs starting 5-12 days after vaccination.

Conclusions

Early MMR is well tolerated with lowest AE-frequencies found in 6-8-month-olds. Thus, it is a safe intervention to protect young infants against measles.

Introduction

Measles is a highly contagious infectious disease, with most severe disease in young infants and adults (1). Measles vaccination was introduced in the National Immunisation Programme (NIP) in the Netherlands in 1976. Since 1987, measles vaccination is given in combined measles-mumps-rubella (MMR) vaccination at 14 months and 9 year of age with corresponding coverage amounting to 96% (first dose) and 93% (first and second dose) (2).

From May 2013 until March 2014, a measles outbreak spread across the Netherlands, mainly among orthodox Protestants living in socio-geographically clustered communities with a low acceptance of vaccination (3). A previous outbreak among the same group occurred in 1999-2000 with more than 3,200 registered cases (4).

To protect infants below the age of routine MMR vaccination in high-risk areas, all infants aged 6-14 months living in municipalities with MMR1 coverage below 90% were invited for an early MMR vaccination. Current vaccination guidelines in the Netherlands already advice to vaccinate infants from 6 months onwards when there is a real risk to contract measles, e.g. when travelling to a country where measles is endemic (5). This is similar to guidelines in the United States (6).

Worldwide licensed MMR vaccines are registered from 12 months of age, while in outbreak settings they can be used from 9 months onwards. In concordance with the Summary of Product Characteristics (SPC) information, infants receiving MMR vaccination before 12 months of age are offered a second MMR vaccination after the age of one year because of the beneficial effects on the cellular and humoral immune response against measles (7). Irrespective of early MMR vaccination, in the Netherlands all children are offered another dose of MMR at the age of nine years. The advice to vaccinate infants aged 6 months and older was based on Dutch population-based seroprevalence data from 1995-1996 and 2006-2007, combined with evidence on age-specific immunogenicity and effectiveness (8-11). The seroprevalence data suggested that most infants of 6 months or older lacked maternal antibodies, especially when they were born to vaccinated mothers.

MMR vaccination from 6 months old onwards is regarded safe based on studies mainly performed during vaccination campaigns in developing countries (12-15). Some studies also show beneficial effects due to a reduced overall mortality after early measles vaccination (16). In the light of continuing measles outbreaks in

developed countries and the need to comply with WHO targets for eliminating measles and rubella, information on effectiveness, safety and impact of early MMR vaccination gives valuable input to policy makers responsible for outbreak control measures.

In this article we describe and discuss results of the tolerability monitoring of the early MMR vaccination campaign in the Netherlands.

Methods

Setting and participants

In response to the measles outbreak among orthodox Protestants, an outbreak management team decided on June 17th 2013 to offer early MMR vaccination to all infants between 6 and 14 months of age living in municipalities with MMR1 coverage below 90%. On July 13th 2013, parents of eligible infants received a personal invitation for early MMR through the routine vaccination programme register. The Netherlands has a very complete national vaccination registration, which allows direct targeting of additional vaccination to risk groups (17). Thereafter, all parents of infants turning 6 months in the previous week and resident in the eligible municipalities received an invitation for early MMR vaccination of their infant. Last invitations were distributed in week 8 of 2014. To avoid interference with the willingness to vaccinate, invitations to participate in our study were sent 4 weeks after the invitation for vaccination. Parents willing to participate could return an application form with their e-mail address. In return, they received a link to an online questionnaire.

Parents who indicated in the past that they do not want to receive regular mail from the vaccination registry were not invited to participate in our study. For this study IRB-approval was not necessary, as checked with the Central Committee on Research Involving Human Subjects of the Netherlands.

Vaccine

The vaccine administered during this vaccination campaign was identical to the MMR vaccine used in the NIP at 14 months and 9 years of age at that time (MMRvaxpro®; Sanofi Pasteur MSD). This vaccine contains at least $1x10^3$ 50% cell culture infectious dose ($CCID_{50}$) measles virus Enders Edmonston strain, $12.5x10^3$ $CCID_{50}$ mumps virus Jeryl Lynn™ strain and $1x10^3$ $CCID_{50}$ rubella virus Wistar RA

27/3 strain. All strains are live attenuated. Measles and mumps strains are produced in chick embryo blasts, whereas the rubella strain is produced in WI-38 human diploid lung fibroblasts. The vaccination is given subcutaneously in the upper arm.

Data collection

The online questionnaire asked for demographics of the infant eligible for early MMR and of the entire household. Furthermore, past and present measles infections and vaccination status of all household members was ascertained. In case early MMR was administered, questions about local and systemic adverse events (AEs) were asked with details on severity, interval with vaccination and duration of symptoms. Tolerability data are only available for infants who received the early MMR vaccination before parents filled in the first questionnaire.

Outcome definitions

Local AEs were classified as mild, moderate or pronounced. Systemic AEs were dichotomized. We defined fever as a temperature ≥38.0°C., measured sublingual, intra-auricular or rectally, based on the Brighton Collaboration case definition (18). Very high fever was defined as a temperature ≥40.5°C. Time between early MMR and start of systemic AEs was divided in three periods, i.e. start on days 0-4, 5-12 or ≥13.

Covariates

All covariates were retrieved from the questionnaire. If parents permitted, their infant's vaccination status was checked in the national vaccination register. All other covariates were self-reported without validation.

Statistics

Frequencies and means of demographics, local and systemic AEs are presented overall and stratified by age, categorizing infants in 3 age groups; 6-8-, 9-11- and 12-14-month-olds. Differences were tested using Pearson's Chi Square or Fisher's exact test (for dichotomous and categorical variables) or student t-test (for continuous variables).

To assess whether age at time of the early MMR was associated with risk for any AE (i.e. local or systemic AE in any risk window) or with systemic AEs starting 5-12 days after early MMR vaccination only, we performed multivariable logistic regression. Hereby age was categorized per month. Covariates with a plausible or known effect on the outcome were part of the multivariable model as possible confounders,

i.e. sex, underlying disease of the infant, ever being breastfed, gestational age, older siblings in the household, maternal age and educational level, measles vaccination status and past measles infection of the mother and reasons to refuse vaccination (see also Table 1). Using stepwise backward selection, all covariates with <10% influence on the estimate of the main determinant, i.e. age in months, were discarded from the model. We also assessed possible interactions. Risks are presented as odds ratios (ORs) with 95% confidence intervals (95%CI).

Analyses were performed using SAS version 9.3. In all analyses, a p-value <0.05 was considered statistically significant.

Table 1. Absolute number (%) of background characteristics per age group.

Age at administration of first MMR		**6-8-months; N=603 n (%)**	**9-11-months; N=239 n (%)**	**12-14-months; N=120 n (%)**	**p-value**
Background characteristics					
Day-care attendance	no	199 (33.0%)	81 (33.9%)	41 (34.2%)	0.9
	yes	404 (67.0%)	158 (66.1%)	79 (65.8%)	
Ever breastfed	no	176 (29.2%)	51 (21.3%)	23 (19.2%)	**0.01**
	yes	427 (70.8%)	188 (78.7%)	97 (80.8%)	
Underlying disease of infant	no	559 (92.7%)	230 (96.2%)	108 (90.0%)	0.06
	yes	44 (7.3%)	9 (3.8%)	12 (10.0%)	
Gender	male	301 (49.9%)	113 (47.3%)	67 (55.8%)	0.3
	female	302 (50.1%)	126 (52.7%)	53 (44.2%)	
Duration of pregnancy	37-44 wk	567 (94.0%)	228 (95.4%)	109 (90.8%)	0.6
	32-36 wk	28 (4.6%)	9 (3.8%)	9 (7.5%)	
	26-31 wk	6 (1.0%)	2 (0.8%)	2 (1.7%)	
	unknown	2 (0.3%)	0	0	
Older siblings in the household	no	273 (45.3%)	116 (48.5%)	47 (39.2%)	0.2
	yes	330 (54.7%)	123 (51.5%)	73 (60.8%)	

Table 1. Continued

Age at administration of first MMR		6-8-months; N=603 n (%)	9-11-months; N=239 n (%)	12-14-months; N=120 n (%)	p-value
Background characteristics					
Refusing vaccination based on life philosophy or religion	no	544 (90.2%)	212 (88.7%)	100 (83.3%)	0.2
	moderate	58 (9.6%)	26 (10.9%)	20 (16.7%)	
	strong	1 (0.2%)	1 (0.4%)	0	
Maternal year of birth	1986-1995	113 (18.7%)	40 (16.7%)	17 (14.2%)	**0.02**
	1976-1985	444 (73.6%)	168 (70.3%)	86 (71.7%)	
	1966-1975	37 (6.1%)	26 (10.9%)	17 (14.2%)	
	unknown	9 (1.5%)	5 (2.1%)	0	
Maternal educational level	no education or only primary/ secondary school	50 (8.3%)	24 (10.0%)	7 (5.8%)	0.5
	intermediate vocational education	230 (38.1%)	98 (41.0%)	51 (42.5%)	
	higher vocational education or university	316 (52.4%)	113 (47.3%)	62 (51.7%)	
	unknown	7 (1.2%)	4 (1.7%)	0	
Maternal vaccination status	unvaccinated	39 (6.5%)	23 (9.6%)	5 (4.2%)	0.3
	vaccinated	511 (84.7%)	194 (81.2%)	103 (85.8%)	
	unknown	53 (8.8%)	22 (9.2%)	12 (10.0%)	
Past maternal measles infection	no	405 (67.2%)	153 (64.0%)	69 (57.5%)	0.2
	yes	81 (13.4%)	32 (13.4%)	17 (14.2%)	
	unknown	117 (19.4%)	54 (22.6%)	34 (28.3%)	

Results

Response

In total 10,097 infants in all (n=29) municipalities with MMR1 coverage below 90% in 2012 were invited for an early MMR. Of these, parents of 9,974 infants were invited to participate in the study (for 123 infants the address was not available or parents had indicated that they do not want to receive regular mail from the vaccination registry). Parents of 1,866 infants (19%) responded. Finally, parents of 1,304 infants (13%) filled in the first questionnaire. By the time parents filled in the first questionnaire, 962 infants (74%) had already received an early MMR. We report tolerability data of these 962 infants.

The median interval between MMR0 and filling out the questionnaire was 49 days (mean 51.4d, range 1-211). For 6-8-month-olds, the median interval was 44d (mean 48.7d, range 1-211), while this was 57.2d (mean 55d, range 9-148) and 51d (mean 53.3d, range 8-144) for 9-11- and 12-14-month-olds, respectively. Differences in median interval between the groups were statistically significant ($p<0.0001$).

Demographics

Median age at early MMR vaccination was 7.0 months (range 5.7-14.9). In total 603 (62.7%) infants received their early MMR at the age of 6-8 months (median 6.3), whereas 239 (24.8%) and 120 (12.5%) infants received their early MMR at age 9-11 months (median 10.0) and 12-14 months (median 12.7), respectively. An equal number of boys and girls (n=481; 50%) received early MMR vaccination during this campaign. Sex distribution between the three age groups were equal ($p=0.3$; Table 1). Furthermore, we found no differences in day-care attendance; underlying disease of infant; duration of pregnancy; presence of older siblings; refusal of vaccination based on life philosophy or religion; maternal educational level; maternal vaccination status and maternal measles infection in the past between the three age groups. In contrast, 6-8-month-olds were less frequently ever being breastfed ($p=0.01$) and less frequently had a mother in the oldest age category, i.e. older than 38 years ($p=0.02$) than infants of older age groups.

Local AEs

Parents of 59 infants (6.1%) reported one or more local AEs following the early MMR (Table 2). There was a trend of an increasing frequency of local AEs with increasing age, but differences were not statistically significant ($p=0.08$). We found no difference in the frequency of any local AE between the early and the late

responders, both in the overall study population (p=0.09) as well as in the three age groups (p=0.2, p=0.5 and p=0.6 for respectively 6-8m, 9-11m and 12-14m). Redness (n=53; 5.5%) was reported most often, followed by pain (n=40; 4.2%) and swelling (n=33; 3.4%). Redness, pain and swelling started within 24 hours after vaccination in 72%, 80% and 82% respectively, whereas symptoms lasted less than three days in 72%, 75% and 70%. Parents of 8 (0.1%), 6 (0.1%) and 4 (0.07%) infants reported that respectively redness, pain and swelling was pronounced.

Table 2. Absolute number and frequencies of local and systemic adverse events per age group and overall.

Age at administration of the early MMR	6-8-months; N=603 n (%)	9-11-months; N=239 n (%)	12-14-months; N=120 n (%)	Difference between age groups; p-value	Total; N=962
Any local AE	30 (5%)	17 (7%)	12 (10%)	0.08	59 (6%)
redness	26 (4%)	15 (6%)	12 (10%)	0.58	53 (6%)
pain	21 (3%)	13 (5%)	6 (5%)	0.15	40 (4%)
swelling	15 (2%)	11 (5%)	7 (6%)	0.67	31 (3%)
Any systemic AE	191 (32%)	108 (45%)	51 (43%)	0.0004	350 (36%)
listlessness	149 (25%)	87 (36%)	38 (32%)	0.68	274 (28%)
fever	106 (18%)	68 (28%)	25 (21%)	0.22	200 (21%)
crying	98 (16%)	59 (25%)	28 (23%)	0.82	185 (19%)
rash	46 (8%)	48 (20%)	22 (18%)	0.0004	116 (12%)
sleeping problems	52 (9%)	27 (11%)	15 (13%)	0.83	94 (10%)
diarrhoea	17 (3%)	10 (4%)	4 (3%)	0.96	31 (3%)
vomiting	12 (2%)	7 (3%)	2 (2%)	0.79	21 (2%)
paleness	11 (2%)	7 (3%)	2 (2%)	0.81	20 (2%)

Systemic AEs

Parents of 350 infants (36.4%) reported one or more systemic AEs (Table 2). Parents of infants who were 6-8 months old at the time of early MMR reported less frequently systemic AEs than older age groups (p<0.001). Overall frequencies were 31.7% (n=191), 45.5% (n=108) and 42.5% (n=51) for those aged 6-8, 9-11 and 12-14 month olds, respectively. We found no difference in the frequency of any systemic AE between the early and the late responders in the overall study population (p=0.1) and in the 12-14-month-olds (p=0.3). In the 6-8-month-olds the frequency of any systemic AE was higher in the early responders than in the late responders (52.8% vs 47.2%; p=0.05). Likewise, among the 9-11-month-olds early responders reported any systemic AE in 56.9% compared with 43.1% in late

responders (p=0.04). No differences in the frequencies of specific systemic AEs were found between age groups, except for rash, which occurred less frequently in the group with the youngest age at vaccination (8%, 20% and 18%, respectively).

Listlessness (n=274; 28%) was reported most often, followed by fever (n=182; 19%), crying (n=185; 19%), rash (n=116; 12%) and sleeping problems (n=94; 10%). Parents of 2 infants reported fever with a temperature of 40.5°C or higher. For one of these fever started within the risk window 5-12 days after vaccination. Most systemic AEs started 5-12 days after the vaccination, with a range of 62% to 75% for specific systemic AEs. A minority of parents reported a start of symptoms within 4 days after vaccination (range of percentages regarding different systemic AEs 13%-26%) or more than 12 days after vaccination (range 5%-24%). In 30%-69% of specific systemic AEs, duration of symptoms was 2 days or less, whereas in 15%-26% and 16%-50% symptoms lasted 3 days or 4 days and more, respectively.

Influence of age on occurrence of local and systemic AEs

After entering all possible confounders in the multivariable logistic regression, for both outcomes stepwise backward selection led to removal of all covariates, i.e. no adjustment was necessary. With 6-month-olds set as reference, ORs for all older ages were above 1 (range 1.1-2.7 and 1.4-4.0 for any AE and systemic AEs 5-12d after vaccination, respectively) except for 14-month-olds (ORs 0.5 and 0.8) for local and systemic AEs (Table 3). For any AE, ORs were not statistically significant in 8- and 14-month-olds, whereas for systemic AEs occurring 5-12 days after vaccination ORs were non-significant in infants aged 7, 8, 12 or 14 months.

Table 3. Logistic regression analysis of risk of any AE and of systemic AEs 5-12d after early MMR and age.

	Infants	**Any AE**			**Systemic AEs 5-12d after vaccination**		
age at time of MMR0		yes N (%)	OR	95%CI	yes N (%)	OR	95%CI
6 months	388	120 (31%)	ref		76 (20%)	ref	
7 months	123	51 (41%)	1.58	1.04-2.4	31 (25%)	1.38	0.86-2.23
8 months	81	27 (33%)	1.12	0.67-1.86	20 (25%)	1.35	0.77-2.37
9 months	72	31 (43%)	1.69	1.01-2.82	24 (33%)	2.05	1.18-3.56
10 months	81	39 (48%)	2.07	1.28-3.37	27 (33%)	2.05	1.21-3.47
11 months	66	30 (45%)	1.86	1.1-3.16	20 (30%)	1.79	1-3.19
12 months	68	31 (46%)	1.87	1.11-3.16	18 (26%)	1.48	0.82-2.68
13 months	55	30 (55%)	2.68	1.51-4.75	27 (49%)	3.96	2.21-7.11
14 months	12	2 (17%)	0.45	0.1-2.07	2 (17%)	0.82	0.18-3.83

Discussion

To our knowledge, this is the first study that assessed tolerability of MMR vaccination administered from 6 months of age onwards in a developed country. We showed that this early MMR was well tolerated and that AEs in infants receiving their first MMR dose at 6-8-month of age were less frequent compared to MMR administered at 14 months, the age when routine MMR1 vaccination is scheduled in the Netherlands.

We found that the occurrence of AEs is age dependent. Frequencies of all local and most systemic AEs were lower in the youngest age group of 6-8 month olds compared with older age groups. For both local as well as most systemic AE frequencies were lowest in the youngest age category. For fever and rash, we found respectively 15% and 7% (6m), 20% and 20% (9m), and 24% and 15% (12m). However, only the frequencies of rash and all systemic AEs combined differed statistically significant between the age groups. Studies performed in Uzbekistan and Malawi found no influence of age on the occurrence of specific AEs with measles containing vaccines administered at 6 and 9 month of age (12, 13). Bolotovski et al. found frequencies of 6-14% for fever and rash after administration of several measles vaccines differing in strain and potency to 6 (n=1202) and 9 (n=1250) month old infants (13). AEs were collected via an interview during a home visit in the second week after vaccination. In the study of Helfand et al. proportions for fever and rash were somewhat lower than in our study (14% and 1%, 6m; n=512 and 11% and 1%, 9m; n=572), following measles vaccination of a HIV-unexposed control group (12). In the study of Helfand et al., parents recorded AEs in a daily log for 21 days after vaccination. The differences in the frequency of AEs between these studies and our study may be attributed to varying methods of AE ascertainment. Furthermore, Bolotovski and Helfand presented no case definition and cut-off for fever, possibly leading to different counting of cases with fever, which perhaps partly explains the differences.

In a study on German infants, receiving MMR, 70% of 9-11-month-olds (n=43) and 76% of 12-14-month-olds (n=29) reported fever (19). This is much higher than the frequencies we found (28% and 21%), but these differences are difficult to interpret giving the small sample size of the German study.

Another possible explanation for the lower frequencies of AEs in younger infants is the presence of maternal antibodies against measles virus that prohibit replication

of vaccine virus and thereby prevent the occurrence of AEs. Dutch seroprevalence data showed that, in the general population, immunoglobulin G antibody levels were below the cutoff for protection in 54% of 3-month-old infants (95% confidence interval, 34%–74%) (9). Among children born to orthodox reformed Protestant mothers who in general were naturally infected, the duration of protection was approximately 2 months longer(10). Furthermore, breastfeeding, maternal vaccination status, and past measles virus infection of the mother were included in the multivariable regression analysis but did not influence the main estimate by >10% and were therefore not considered as confounders. Therefore, we think the influence of maternal antibodies is limited. However, we cannot exclude a possible influence of nondetectable, residual maternal antibodies. Furthermore, young infants are immunologically immature, which may also lead to less reactogenicity.

Two other Dutch surveys on the tolerability of MMR1, given at children aged 14 months, found different frequencies of local and systemic AEs than we assessed in our 12-14-month-olds (20, 21). Kroesbergen et al. (n=863) found 9% local reactions and 32%, 38% and 24% for fever, crying and rash, respectively (20), while Jongerius et al. (n=391) found 24%, 20% 17% and 17% for the respective AEs (21). In our study, frequencies were 10%, 14%, 18% and 13%. The lower frequencies we found may be explained by study logistics: study participation was asked 4 weeks after the invitation for vaccination and infants received the vaccination before parents filled in the survey and maybe they did not remember all AEs, in particular the less severe symptoms. Another possible explanation for the lower frequency of AEs found in our study is that our primary aim was to assess vaccine effectiveness with additional questions on AEs, while both MMR1 surveys exclusively assessed tolerability. Therefore, frequencies found probably suffer less from over-reporting compared with the two tolerability surveys.

Apart from this survey, parents were asked to report AEs after vaccination to the Dutch Pharmacovigilance Center, Lareb. Lareb received 11 reports, of which 2 involved serious systemic AEs (1 infant had febrile convulsion and 1 experienced crying and dehydration).

Our study has several limitations. First, only 13% of the parents of eligible infants completed the questionnaire, which may hamper generalizability. However, the overall early MMR vaccination coverage in the 29 municipalities was 66%, while 74% of the infants in our study received early MMR vaccination. These percentages do not differ very much. Therefore, we think the risk of bias is low, despite the low

response rate. Furthermore, the sex distribution in our study is comparable to the distribution in the general population.

The overall median interval between early MMR vaccination and questionnaire completion was >1.5months. This possibly influenced the reported AEs, resulting in an underestimation. However, because the age group in which this interval was shortest also had the lowest AE frequencies, recall bias may be limited.

Because this outbreak occurred in a high-income country, results may be less applicable to developing countries. The latter countries often have a less developed healthcare system and a greater prevalence of malnutrition, possibly (1) resulting in an impaired immune response and (2) influencing the occurrence of AEs.

Furthermore, all AEs were self-reported without additional validation. This may have led to an overestimation of AE frequencies. As known from the twin study by Peltola et al on MMR vaccine–associated AEs, the vast majority of AEs following MMR vaccination are temporally associated but not causally related (22). Therefore, most AEs reported in our study were probably not caused by MMR vaccination. Since we did not compare results with the occurrence of symptoms in age matched unvaccinated children, we could not assess causality. We also were unable create an internal control group by monitoring the occurrence of the AEs prior to vaccination, because we sent invitations to participate 4–5 weeks after the invitation for vaccination so that there would be no interference with parent's decision regarding the vaccination. However, the rates found are useful for monitoring variation in AE frequencies between groups and over time and an efficient and easy way to monitor tolerability.

To conclude, our results show that early MMR vaccine administration during an outbreak is safe to protect infants aged 6–14 months against measles. Frequencies of local and common systemic AEs were lowest in younger age classes.

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Chapter 8

Additional evidence on serological correlates of protection against measles: an observational cohort study among once vaccinated children exposed to measles

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Abstract

To assess correlates of protection against measles and against subclinical measles virus (MV) infection, we recruited once-vaccinated children from geographic regions associated with increased MV circulation and/or at schools with low vaccination coverage in The Netherlands.

Paired blood samples were collected shortly after onset of the measles outbreak and after the outbreak. A questionnaire was used to document the likelihood of exposure to MV and occurrence of measles-like symptoms. All blood samples were tested for MV-specific antibodies with five different assays. Correlates of protection were assessed by considering the lowest neutralizing antibody levels in children without MV infection, and by ROC analyses.

Among 91 participants, two seronegative children (2%) developed measles, and an additional 19 (23%) experienced subclinical MV infection. The correlate of protection against measles was lower than 0.345 IU/ml. We observed a decreasing attack rate of subclinical MV infection with increasing levels of specific antibodies until 2.1 IU/ml, above which no subclinical MV infections were detected. The ROC analyses found a correlate of protection of 1.71 IU/ml (95%CI 1.01-2.11) for subclinical MV infection.

Our correlates of protection were consistent with previous estimates. This information supports the analyses of serosurveys to detect immunity gaps that require targeted intervention strategies.

Introduction

Measles is a highly contagious viral disease. Around 10 days after exposure the first clinical symptoms occur, consisting of fever, cough, coryza and conjunctivitis. About three days after the onset of fever, a maculopapular rash spreads from the face and the neck to the extremities [1]. Complications due to opportunistic pathogens most often present in the respiratory tract. Measles virus (MV) infects cells of the immune system and causes lymphopenia leading to immunosuppression, which can last up to 2 to 3 years, leaving individuals vulnerable to secondary infections [2,3].

Measles vaccination programs have led to a significant decrease in measles incidence, resulting in corresponding reductions in measles mortality and morbidity [4]. Worldwide coverage with the first dose of a measles-containing vaccine increased to around 85% but stabilized since 2009. As the incidence of measles is decreasing, population immunity will gradually become more dependent on vaccine-induced immunity.

Vaccine-induced immunity provides lower measles antibody concentrations than natural-induced immunity [5-8]. While natural infection has been shown to provide life-long immunity [9], 2 – 10% of individuals with vaccine-induced immunity may not develop or sustain protective humoral immunity [10-12]. As a result, outbreaks of measles, generally initiated by unvaccinated index cases, have been observed in vaccinated populations [13-15]. In vaccinated subjects, clinical symptoms following MV infection may range from being absent (subclinical MV infection associated with secondary immune responses) to full-blown measles. MV transmission from twice-vaccinated individuals has rarely been observed [13,16]. From the perspective of measles elimination, monitoring the immunity against measles of vaccinated populations is essential.

Immunity against measles consists of both humoral and cellular immune responses. Humoral immunity is mostly involved in the prevention of MV infection whilst cell-mediated immunity is required to clear the virus once infection has occurred. Tests for humoral immunity are more widely available and standardized than those for cellular immunity and are therefore most often used to assess measles immunity. The presence of neutralizing antibodies, commonly demonstrated by the plaque reduction neutralization, is considered the most reliable assay for serological immunity.

Measuring antibody levels prior to and after exposure to MV combined with surveillance of measles has provided some insight in the correlate of protection, i.e. the antibody level needed to prevent against disease or infection [17]. However, this evidence is limited to a few studies with a small number of participants. In the US [18], 7 out of 9 students with neutralizing antibody concentrations equivalent to values below 0.12 IU/ml measured prior to an outbreak developed measles compared with none of 71 students with titers above 0.12 IU/ml. In Senegal, where a measles outbreak occurred during a vaccine trial, 13 of 36 (36%) children with titers of 0.04 to 0.125 IU/ml developed measles, compared with 7 out of 258 (3%) of those with titers higher than 0.125 IU/ml [19]. However, the authors noted that many seronegative vaccinated children were also protected against measles, most likely indicating the presence of cellular immunity [19]. In the Netherlands, during a measles outbreak among health care personnel, antibody titers up to 0.146 IU/ml were insufficient to protect against measles [14].

Correlates of protection against subclinical infection (as measured by secondary immune responses) were studied during an outbreak among children in Luxembourg. Exposed parents who previously experienced measles were boosted when their pre-exposure neutralization titers were below 64. None of these parents reported any symptoms [20]. Among vaccinated individuals, estimates of correlates of protection against subclinical MV infection range from approximately 1.0 IU/ml [18,21] to 4.0 IU/ml [19].

In the Netherlands, immunization programs against measles were introduced in 1976 (monovalent measles vaccine), followed by the introduction of measles-mumps-rubella (MMR) vaccination in 1987. The first MMR is given to infants at 14 months of age and the second dose to children at 9 years of age. Vaccination coverage was around 95% for a significant period of time for the first dose [22], and measles has become a rare disease. However, large outbreaks continue to occur among unvaccinated children in the orthodox Protestant community, as observed in 1983, 1988, 1993, 1999/2000, and 2013/2014 [23,24]. Orthodox Protestants form a socially and geographically clustered minority group in the Netherlands of about 250.000 individuals among whom vaccination coverage is approximately 60% [25]. Foreseeing an outbreak based on a serosurvey and mathematical modelling [5,26], we designed a study to assess immunological correlates of protection against measles, assuming that vaccinated children attending schools with low vaccination coverage would likely be exposed to MV. The outbreak, which included an estimated 30,000 measles cases, started in May 2013 and lasted until March

2014 [27]. The main objective of our study was to identify serological correlates of protection against measles and MV infection among once vaccinated children.

Materials and Methods

Study design

We performed an observational cohort study among once vaccinated children aged 4-8 years during the 2013-14 measles epidemic in the Netherlands. Pairwise blood samples were collected. Four serological tests were used to determine MV infection. Neutralizing antibody concentrations were determined in the fifth assay to assess the correlate of protection.

Participants

Eligible children had received one MMR vaccination, M-M-RVAXPRO (Merck & Co., Inc.) containing more attenuated MV Enders' Edmonston strain, and were enrolled in an orthodox Protestant primary school. Children who received a second MMR during the study period were excluded from the analyses. Eligible children were invited by two approaches. The first approach consisted of the identification of MMR-1 vaccinated children of 4 to 8 years of age from the national vaccination register resident in municipalities with orthodox Protestant schools. These municipalities were chosen to ensure that children had a probability to be enrolled in an orthodox Protestant primary school. After the number of participants from the first approach remained unsatisfactory low, an additional approach was used, in which participants were invited directly via orthodox Protestant primary schools. This was only feasible when the management team of the school gave permission for this to the local municipal health service.

Data collection

Parents of all potentially eligible children were sent an invitation letter, an informed consent form and a questionnaire. The latter ascertained their eligibility and consisted of questions including sex, date of birth, and whether the child experienced measles in the past. Parents of eligible children were subsequently invited to attend a clinic where their child was asked to give a blood sample through a venipuncture. This blood sample was taken shortly after onset of the outbreak.

After March 2014 when the outbreak had ended [27], children were invited to attend the clinic for a finger stick blood sample. Parents of children were then asked to fill in a second questionnaire, which documented potential exposure to MV and occurrence of clinical symptoms potentially related to MV infection (rash, fever, cough, conjunctivitis, sore throat, coryza, Koplik spots, headache, listlessness, vomiting, diarrhea, swollen glands in neck) in the period between the two blood samplings. We defined exposure to MV at school by measles cases reported from the school to the national register of notifiable diseases during the measles outbreak. Exposure to MV elsewhere was ascertained from information provided by parents in the questionnaire. Children who were enrolled in an orthodox Protestant school without reported measles cases and who were not exposed to measles by parental recall were excluded from all analyses.

Laboratory tests

All serum samples were tested pairwise for measles specific antibodies with five serological assays: A bead-based multiplex immunoassay (MIA) for total MV specific IgG [28], an immunofluorescence assay to detect antibody levels specific for MV-F (FIgG) or MV-H (HIgG) [29], an indirect EIA to detect antibodies to MV-N (NIgG) [30] and by an in-house focus reduction neutralization test (FRNT). Laboratory tests are further specified in Supplement 1.

Case classification

We decided a priori that children could be classified into three classes: those having had MV infection prior to the study period, MV infection during the study period, or no MV infection. We defined clinical measles by fever, rash, and at least one out of cough, coryza, and conjunctivitis, as reported in the questionnaire filled in by parents [31]. We identified MV infections by calculating the ratio between pre- and post-test results of four out of the five immunoassays used (the MIA, HIgG, FIgG and NIgG). The fifth assay (FRNT) was used independently to assess the correlates of protection. The 10log-transformed normalized ratios of the four immunoassays were used to classify children using k-means cluster analyses. This involves applying an algorithm to differentiate groups while minimizing the within-cluster sum of squares [32]. Normalizing the ratios ensured that the deviation from the average per sample was considered equally for all four immunoassays in the k-means cluster analyses.

Statistical methods

We estimated the overall attack rate of MV infection by including infections occurring prior to and during the study period. Univariable logistic regression was used to assess determinants of MV infection and to determine whether the occurrence of symptoms differed between those who experienced MV infection during the study period and those who did not.

Correlates of protection against measles or subclinical MV infection were assessed among all participants except those who experienced MV infection prior to the first sampling. First, we considered as the correlate of protection the lowest concentration in pre-sera among children who did not develop clinical measles or subclinical MV infection during study period. Second, we assessed the correlates of protection using receiver operator characteristics (ROC) analyses. The FRNT antibody level corresponding with the highest sum of the sensitivity and specificity on the ROC curve was considered the correlate of protection. The area under the curve (AUC) was estimated as reported previously [33]. We also assessed whether a relationship existed between pre-exposure antibody concentrations and the attack rate of MV infection during follow up using Fisher's exact test.

An assumption in the assessment of the correlate of protection is that all children were exposed during the outbreak. We performed a sensitivity analysis to assess whether our estimates would hold with a selection of children who were most likely exposed (enrolled in a school with reported measles cases and exposed to measles according to their parents).

Data visualization and data analyses were carried out using R (version 3.4.0). Package "pROC" was used to visualize the ROC curve and to assess the optimal cut-off [34].

Ethics statement

The Central Committee on Research involving Human Subjects (CCMO) provided ethical permission to perform the study (CCMO 13.0520). Informed consent was obtained from the parent(s) of the children.

Results

Of 13344 parents invited through the national vaccination register, 2579 submitted the initial questionnaire. Of these, 279 of their children were eligible and were invited for the first blood sampling. Blood samples were taken from 27 children. Via orthodox Protestant schools, parents of 738 children were invited to participate. Blood samples were collected from 92 children. In total, 119 children were enrolled in the study. Of these, 28 children were excluded from the analyses: Sixteen because they received a second MMR during study period, three because they did not participate to the second sampling, and nine because they were not enrolled in an orthodox Protestant school with reported cases of measles nor experienced exposure to MV according to their parents. Thus, the analyses included 91 children; of whom 21 were enrolled via the national vaccination register and 70 via orthodox Protestant schools.

Descriptive results

Of the 91 participants, 41 were boys (Table 1). Median age at the first sampling was 6.5 years (IQR 5.5 – 7.5). Median follow-up period was 8.4 months (IQR 6.6 – 8.4). The distribution of antibody concentrations measured with the four immunoassays of the first blood sampling against the ratio between the first and second sampling are shown in Figure 1. From the 10log-transformed normalized ratios deduced from pre- and post-scores of four immunoassays, we instructed the k-means clustering algorithm to identify three groups. One group consisted of eight children with relatively low ratios indicating MV infection prior to study period. Another group consisted of thirteen children with relatively high ratios indicating MV infection during study period. Children with ratios around the value of 1 (n=70) were assigned to the group no MV infection. The overall attack rate of MV infection in the study sample was 23% (21/91). Sex, age, and moment of inclusion were not predictive of the attack rate.

Two children developed symptomatic measles during the study period. Both were also retrieved as reported cases in the Dutch national register of notifiable infectious diseases. These two children had no detectable virus neutralizing antibodies at the first sampling (⩽ 0.06 IU/ml) and had neutralizing antibody levels of 2.96 IU/ml and 6.40 IU/ml at the second sampling.

Table 1. Characteristics of once-vaccinated participants (n=91) included in an observational cohort study to assess correlates of protection against measles, The Netherlands, 2013-14.

Characteristic	N (%)
Sex	
Boy	41 (45)
Girl	50 (55)
Enrollment	
School	70 (77)
National vaccination register	21 (23)
Age at first MMR in months (IQR)	14.5 (14.4 – 15.1)
Median follow-up time in months (IQR)	8.4 (6.6 – 8.4)
Self-reported symptoms along the follow – up	
Fever	38 (42)
Rash	3 (3)
Cough	46 (51)
Runny nose	42 (46)
Conjunctivitis	8 (9)
Exposure to MV*	
High	82 (90)
Medium	9 (10)
Age at first sampling in years (IQR)	6.5 (5.5 – 7.5)
Age at second sampling in years (IQR)	7.3 (6.2 – 8.2)

* Exposure to MV was divided into two categories. Category 'high' comprised children enrolled in a school with reported cases and exposure according to the parents. Children in category 'medium' were enrolled in a school without reported measles cases but with exposure according to the parents.

Those who experienced subclinical MV infection during the study period (n=11) had antibody concentrations ranging from 0.345 IU/ml to 2.060 IU/ml in the FRNT assay in their first sample. The following symptoms were reported among these 11 children during study period: Rash (0 children), fever (3 children), cough (2 children), conjunctivitis (1 child), and coryza (2 children). These children did not differ with regard to the frequency of reported measles compatible symptoms compared with children who did not experience MV infection.

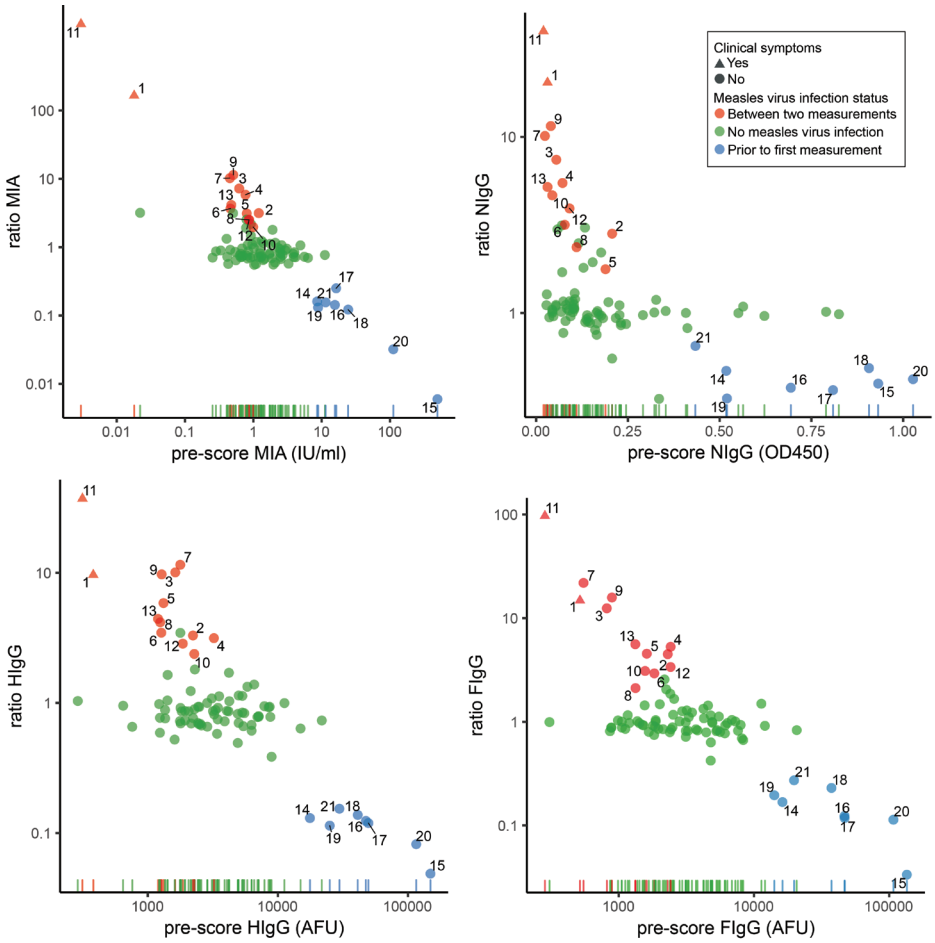

Figure 1. Ratios of pre- and post-measurements of measles specific antibody concentrations by pre-outbreak results, of 91 children. The colors indicate the classification based on the k-means clustering analyses. Numbers provide a comparison of samples across the different tests. MIA: (bead-based multiplex immunoassay), FIgG: MV-F specific antibodies, AFU: arbitrary fluorescence units, HIgG: MV-H specific antibodies, NIgG: MV-N specific antibodies.

Correlates of protection

Three children had no detectable (neutralizing) antibodies in their first blood sample (Figure 2). Two of these developed measles including seroconversion. No measles was observed in participants other than these two. We consider these children to have had primary vaccine failure of the first measles vaccination. Due to the low number of measles cases, we unfortunately could not assess the correlate of protection using a ROC curve nor the relationship between the attack rates

and neutralizing antibody levels. The lowest measurable FRNT concentration in pre-sera of children without measles during study period was 0.345 IU/ml (dashed line in Figure 2).

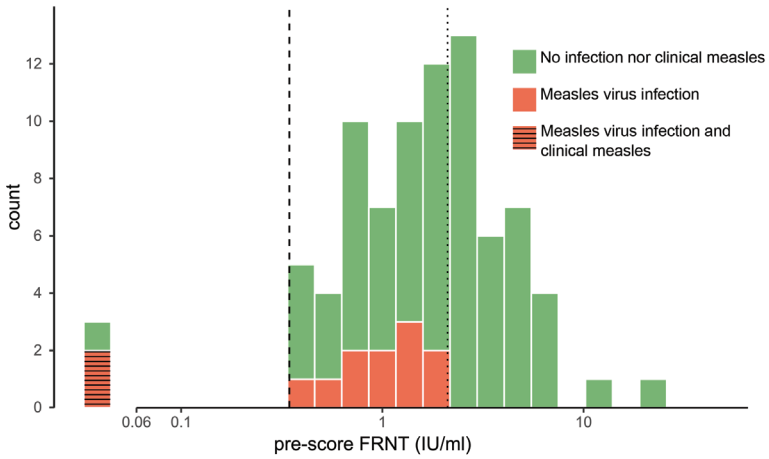

Figure 2. Distribution of FRNT log antibody concentrations at the first sampling in participants excluding those with evidence of MV infection prior to the first sample (n=83) taken shortly after the onset of a measles outbreak in the Netherlands, 2013 - 2014. Colors indicate MV infection status. The vertical dashed line depicts the correlate of protection against measles (0.345 IU/ml) and the vertical dotted line the correlate of protection against MV infection (2.06 IU/ml). Three children had antibody concentrations below the lower limit of detection (0.06 IU/ml). FRNT: focus reduction neutralization test Figure 3. Receiver operator characteristic of the predictive value of measles neutralizing antibody concentrations measured prior to the measles outbreak in the Netherlands, 2013-2014 to protect against MV infection. The green dot corresponds with antibody levels of 1.71 IU/ml and yields the highest sum of the sensitivity and specificity. The AUC is 0.76 (95% CI: 0.65 – 0.88).

The lowest concentration of FRNT antibodies observed above which no MV infection was observed among children was 2.06 IU/ml (dotted line in Figure 2). The ROC analyses indicated that the sum of the sensitivity and specificity was highest at a correlate of protection of 1.71 IU/ml (95%CI 1.01 – 2.11 IU/ml) against MV infection, which corresponds to a sensitivity of 92% (95% CI 77 – 100) and a specificity of 59% (95% CI 43 – 78) in our study population (Figure 3). The lower value derived from the ROC analyses results from the optimization for both the sensitivity and the specificity whereas the other approach seeks a sensitivity of

100%. The AUC (area under the curve) was 0.76 (95% CI: 0.65 – 0.88). The attack rate for MV infection was inversely related to the antibody concentrations measured before exposure (p<0.001, Fisher's Exact test) (Table 2), although the attack rate was approximately similar between children with antibody concentrations ranging from 0.345 – 1.205 IU/ml and 1.206 – 2.540 IU/ml.

Sensitivity analysis
Limiting the analyses to children who were enrolled in a school with reported measles cases and experienced exposure to MV according to parental recall (n=75), we found that the correlate of protection against measles (lower than 0.345 IU/ml) or MV infection (2.1 IU/ml) remained the same. The correlate of protection against MV infection found with the ROC decreased slightly to 1.59 IU/ml (95% CI 0.58 – 2.14 IU/ml), which corresponded with a sensitivity of 91% (95% CI 64 – 100) and specificity of 61% (95% CI 44 – 94).

Discussion

An anticipated large measles outbreak in the Netherlands provided a unique opportunity to assess correlates of protection against measles and subclinical MV infection, an area for which existing evidence is scant.

Two out of 3 children who tested negative developed measles. None of the children with detectable antibodies developed measles. All of these had antibody levels above the previously established correlate of protection of 0.12 IU/ml [18]. The lowest antibody concentration observed among children with detectable antibodies was 0.345 IU/ml. Lower concentrations may still provide clinical protection, but children with these concentrations were unfortunately not present in our study.

We found that antibody concentrations of 2.1 IU/ml and above completely protected against MV infection. This was substantially higher than the neutralizing titers of approximately 1.0 IU/ml found to prevent MV infections among students in the US and Taiwan [18,21]. However, antibody titers of approximately 4.0 IU/ml (HI titers > 1:256) were needed to protect children in Senegal against MV infection[19]. These differences can be caused by differences in the intensity of exposure to MV [35,36]. Differences in neutralization assays that have been used in the past further complicate the comparison of correlates of protection [37]. The recent standardization of the neutralization assay greatly facilitated the comparison

between different studies [38], but most of the previous studies which assessed correlates of protection lacked standardization [18,19].

The relationship observed here and in other studies [19,21,39] between antibody levels and MV infection attack rates underlines that correlates of protection against measles are relative rather than absolute. They depend on the level of exposure to measles virus and presence of cellular immunity. Cellular immunity is thought to be protective in individuals with low levels of antibodies [19,40], although it is considered to control and/or eliminate virus-infected cells rather than blocking infection [41]. T cells could therefore have played a role in preventing the occurrence of symptoms among those who experienced boosting of antibodies in our study population.

Symptoms reported by those who experienced specific boosting of antibodies did not differ from those without antibody boosting. This suggests that the boosting of antibodies we observed was caused by a subclinical secondary infection.

Three of the children in our cohort had not responded to the first immunization, two of which developed measles during the outbreak, and one that will likely experience a primary immune response with the second MCV at 9 years of age. For those who already showed a primary immune response, a second vaccine dose will result in only a transient increase in the antibodies [7,10,42].

One limitation of our study was the laborious enrollment of children, which resulted in low number of respondents and a delayed enrollment to such an extent that children were enrolled after the onset of the outbreak. As a result, some participants already experienced MV infection prior to their inclusion in our study. However, we were fortunate to identify the infected individuals well by measuring significant antibody decay. Timing between the first and second blood sampling is an important prerequisite to measure this decay, as was recently shown by others [43] and us [14], which enabled us to distinguish those who experienced regular waning immunity of vaccine-induced immunity from those that experienced a steep decay indicative of a recent infection.

The low response rate limited the possibilities and precision of estimating correlates of protection. However, we do not think that the low response biased our results: we have no reason to believe that those that participated were different from non-participants in terms of exposure to MV or measles immunity.

Another limitation is the assumption that all children were exposed to measles. As 1 out of 3 fully susceptible children did not develop measles, this assumption was not entirely correct. Yet, the attack rate of MV infection in our study was high and our study participants were enrolled in orthodox Protestant primary schools. As orthodox Protestants between 4 and 12 years of age were the most affected group during an outbreak of about 30,000 MV infections [44], we can assume that the majority in our study population was indeed exposed to measles. Furthermore, the results did not change when we limited the analyses to children with highest likelihood of exposure to MV.

To prevent outbreaks among high vaccination coverage populations, immunity gaps can be found by monitoring antibodies in populations to guide the implementation of immunization strategies. High vaccination coverage alone does not guarantee adequate population immunity due to for example gaps in cold-chain quality or waning immunity and should be supported by serosurveys. Our new evidence about the level of antibodies that are protective against measles and MV infection is crucial here.

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Conflicts of Interest

The authors declare no conflict of interest

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Chapter 9

Measles seroprevalence assessed by plaque reduction neutralization to assess immune protection in birth cohorts with low levels of antibodies

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Abstract

While virus neutralization is considered the gold standard test for assessment of measles immunity, enzyme immune assays (EIAs) are preferred given their efficiency. In The Netherlands large cross-sectional seroprevalence studies are conducted using a bead-based multiplex immunoassay (MIA), which enables high-throughput screening of virus-specific antibody levels. We aimed to examine results obtained by MIA with results in the plaque reduction neutralization test (PRNT) in a cohort with suboptimal immunity. To this end, we re-analyzed individuals previously identified to contain serum antibody levels below 0.3 IU/ml and a representative number of individuals with higher antibody levels. In comparison with the PRNT, the MIA had a sensitivity of 95% (95% CI: 94 - 96) and specificity of 88% (95% CI:(64 – 97). Based on PRNT and MIA results, protective antibody levels of birth cohort 1972-1990 were 99% and 94%, respectively. The discrepancy between the two assays was largely caused by a fraction of vaccinated individuals with low IgG levels, but with sufficient neutralizing capacity to confer immune protection (> 0.12 IU/ml). Individuals that tested negative in both assays predominantly belonged to unvaccinated individuals born around 1976. In conclusion, our data demonstrate that when seroprevalence data of a study population are characterized with low IgG antibody levels, testing this subset with a neutralization assay is a valuable addition. We conclude that the oldest vaccinated cohorts in The Netherlands are well-protected against measles, although small pockets of unvaccinated individuals remain susceptible.

Introduction

Measles remains an important cause of morbidity and mortality (1), despite the availability of safe and effective live-attenuated measles virus (MV) vaccines (2). To eliminate measles, WHO recommends a vaccination program of two doses of a measles-containing vaccine with 95% coverage (3). The aim of measles immunization is to provide long-term immunity against disease and complications, the presence of which is usually assessed by the detection of measles-specific antibodies in serum.

Based on a limited number of studies, neutralizing antibody concentrations above 0.12 international units (IU)/ml have been identified as a correlate of protection from measles (4, 5). The plaque reduction neutralization test (PRNT) is regarded as the gold standard for measurement of neutralizing antibodies (standardized against a reference serum, currently the WHO 3rd international standard; NIBSC 97/648) (6, 7). However, PRN assays are technically demanding and expensive whereas enzyme immune assays (EIA) are easier to perform and cheaper, whilst less blood is needed and antibodies against various diseases can be analysed at the same time. EIAs have therefore been the preferred type of assay to study large number of samples in population-based seroprevalence studies (8). Neutralizing antibodies are exclusively directed to the hemagglutinin or fusion protein, the two transmembrane glycoproteins of MV (9). Although validated EIAs may show a good correlation with the PRN assay, they measure antibodies directed to a wider spectrum of viral proteins. EIAs have repeatedly been shown to display suboptimal sensitivity for detection of measles IgG in cohorts with vaccine-acquired immunity (7, 10, 11). Using an EIA could therefore lead to overestimating the percentage of susceptibles in a population where the majority of individuals have vaccine induced measles immunity (12, 13).

In the Netherlands, a population-based cross-sectional seroprevalence study was conducted in 2006-2007 (n = 7900) (14). Serum samples were analyzed by a multiplex immunoassay (MIA), which revealed that immunity levels of birth cohort 1972 – 1986 were below the target immunity levels of the WHO of 95%. The applied MIA is a bead-based EIA, and was previously shown to display higher sensitivity in this context when compared to commercial EIAs (97% versus 90%). However, this assessment was performed among health care workers of different age, with less precise information on vaccination history (11), and with relatively high antibody levels. We aimed to evaluate the performance of the MIA in comparison with a virus

neutralization assay in a larger population setting with a registered documentation on vaccination history by comparing the results of these two assays in selected samples with a relatively high proportion of individuals with antibodies around the cut-off for protection. Subsequently, we set out to re-assess the seroprevalence of MV-specific antibodies in this cohort based on the levels of virus neutralizing antibodies.

Methods

Study design

We used serum samples from a cross-sectional seroprevalence study designed to evaluate the Dutch national immunization program, which was carried out in 2006 (15). This study assessed antibody levels against several vaccine-preventable diseases, including measles, in a nationwide sample and in eight municipalities with low vaccination coverage. In short, participants were requested to donate a blood sample, to complete a questionnaire, and to bring their vaccination certificates. For this study, we limited samples to birth cohort 1972 – 1990. Serum samples from this cohort revealed that antibody levels as measured by EIA were sometimes below the target of 95%, whereas in other birth cohorts this was close to 100%. We selected all samples with an EIA-based MV-specific IgG concentration below 0.3 IU/ml of this cohort and re-analysed these for the presence of virus neutralizing antibodies. We also obtained a random sample of 100 individuals with an EIA-based MV-specific IgG level above 0.3 IU/ml of birth cohort 1972 – 1990. We assessed the lower limit of detection (LoD) of both assays by testing the sera of true measles seronegative persons to reduce the number of false-positive results. For this assessment, we used sera of 13 month-old infants taken just before their first measles immunization (n=41) (16) and plasma samples from unvaccinated children aged 6 to 12 years of age prior to confirmed measles virus infection (n=61) (17). These individuals were regarded truly measles seronegative. We defined the LoD as the 95% percentile of merged test results of both cohorts (n=102).

Laboratory analyses

Measles-specific IgG was determined with a fluorescent bead-based multiplex immunoassay (MIA) as described before (18). An in-house MV lysate (strain Edmonston, genotype A) was used (19). Briefly, serum samples were diluted 1:200 and 1:4000 in phosphate buffered saline (PBS) containing 0.1% Tween and 3% bovine serum albumin. Fluorescent intensity of the samples was interpolated on

the standard curve to determine measles-specific IgG concentrations expressed in IU/ml. A concentration of ≥0.2 IU/ml was based on cross-bridging with previous seroprevalence data using the 2nd international serum standard for measles antibody (18, 20), was assumed to be in agreement with protective titers (4, 14).

MV-specific neutralizing antibodies were measured in a plaque reduction neutralization test (PRNT) endorsed by WHO (Cohen 2006/2007), but modified to a more practical 96-well culture standard with a specific staining of MV-infected foci as recently described (16). Measles-neutralizing antibody concentrations were expressed in IU/ml based on the 50% plaque reduction dilution of the serum, standardized against the 3rd international serum standard (3 IU/ml). A concentration ≥0.12 IU/ml was considered to be protective (4, 21). Chen et al (1990) found that a titer of 1:120 correlated with protection against measles among a group of students during a measles outbreak (4). Calibrating the neutralizing titer of 1:120 to the second and third international standard established antibody levels of 0.12 IU/ml to be the correlate of protection, as described by Cohen et al (2007) (21).

Statistical methods

Continuous variables were described by the mean and the interquartile range. Categorical variables were described by frequencies and percentages. We used a t-test to test differences between means and a chi-squared test to assess distributions between groups. Confidence intervals around sensitivity, specificity, positive and negative predictive power, and the concordance were estimated using Wilson's method.

We assessed the correlation between the PRNT and the MIA using Pearson's correlation coefficient. To estimate Pearson's correlation coefficient we took into account the sampling strategy. We selected all samples with a MIA result above 0.3 IU/ml and a random subset of the samples below 0.3 IU/ml. The subset was based on a proportion similar to the sampling proportion, which we used to sample the 100 out of the 1429 samples above 0.3 IU/ml (7%). This selection and the estimation of Pearson's correlation coefficient was bootstrapped 1000 times to estimate confidence intervals, which were the 5th and 95th percentile of Pearson's correlation coefficients. Values observed below the limit of detection were excluded. The limit of detection was determined as the 95th percentile of the test results of the individuals regarded truly measles seronegative.

We also assessed the performance of the MIA in comparison with the PRNT by creating a receiver operator characteristics (ROC) curve. The ROC curve visualizes the sensitivity and specificity of identifying seropositive individuals reflecting the range of possible cutoff values. The cutoff for the MIA used in the previously published seroprevalence study was 0.2 IU/ml (14). We re-assessed this cut-off value in relation to PRNT cutoff of 0.12 IU/ml by maximizing the sum of the sensitivity and specificity empirically for the complete range of possible cutoff values. The 95% confidence intervals were created by bootstrapping the sample 2000 times.

We subsequently re-estimated the seroprevalence based on the results obtained with PRNT with the cut-off of 0.12 IU/ml of the specific birth cohorts from the nationwide sample as described earlier (14). In short, seroprevalence was estimated by weighting age, gender, ethnicity, and degree of urbanization to take into account the survey design in relation to the Dutch population distribution to that of 1 January 2007.

Data visualization and data analyses were carried out using R (version 3.4.0). Package "pROC" was used to visualize the ROC curve and to assess the optimal cutoff (22).

Results

Participants

We selected a total of 1595 participants born between 1972 and 1990, as serum samples from these birth cohorts revealed that antibody levels as were sometimes below the target of 95%, whereas in other birth cohorts this was close to 100% (14). Their specific serum IgG antibody concentrations have been assessed previously with the MIA, a bead-based immunoassay. Yet this assay has not been directly compared to virus neutralizing antibodies, which is considered to correlate with immune protection at levels > 0.12 IU/ml. We first set out an analysis by comparing the sensitivity of the MIA with a plaque-reduction neutralization test (reaching out for all participants with IgG antibody levels < 0.3 IU/ml by the MIA assay (n = 166). For those that had IgG levels ≥ 0.3 IU/ml, we took a random sample of those (100 out of 1429) to be analyzed with the PRNT to avoid unnecessary testing of all individuals that we consider to be PRNT positive. Concerning sex, age, age at first vaccination, time since last vaccination, and vaccination status, the random

sample was not different from all that had antibody concentrations above 0.3 IU/ml (Table 1). In total, we retested 263 samples with the PRNT, as 3 samples had insufficient material to be tested.

Table 1. Demographic characteristics and vaccination status of the study population. The median and the interquartile range describe continuous variables. Categorical variables are described by frequencies and percentages. *There were no significant differences in characteristics between samples above 0.3 IU/ml and the subset of this sample, tested by either a t-test or chi-squared test.

Characteristic	Samples with antibody concentrations ≥ 0.3 IU/ml (n = 1429)*	Random sample of participants with antibody concentrations ≥ 0.3 IU/ml (n = 100)*	Samples with antibody concentrations < 0.3 IU/ml (n = 166)
Sex			
Female	561 (39)	37 (37)	63 (38)
Male	868 (61)	63 (63)	103 (62)
Birth cohort	1981 (1976 - 1986)	1980 (1976 - 1986)	1980 (1977 - 1985)
Vaccination status			
Unvaccinated	414 (29)	27 (27)	37 (22)
1 x MCV	285 (20)	21 (21)	34 (21)
2 x MCV	570 (40)	43 (43)	69 (42)
3 x MCV	160 (11)	9 (9)	26 (16)
Age at first vaccination (days)	456 (434 - 500)	448 (428 - 471)	445 (423 – 470)
Time since last vaccination (days)	5307 (3992 - 7165)	5454 (4142 - 7314)	5627 (4211 - 7147)

The antibody concentrations measured by the MIA of individuals born between 1972 and 1990 are shown in Figure 1A. Samples selected to be retested with the PRNT are shown in Figure 1B. Their neutralizing antibody concentrations are shown in Figure 1C.

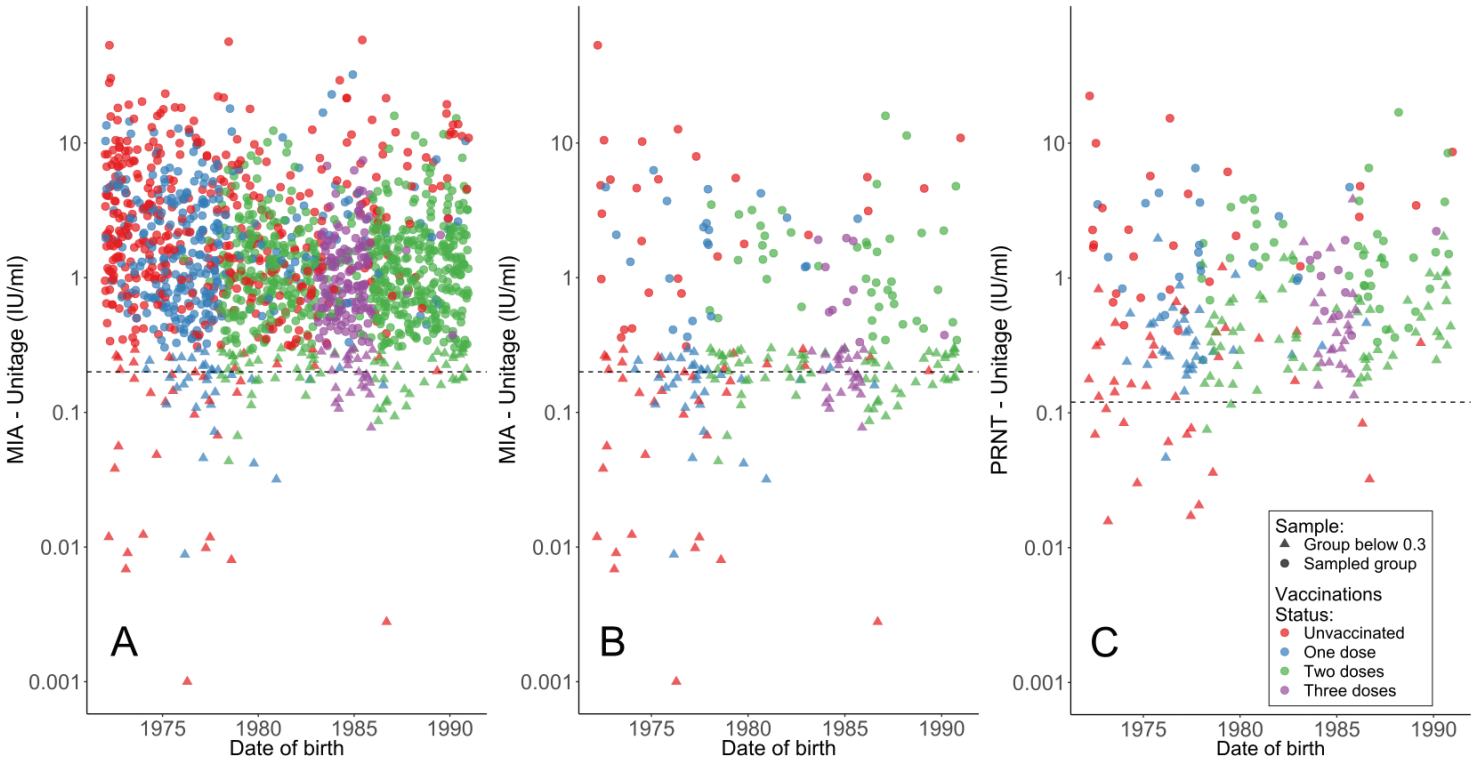

Figure 1. Measles antibodies by date of birth. Colors depict vaccination status and shape visualizes the sampling strategy. Dashed lines depict the cut-off concentration of 0.2IU/ml used with the MIA and 0.12IU/ml with the PRNT. Dotted lines depict the LoD (A) Serum, IgG antibody concentrations measured with the MIA of all samples born between 1972 and 1990 (N = 1595) (14). (B) Serum IgG antibodies measured with the MIA of those selected to be retested with the PRNT (N = 263). (C) Neutralizing antibodies of those retested in the PRNT (N = 263).

To assess antibody levels of truly measles negative persons, we estimated the limit of detection by testing the serum samples from 102 unvaccinated children up to 12 years of age proven not to be exposed to measles virus nor being vaccinated against. The LoD for the PRNT was estimated to be 0.061 IU/ml and 0.080 IU/ml for the MIA (Figure 2A).

All samples (n = 99, one sample had insufficient material to be tested) with antibody concentrations above 0.3 IU/ml in the MIA were above 0.12 IU/ml in the PRNT (Figure 1B and 2B).

Of the samples below 0.3 IU/ml (n = 164, 2 had insufficient material), 86 were below 0.2 IU/ml and 78 between 0.2 and 0.3 IU/ml in the MIA. Among those who had antibody levels below the previously established cut-off of 0.2 IU/ml in the MIA, 72 out of 86 (84%) were protected according to their levels of neutralizing antibodies (> 0.12 IU/ml, Figure 1C and Table 2). The majority among these were vaccinated (60 out of 72, 83%), 18 were vaccinated once, 28 were vaccinated twice, and 14 were vaccinated three times. Among those who were below the cut-off values in both assays (n = 14), 12 were unvaccinated (86%), one was vaccinated once and one was vaccinated twice (14%). Of the 78 samples that were between 0.2 and 0.3 IU/ml in the MIA, all tested above 0.12 IU/ml in the PRNT except for two, who had neutralizing antibody concentrations of 0.115 IU/ml and 0.08 IU/ml, respectively (positive predictive value of 99%) (Figure 1A and 2C). The neutralizing antibody concentrations showed a positive correlation with the test results from the MIA (Pearson's correlation coefficient: 0.86, 95%CI 0.83 – 0.88).

Table 2. Performance of the bead-based multiplex immunoassay (with a threshold of 0.2 IU/ml) against neutralizing antibody concentrations (threshold of 0.12 IU/ml) of 263 samples (three had missing values on the PRNT).*The performance of the MIA is based on a population wide sample by including the additional 1329 who were assumed protective based on a representative sample of 100.

	PRNT ≥0.12	**PRNT < 0.12**	**Sensitivity (%)**	**Specificity (%)**	**Positive predictive value**	**Negative predictive value**	**Agreement (%)**
MIA ≥ 0.2*	1504 (175 tested)	2	95 (94 - 97)	88 (69 - 100)	100 (100 - 100)	12 (16 - 21)	95 (94 - 96)
MIA < 0.2	72	14					

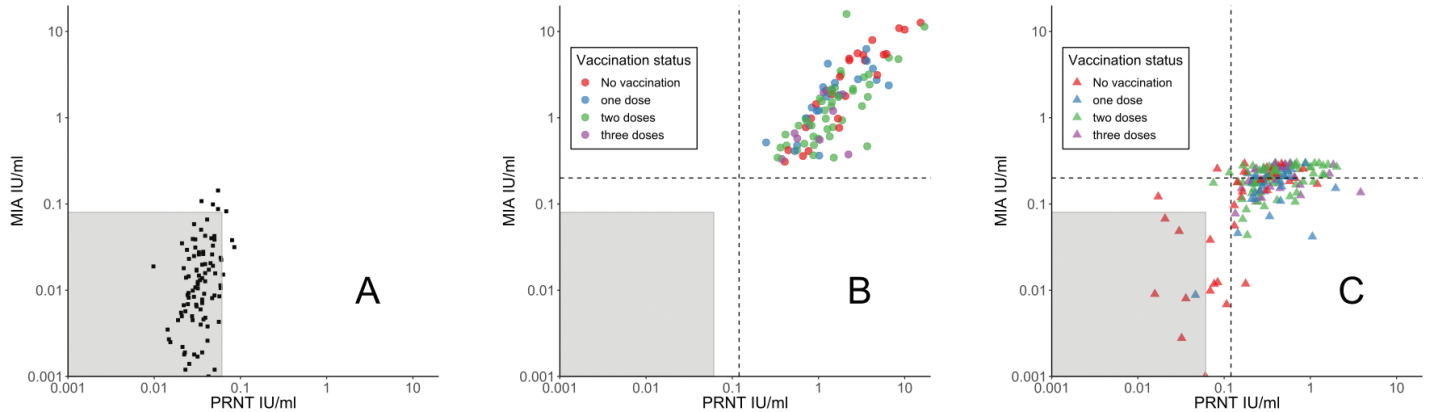

Figure 2. Plots of the antibody levels measured with the bead-based multiplex immunoassay (MIA) against the scores obtained with the PRN test. (A) Plot A visualizes the antibodies of 102 seronegative individuals, consisting of 41 unvaccinated 13-month-old infants and 61 unvaccinated 6-12 year-old children prior to their confirmed measles infection. The limit of detection was determined as the 95th percentile of the test results, represented by the grey square. (B) Plot B contains a random sample (n = 100) of 1429 samples with antibody concentrations above 0.3 IU/ml and plot C consists of all samples below 0.3 IU/ml in the MIA (n = 163). The cutoff value of the PRN test (0.12 IU/ml) and the cutoff value of the MIA (0.20 IU/ml) are depicted as dashed lines in plot B and C. Colors indicate type of sample in plot A and vaccination status in plot B and C.

Assuming that all the samples above 0.3 IU/ml (n = 1429) had protective concentrations of neutralizing antibodies above 0.12 IU/ml, the sensitivity for the whole cohort was 95% (95% CI:94 - 96) and the specificity was 88% (95% CI 64 - 97).

Based on receiver operator characteristics (ROC), the optimized cutoff for the MIA, which corresponded with the highest combined sensitivity and specificity in relation to the correlate of protection of 0.12 IU/ml in the PRNT, was 0.26 IU/ml (95% CI: 0.12 – 0.29). The corresponding sensitivity was 93% (95% CI: 92 - 99) and the specificity was 100% (95% CI: 100 – 100). The empirical ROC curve is visualized in Figure 3 and has an area under the curve of 0.99 (95%CI: 0.97 – 1.00).

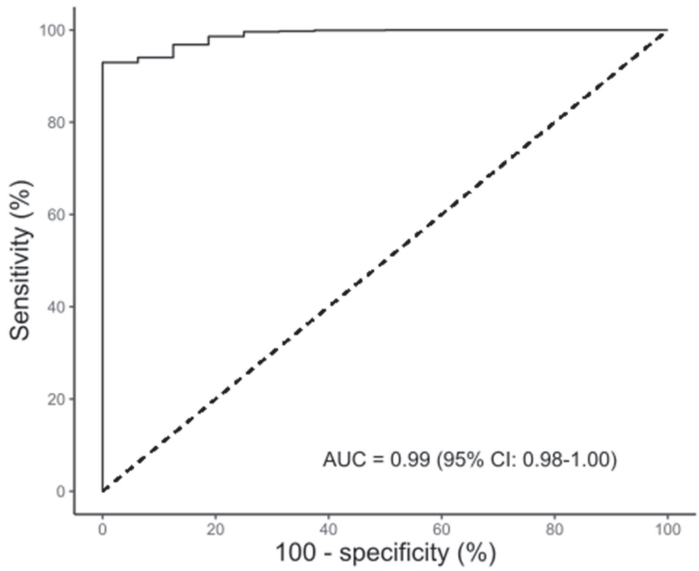

Figure 3. Receiver Operating Characteristic Curve and Area Under the Curve of the bead-based multiplex immunoassay (MIA) predicting antibody levels to be protective in relation to the PRNT with cutoff of 0.12 IU/ml (n = 1592).

Re-assessment of seroprevalence among the study population

The weighted susceptibility among birth cohort 1972 – 1990 decreased from 6.72% to 1.21% when we assessed the seroprevalence by the neutralization assay instead of the MIA (Table 3). The decrease was strongest among birth cohort 1982 – 1986, due to the relatively high presence of vaccinated individuals who tested below the cutoff in the MIA but above the cutoff in the neutralization assay.

Table 3. Number of susceptible individuals and weighted seroprevalence stratified by birth cohort. Participants are from a population-based seroprevalence study in the Netherlands conducted in 2006/2007.

Birth cohort	**1972 – 1976 (n = 406)**	**1977 – 1981 (n = 437)**	**1982 – 1986 (n = 452)**	**1987 – 1990 (n = 300)**	**Total (n=1595)**
Susceptible individuals according to MIA (cutoff 0.2 IU/ml) (%)					
Total	22 (5.4)	28 (6.4)	28 (6.2)	10 (3.3)	88 (5.5)
Vaccinated	7	20	27	10	64
Unvaccinated	15	8	1	0	24
Susceptible individuals according to PRNT (cutoff 0.12) (%)					
Total	7 (1.7)	7 (1.6)	2 (0.4)	0 (0)	16 (1.0)
Vaccinated	1	2	0	0	
Unvaccinated	6	5	2	0	
Weighted* percentage susceptible MIA‡ (14)	6.60 (3.35-9.84)	8.32 (4.97-11.66)	7.22 (4.49-9.95)	3.97 (1.42-6.52)	6.72 (5.17-8.26)
Weighted* percentage susceptible PRNT‡	1.91 (0.45-3.37)	2.08 (0.50-3.66)	0.55 (0.00-1.36)	0.00 (-)	1.21 (0.72-1.69)

*Weighted by age, gender, ethnicity and degree of urbanization.
‡ Weighted seroprevalence estimates are representative of the national sample from the seroprevalence study.

Discussion

A bead-based multiplex immunoassay (MIA) employed with a cutoff of 0.2 IU/ml was previously used to distinguish immune from susceptible individuals during a serosurvey in The Netherlands (14). Here, we assessed the performance of MIA in relation to virus neutralizing antibodies by testing a subset of these samples by PRNT, for which the cutoff for protection has been previously determined to be 0.12 IU/ml (4, 5). We have specifically chosen to test all serum samples with specific IgG concentrations below 0.3 IU/ml, and a selection of samples that were expected to have antibody concentrations well above this cutoff level to reduce the workload. This way, power was retained to both re-assess the seroprevalence with the neutralization assay as well as to assess the performance of the MIA.

In comparison with the PRNT, we found a sensitivity of 95% (95% CI:94 - 97) and a specificity of 88% (95% CI:64 – 100) for the MIA assuming that the random sample of 100 participants were representative for all samples above 0.3 IU/ml in the MIA. Low numbers involved in the estimation of the specificity are reflected in the wider confidence interval.

This sensitivity is comparable with a previous serosurvey among healthcare workers (11), where the MIA was found to have a sensitivity of 97% in relation to the neutralization assay. However, the specificity of the MIA among the health care workers appeared higher when compared to this study. This performance, however, was established with a different population sample, consisting of health care workers with relatively high antibody concentrations (149 out of 154 tested positive on both tests), and only one sample below the correlate of protection. The current selection comprised a more consistent and larger study population that harboured more individuals with antibody levels close to the cutoff value of 0.2 IU/ml in the MIA. Yet, the lower sensitivity was still based on only 2 individuals with disconcordant results between the two immunoassays. More serum samples that are close to the cutoff value are needed to increase the probability of a lower performance of the enzyme-based immunoassays as was observed earlier among populations with relatively low antibody concentrations (7, 10, 13).

A cutoff value of 0.26 IU/ml, derived through ROC-analyses, slightly decreased the sensitivity to 93% (95% CI:92 - 99) but increased the specificity to 100% (95% CI:100 - 100). The cut-off value of 0.26 was estimated by optimizing the sum of the sensitivity and the specificity. Due to the low number of sera with neutralizing antibodies below the cut-off of 0.12 IU/ml, the specificity was determined on 16 samples only, and a considerable increase in the specifity (12%) coincided with a small decrease (2%) in sensitivity. As a result, we observed a value of 0.26 as the cut-off with the highest sum of sensitivity and specificity.

We re-assessed the seroprevalence of birth cohort 1972-1990 using the correlate of protection of 0.12 IU/ml. The previous estimate of this seroprevalence for these birth cohorts was estimated to be 93.28% using the cutoff of 0.2 IU/ml in the MIA (14). Here, we found a seroprevalence of 98.79% determined from measuring neutralizing antibodies. The discrepancy is largely caused by vaccinated individuals with IgG antibody concentrations below 0.2 IU/ml but which showed sufficient levels of neutralizing antibodies that are considered protective against measles (12, 13). Those defined negative in both tests appeared to be mostly unvaccinated

individuals. This contrasts the first assessment of the seroprevalence which showed a miscellaneous group of both vaccinated and unvaccinated persons susceptible to measles, but which, according to the assessment with the PRNT, mostly concern unvaccinated persons born around the introduction of measles vaccination in The Netherlands in 1976. Important to note is that we only re-assessed the seroprevalence of birth cohort 1972-1990.

One caveat of our study is that our re-assessed seroprevalence is based on the putative correlate of protection of 0.12 IU/ml in the neutralization assay which is based on only two studies (4, 23). Besides that the neutralization assay is a biological assay and more subject to variation, the unitage value is not an absolute indicator for immune protection, based on observations that individuals with higher concentrations can still be infected with measles virus, albeit with a lower chance of developing measles symptoms, which could be related to the intensity of exposure (24, 25). Using this correlate of protection as the benchmark of a seroprevalence study should therefore be interpreted with some caution. Despite these setbacks, it is the preferred test as it measures an important functional aspect of the antiviral serological response against measles virus infection in contrast to EIAs that measure the presence of antibodies not necessarily involved in immune protection. More importantly, suboptimal sensitivity for detection of measles IgG by EIA is a particular concern in cohorts with vaccine-acquired immunity (12, 13). The bead-based MIA proved much more sensitive in this context (11).

Another caveat of our study is the low number of individuals (n = 16) below the protective level of 0.12 IU/ml, we therefore lacked precision in the determination of the specificity Among these 16, we observed only three vaccinated individuals out of a total of 1144 vaccinated individuals born between 1972 and 1990. From a public health perspective, this is reassuring. We can conclude that the oldest vaccinated individuals in the Netherlands have sufficient neutralizing antibody concentrations to be protected against measles, at least within the time frame of investigation in the Netherlands (1976-2006). Whether the oldest vaccinated individuals are still protected in the future should be investigated in future seroprevalence studies (26).

In conclusion, the MIA performed satisfactorily when testing the antibodies of a study population at concentrations > 0.3 IU/ml. However, when the study population is characterized by low levels of antibodies, we suggest to validate a subset of EIA results with a neutralization assay. Consequently, in the Netherlands

for birth cohort 1972 - 1990, we can conclude that our oldest vaccinated cohorts are protected well against measles based on the correlate of protection of 0.12 in the neutralization assay, and that the majority of susceptible individuals in birth cohort 1972-1990 concern unvaccinated individuals at an age that comes with high risk of complications. Tailored immunization programmes should be considered, especially for health care workers. More recent serosurveys are ongoing, to investigate how well protective antibodies are sustained in the oldest birth cohorts within a time frame of 40 years of established measles vaccination in The Netherlands (1976-2016*)*.

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Chapter 10

Economic Costs of Measles Outbreak in the Netherlands, 2013–2014

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Abstract

In 2013 and 2014, the Netherlands experienced a measles outbreak in orthodox Protestant communities with low measles, mumps, and rubella vaccination coverage. Assessing total outbreak costs is needed for public health outbreak preparedness and control. Total costs of this outbreak were an estimated $4.7 million.

During May 2013–March 2014, the Netherlands was affected by a large measles outbreak [1]. The outbreak began in the center of the country in an orthodox Protestant community and spread mainly to regions with low vaccination coverage. Overall, the Netherlands has high measles–mumps–rubella (MMR) vaccination coverage, with >95% coverage for the first dose of MMR for children. However, some orthodox Protestant and anthroposophic communities opt out of childhood vaccination programs on religious grounds or personal beliefs [2]. In addition to the effects of disease on a society, measles outbreaks have economic consequences, including direct medical costs and productivity losses. Moreover, a measles outbreak demands a range of responses from the National Institute for Public Health and the Environment (RIVM) and municipal public health services (MHS). Assessing outbreak costs, including costs of response activities by public health authorities, can help in planning for future outbreaks and in optimizing allocation of public resources. Recent research on measles outbreak costs in industrialized countries is scarce and has addressed hospitalizations costs [3], costs of imported cases of measles [4-7] or small outbreaks [8, 9]. We assessed the economic costs of a large measles outbreak in the Netherlands.

The Study

All physicians and laboratories are mandated to report measles to MHSs in the Netherlands. Each MHS records patient information in a national database, which includes information on age, postal code, date of symptoms, complications, hospitalization, and source of infection. Notifications of measles cases were used to assess medical costs and productivity losses (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/21/11/15-0410-Techapp.pdf). Information on additional serologic tests and extra vaccinations among health care workers in hospitals were obtained from a study on the implementation of measles guidelines for hospitals (online Technical Appendix). Information about vaccinations of infants

and older unvaccinated children in response to the outbreak was retrieved from the national immunization register. We interviewed staff at MHSs and the RIVM to assess the amount of personnel time related to outbreak response activities (online Technical Appendix).

Table 1. Estimated direct health care costs during measles outbreak, the Netherlands, 2013–2014*

Type of cost	Total no. patients	Unit cost, $	Average health care utilization	Total cost, $
Physician consultation				
Uncomplicated measles, no visits	2,320	37.35	0.2	17,330
Uncomplicated measles, no phone calls	2,320	18.07	0.1	4,192
Hospitalization, no. cases	181	37.35	1.0	6,760
Other complicated measles, no cases	199	37.35	2.0	14,865
Treatment for pneumonia in general practice, no. cases	75	16.02	.01	1,202
Length of hospitalizations, d				
General ward	174	600	4.6	480,240
Intensive care unit	7	2866	13.1	262,812
Rehabilitation	1	447	245	109,515
Serologic test results, no. cases†			.	
Positive tests	139	21.37	1.0	2,970
Negative tests	854	21.37	1.0	18,250
DNA/RNA amplification, no cases‡				
Positive tests	765	251.55	1.0	192,436
Negative tests	577	251.55	1.0	145,144
Total				**1,255,718**

*Costs are calculated in 2013 US dollars ($). Total number of measles cases = 2,700. Total cost differs from sum of category costs because of rounding. †IGM. ‡ PCR.

During the epidemic, 2,700 measles cases were reported, mostly among children 5–14 years of age (Table 1). In 329 patients, complications such as otitis media, pneumonia, and encephalitis developed. One child died from measles complications, and 181 patients were hospitalized. One patient with encephalitis spent 8 months in a rehabilitation clinic. Of patients who consulted a physician but were not hospitalized, 199 experienced measles complications, mostly otitis media (104 patients) or pneumonia (75 patients). Total estimated cost for direct health care was $1,255,718 (mean $465/case). An additional $365,855 ($136/case) was attributed to productivity losses and informal child care losses (online Technical Appendix Table

1). In 2013, most (85%) of the responding hospitals in the Netherlands offered a serologic test to employees to ensure that they were sufficiently protected against measles (online Technical Appendix). Employees identified as being at risk for measles infection were offered an MMR vaccination. On average, 80 serologic tests led to 63 vaccinations per hospital for a total estimated cost of $222,203 (Online Technical Appendix Table 2).

At the start of the outbreak, the RIVM convened a national outbreak management team to discuss a strategy regarding targeted vaccination campaigns for infants living in communities with low vaccination coverage and for previously unvaccinated persons. A total of 6,652 infants received a complementary MMR vaccination. Among children 18 months–19 years of age, 6,948 received an MMR vaccination during July 2013–March 2014. Costs for these vaccinations were $299,840. During this outbreak, the RIVM also coordinated outbreak control, conducted enhanced surveillance, and responded to extensive media attention (online Technical Appendix). Total costs for outbreak response activities by the RIVM were an estimated $698,280 ($259/case). In addition, we collected information from 6 MHSs that together had recorded more than half of all notified measles cases nationally. Their response activities included registration and processing of cases, vaccination activities, and advising of local authorities, professionals, and the general population (Technical Appendix). Total estimated costs for all MHSs were $1,852,470 ($686/case).

Table 2.

Category	Costs, $	% of total costs
MHS	1,858,470	39.5
Hospitalization	852,567	18.2
RIVM	698,280	14.9
Production losses	365,885	7.8
Laboratory tests	358,801	7.6
Vaccination of children	299,840	6.4
Vaccination of health care workers	222,203	4.7
General practitioner consultation	44,350	0.9
Total	**4,694,395**	**100**

*Costs are calculated in 2013 US dollars ($). Total costs and % does not equal because of rounding. MHS, municipal public health services; RIVM, National Institute for Public Health and the Environment, the Netherlands.

The MHSs incurred most of the costs of the outbreak, followed by costs for hospitalizations (Table 2). Costs of outbreak response activities by the RIVM were also considerable. Costs classified as other medical costs (i.e., consultations with general practitioners), productivity losses, and costs for vaccination campaigns were among the lowest costs (Tables 2; online Technical Appendix Table 3).

Conclusions

The measles outbreak occurring in the Netherlands during 2013–2014 is associated with substantial costs of ≈$4.7 million (€3.9 million) or 0.0042% of overall health care costs ($113 billion in 2013) in the Netherlands. The 2,700 reported measles cases during this outbreak resulted in an estimated $1,739 per case. Outbreak management costs were the primary cost, probably because of demands for expert advice, response to extensive media attention, registration of notified cases, and more surveillance activities than usual.

Despite being substantial, the outbreak costs in our study are underestimated. Because of data limitations, we were unable to estimate normal human immunoglobulin costs, patients' traveling costs, or costs of vaccinations of adults or of long-term complications of disease. Also, cases in other countries have been linked to this outbreak, including Canada, United States, and Belgium; associated costs for cases imported to other countries are not included in our calculations. Furthermore, surveillance systems are affected by a degree of underreporting; therefore, uncertainty exists about the "true" economic costs of disease [10]. In a previous measles outbreak in the Netherlands, the estimated true number of measles cases was ≈10 times the number of cases reported in the surveillance system [11]. Moreover, only 47% of hospitalized cases in the previous outbreak were reported [12]. Applying these data to our results, the estimated total outbreak costs would be ≈$0.9 million higher. Further research into the extent of underreporting in this outbreak is planned.

In Australia, the public health unit cost for responding to a single case of measles was $1,701 [7], a similar amount to our results. In the United States, costs of containing an outbreak were estimated at $6,180 per case. Additional U.S. studies report that containment of a single imported measles case resulted in even higher costs per case [5, 6]. Explanations for the higher costs in the United States include more extensive contact tracing and higher medical care expenses.

The 2013–2014 measles outbreak posed considerable logistical challenges for MHS staff. Registration of reported cases contributed especially to the increased workload and costs created by this measles outbreak. To reduce this workload during a large outbreak, information considered to be critical for review could be collected for most patients, who usually recover within a few days or weeks, while more detailed information should continue to be collected for patients with complications or serious illness.

Measles substantially affects patients' quality of life [13] and their ability to perform their usual daily activities. Complications resulting from measles, such as pneumonia, encephalitis, and subacute sclerosing panencephalitis, sometimes occur a few years after the illness [14]. Complications from measles also affect quality of life and incur high financial costs, as shown in the extensive rehabilitation care needed by a patient with encephalitis that resulted from this outbreak. In the Netherlands, because religious arguments affect vaccination rates [15], elimination of measles will be challenging. For the foreseeable future, measles outbreaks are expected to continue to cause substantial effects from disease and economic costs. To prepare for new outbreaks, medical costs, productivity losses, and containment costs should be considered.

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Technical Appendix

Additional Methods and Results

Case Definition

The measles case definition used is based on the presence of clinical symptoms and laboratory confirmation of diagnosis. An epidemiologically linked case is defined as someone with a matching clinical presentation who had contact with a laboratory-

confirmed case. The measles case definition used is consistent with that of the European Centre for Disease Control and Prevention [16].

Assessment of Outbreak Costs

Direct Medical Costs

Information on health care use regarding the number of general practitioner (GP) visits due to measles infection has been reported [11]. In a previous Dutch outbreak, van Isterdael et al. (2004) estimated that 30% of the measles patients consulted their GP. We used this average number of consultations for patients without complications and assumed that 1 third of the patients contacted their GP by phone and the remainder by a GP visit (unpub. data, Tom Woudenberg, RIVM, Bilthoven, the Netherlands). In line with the Dutch health care system, we assumed that all hospitalized patients had consulted a GP once. Patients for whom complications developed (but who were not hospitalized) were assumed to have visited their GP twice. According to Dutch guidelines, apart from pneumonia, most measles complications reported by GPs (i.e., otitis media, dehydration, and upper respiratory infection) do not require further treatment [17]. Medical costs were gathered from standard unit cost lists [18] and list prices available online [19]. The unit cost per hospitalized day comprises treatment in hospital, treatment in an intensive care unit, and clinician consultation fees. Since the database of notified cases did not include negative diagnostic tests, we estimated the total number of diagnostic tests by applying the ratio of positive and negative diagnostic tests of the RIVM laboratory to all positive tests recorded in the national database. All costs are expressed as U.S. dollars as of 2013. Euros were converted to US$ by using data on purchasing power parity of the Organization for Economic Co-operation and Development: 1 US$ = 0.83 euro.

Productivity Losses

Almost all notified measles cases were unvaccinated orthodox Protestants. Orthodox Protestants constitute a Calvinistic religious minority in the Netherlands who believe in predestination and divine providence. Their lifestyle is based on the scripture and religion, which play an important role in daily life. Many of them reject vaccination for religious reasons. Since most orthodox Protestant women do not have a paid job to take care of their children, productivity losses of women with measles were calculated as loss of informal care [18, 20].To calculate productivity losses for men, we used standard tariffs (mean, all ages) [18], adjusted

for work participation in all age groups [21]. We did not calculate productivity losses for parents taking care of sick children. The duration of productivity losses was calculated for the average duration of illness (i.e., 14 days).

Costs of Targeted Vaccination Campaigns

In the beginning of the outbreak, health care workers born after 1965 were encouraged to check their vaccination and measles infection status and complete their MMR vaccination if necessary [22]. Data about serologic tests and extra vaccinations among health care workers in hospitals were obtained from a study on the implementation of measles guidelines for hospitals (unpub. data, Lydia Fievez, RIVM). In this study 85% (69) of responding hospitals (81 of 88 hospitals in the Netherlands) offered a serologic test to employees.

In July 2013, parents of children 6–14 months of age who lived in communities with vaccination coverage <90% or who belonged to orthodox Protestant communities received a personal invitation for an early MMR vaccination. The normal schedule for MMR in the Netherlands is to receive the MMR-1 at 14 months and the MMR-2 at 9 years of age. Children 6–12 months of age received an MMR-0 vaccination, and those 12–14 months of age received an early MMR-1 vaccination. In addition, the MHSs offered an MMR vaccination to children and adolescents within the vaccination program up to 19 years of age if they had not yet received an MMR vaccination. To avoid including routine MMR-1 vaccinations at 14 months in the outbreak costs, we selected children who received their MMR-1 during the outbreak period who were >18 months–19 years of age. All vaccinations used in this study were recorded in the national immunization register (unpub. data; Praeventis database for registering vaccinations in the Netherlands). Vaccine price and administration costs were gathered from the Dutch Healthcare Authority [19].

Costs of Outbreak Response Coordination at the National Level (RIVM)

Personnel time spent on outbreak control and on investigating and processing the outbreak was also estimated. Given the limited resources, this reallocation of personnel time represents the loss of other production (i.e., opportunity costs). Personnel time of the RIVM was determined by hours allocated to surveillance, response, laboratory, and vaccination activities at the national level. This time estimate was obtained from personal interviews with the personnel in the relevant departments within the Institute. We calculated personnel costs by multiplying a person's salary tariff by the time spent on the measles outbreak.

Costs of Outbreak Response Coordination at the Regional Level (MHS)

To estimate the amount of personnel time associated with local outbreak response activities, we developed a questionnaire for semistructured interviews of MHS staff (i.e., doctors, nurses, and managers) in some of the regions with the highest number of notified measles cases. The interviews explored all MHS activities and the associated time investment of the personnel involved. All possible local reports and registries were collected for additional information. The estimated time investment of physicians, nurses, nursing assistants, managers, and communication employees involved in the outbreak were converted to costs by using an average salary tariff per hour of MHS staff. We calculated time and costs per each notified case in these regions and extrapolated these estimates to all notified cases in the Netherlands.

Additional tables

Technical Appendix Table 1. Estimated indirect costs and productivity losses for men and women during measles outbreak, the Netherlands, 2013–2014*

	Sex	
Category	**M**	**F**
Adult cases, no.	100	109
Employment or provision of informal child care, %*	72.3%	100%
Employment or provision of informal child care, h/wk*	36.1	40.0
Productivity costs/h, $†	42.67	16.42
Total productivity losses, $†	222,740	143,145

*Productivity losses were calculated for 2 weeks (10 working days) as average duration of illness. Because orthodox Protestant women tend to stay at home taking care of their children, we calculated production losses of work absenteeism for men and production losses of informal child care provided by women.
†Production costs and losses are calculated in 2013 US dollars ($).

Technical Appendix Table 2. Costs of targeted vaccination campaigns during measles outbreak, the Netherlands, 2013–2014*

		Costs and factors affecting costs		
Population/category of cost	**Unit costs, $**	**Hospitals, no.**	**Average no.**	**Total costs, $**
Health care workers				
Serologic test	21.37	69	80	117,962
Vaccination	8.33	69	63	36,211
Administration costs	15.65	69	63	68,031
Total				222,203
Children 6–14 mo		MMR-0, no.	Early MMR-1, no.	
Vaccination	8.33	5,238	1,414	55,380
Administration costs	14.76	5,238	1,414	98,177
Total				153,557
Children 18 mo–19 y			MMR-1, no.	
Vaccination	8.33		6,948	57,877
Administration costs 0–5 y	14.76		2,764	40,797
Administration costs 5–19 y	11.37		4,184	47,572
Total				146,246

*Costs are calculated in 2013 US dollars ($).Total cost differs from sum of category costs because of rounding. MMR, measles–mumps–rubella; MMR-0, extra MMR vaccination given to children 6–12 months of age; Early MMR-1,MMR vaccination given to children 12–14 months of age; MMR-1, MMR vaccination given to children 18 months to 19 years of age.

Technical Appendix Table 3. Main cost categories of measles outbreak, the Netherlands, 2013–2014*

Category	**Costs,$**	**% of total costs**
Outbreak management	2,556,750	54.3
Medical costs	1,255,718	26.8
Prevention (vaccination)	522,044	11.1
Production losses	365,885	7.8
Total	4,694,395	100

*Costs are calculated in 2013 US dollars ($). Outbreak management and medical costs differ slightly from itemized costs in Table 4 due to rounding.

Additional Results of Costs of Outbreak Response Coordination

Costs of Outbreak Response Coordination at the National Level (RIVM)

Four departments at the RIVM were involved with the measles outbreak: the Centres for Epidemiology and Surveillance; Communicable Disease Control; Infectious Diseases Research Diagnosis and Screening; and Policy and Regional Support. During the outbreak, representatives of these departments participated in a weekly response meeting at which the current outbreak and national containment strategies were discussed. Online Technical Appendix Table 4 shows the total labor time and costs for all personnel involved. Total costs were estimated at $698,280.

The interviewed MHS staff confirmed that measles response activities were time consuming, especially registration and processing of new measles cases in their region. On average, these activities required 2–3.5 hours per case. At the beginning of the outbreak, numerous internal staff meetings were held to organize regional response activities adequately. Vaccination activities were limited because the targeted group of orthodox Protestants is generally unwilling to accept measles vaccination. Of the different personnel categories, public health nurses spent most of their time performing outbreak response activities (online Technical Appendix Table 5). Based on these data, the total cost for all MHSs was estimated to be $1,858,470 ($686.1 for each of 2,700 notified cases).

Technical Appendix Table 4. Labor time and costs for personnel involved in outbreak management at the national level (RIVM) during the outbreak of measles, the Netherlands, 2013–2014*

Department	Task	Labor time, h	Costs, $
Disease control	Coordination of outbreak control and communication	2,730	300,723
Support	Organization of MMR-0 and MMR-1 vaccination campaigns	1,754	177,372
Surveillance	Analysis and reporting of outbreak data	996	118,257
Diagnostics	Advice and Interpretation of laboratory results†	846	101,928
Total			698,280

*Costs are calculated on the basis of 2013 US dollars ($). RIVM, Ministry of Health, Welfare and Sport, the Netherlands; MMR, Measles–Mumps–Rubella. MMR-0, extra MMR vaccination given to children 6–12 months of age; Early MMR-1,MMR vaccination given to children 12–14 months of age; MMR-1, MMR vaccination given to children 18 months to 19 years of age. †Costs of laboratory tests are presented in Table1 of article.

Technical Appendix Table 5. Labor time and costs for personnel involved in outbreak management at regional level MHSs in measles outbreak, the Netherlands, 2013–2014*

Employee	Tariff, $	Labor time per notified case, h	Costs per notified case, $
Nurse	69	5.2	359
Physician	107	1.9	203
Manager	103	0.6	62
Communication employee	88	0.4	35
Nursing assistant	54	0.5	27
Total		8.6	686

*Costs are calculated in 2013 US dollars
($).Total cost differs slightly from sum of category costs because of rounding.

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Chapter 11

The Reduction of Measles Transmission During School Vacations

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Epidemiology, 2018

Abstract

Background: Historically, measles incidence has shown clear seasonal patterns driven by the school calendar, but since the start of mass vaccination in developed countries there are only occasional outbreaks, which may have changed the effect of school vacations on transmission. In 2013–2014, a large measles epidemic took place in a low vaccination coverage area in The Netherlands, allowing us to quantify current-day measles transmission and the effect of school vacations.

Methods: We fitted a dynamic transmission model to notification and hospitalization time series data of the Dutch 2013–2014 measles epidemic. Our primary aim was to estimate the reduction in contact rate during school vacations and the number of cases averted due to the vacation. In addition, because the summer vacations were timestaggered in three regions, we could distinguish within-region from across-region effects of school vacations.

Results: We estimated a 53% (95% credible interval: 45%, 60%) reduction in contact rate during school vacations, resulting in 4900 (3400–7100) averted cases (estimated outbreak size: 16,600 [12,600–23,200]). There was a shift from mainly local transmission during school term to mainly cross-regional transmission during vacations. With seroprevalence data, we derived a current-day estimate of 15 to 27 for *R*0 (number of secondary cases per primary case in a susceptible population).

Conclusions: School vacations are associated with greatly reduced overall measles transmission. However, transmission is not eliminated, and increased long-distance travel may even promote spread to other areas. Therefore, we estimate that school closure is unlikely to prevent measles epidemics unless there are still few cases and the community is well vaccinated.

Background

Measles is a highly infectious childhood disease caused by the measles virus (morbilliviruses). Infection causes fever, a typical rash, and immune suppression and may progress to more severe conditions such as pneumonia, meningitis, and even death. Before the introduction of mass vaccination in developed countries, measles was endemic and incidence showed a clear seasonal pattern. Prevaccination measles dynamics has been studied extensively and could be well described by fairly simple transmission models assuming homogeneous mixing, driven by (summer) school calendars and birth rates.[1–3] Since the introduction of mass vaccination in the 1960s and 1970s, endemic measles circulation has come to an end with a large reduction in measles burden,[4,5] only to be interrupted by occasional measles outbreaks in insufficiently vaccinated populations.[6–11] As seasonality has disappeared and the mean age of infection increased, dynamics in present-day populations is far less understood, in particular, the role of school vacations in transmission.

School vacations disrupt measles transmission because children have reduced contact rates when not in school. This is not specific to measles: the reduced transmission is also observed in endemic and epidemic dynamics of influenza,[12–15] and it is the reason why schools are sometimes closed when measles is diagnosed[16,17] and why school closure is suggested as a public health measure for other close-contact infectious diseases, such as influenza. [12,15,18] Closing schools is costly and disruptive for children, parents, and society,[18,19] so understanding its effect on a variety of infections is important for decision-making in control of epidemics.

The Netherlands has a high vaccine uptake, but a small Orthodox Protestant community has a lower vaccine uptake for religious reasons.[5] This community mostly lives concentrated in the so-called bible belt, stretching from the Southwest to the Northeast of the country. Early in 2013, measles started to spread in that community, which had been almost free of measles since a large epidemic in 1999–2000.[10] The 2013–2014 epidemic occurred in large part during the summer vacation, when a temporary decline in incidence was observed followed by a resurgence in the autumn.[10,20] No interventions were implemented with the exception of inviting parents of 6- to 14-month-old infants in the affected region for a preschedule vaccination (with 57% uptake) and offering vaccintion to contacts and unvaccinated Orthodox Protestants, with limited uptake.[20] The epidemic

was large (2700 notified cases), and because measles is a notifiable disease, it was relatively well monitored, providing an excellent opportunity to update our quantitative knowledge of measles transmission in relation to school vacations.

In this study, we analyze the Dutch measles epidemic to assess how the summer school vacation affected transmission and outbreak size. The fact that the summer vacations were time-staggered in three regions created a natural experiment to differentiate within-region from across-region effects of vacations on measles transmission; to assess sensitivity of the estimated effects to the completeness of notification, in particular during the summer; and to assess sensitivity to the exact timing of the school vacations, thus, ruling out other seasonal factors that could have led to reduced transmission during summer.

Methods

Data

Measles is notifiable in The Netherlands, and the National Institute for Public Health and the Environment maintains the electronic notification system. A detailed description of the measles outbreak in the Netherlands in 2013–2014 is given in the work by Woudenberg et al.[20] The first case was reported on May 27, 2013 (week 21), which was the start of the outbreak, with ultimately 2700 reported cases until 12 March 2014 (week 11). The large majority (72%) were children between 4 and 15 years old, of whom 96% were not vaccinated. The outbreak was concentrated in the population of Orthodox Protestant Individuals (OPIs): 2314 cases (86%) were explicitly identified as OPI because they gave Orthodox Protestant religion as reason for vaccine refusal (2162 cases) or because they were linked to orthodox schools (152 cases); in total, 28 (1%) cases were explicitly non-OPI, and for the remaining 365 (13%), it was unclear, but most of them did reside in low vaccine coverage areas with a high proportion of OPIs.

For this analysis, we partitioned the cases into three regions—North, Middle, and South—according to the timing of the summer school vacation, and we used for each case the reported day of symptom onset (Figure 1). The epidemic in the South had 132 of the 416 reported cases before start of the vacation in week 27, with a slow increase in incidence after the vacation. In the Middle region, 561 out of 1810 cases were reported before the vacation started in week 30, with a clear rebound

after summer. In the North, only five cases occurred before the vacation started in week 28, and most of the 474 cases were observed after the vacation.

We constructed two time series of weekly incidences (Monday–Sunday) per region, one with all notified cases and one with only hospitalized cases, allocated into weeks by day of symptom onset. The dataset consisted of 44 weeks including 1 week without notifications at the start. Data to estimate the initial proportion of susceptibles in the affected population at the start of the epidemic came from a large cross-sectional seroprevalence study in The Netherlands, carried out in 2006–2007.[21] In that study, eight municipalities with low vaccination coverage due to a high proportion of OPIs were oversampled, and we used the data from these municipalities. For each of the 1518 sampled individuals, we used OPI status, vaccination status, age, and serologic result. To translate the proportion of susceptibles to actual numbers, we assumed a population size of 250,000 OPIs,22 of whom 38,717 were children between 4 and 11 years old (Orthodox denomination in primary school database23), which translates to 4840 children per age cohort. Assuming 20 of such age cohorts, there were 153,200 adult OPIs.

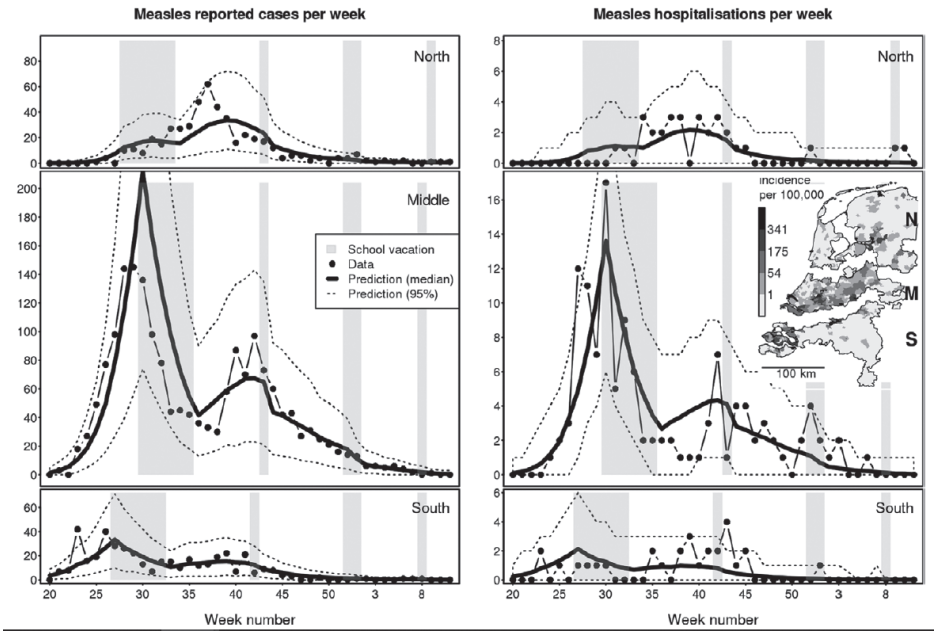

Figure 1. Epidemic curves in the three regions, observations with model fit and prediction. Left, Notified cases; Right, hospitalized cases. The inset shows incidence maps in the three vacation regions.

Main Outcome

Our main aim was to estimate the impact of the school vacation on the contact rate, expressed as a reduction of reproduction number *R*, defined as the mean number of secondary cases per primary case, and the number of averted cases. To this end, we built a stochastic transmission and notification model, which we fitted to the notification and hospitalization time series data. This resulted in an estimate for the parameter φ, which is the relative reduction in *R* during school vacations. For the number of averted cases, we compared the estimated total number of measles cases with the counterfactual epidemic size without school vacation, which we simulated with our fitted model.

Transmission and Observation Model

Full details of the transmission and observation models are given in the eAppendix; http://links.lww.com/EDE/B343. We assumed that transmission could be described by a discrete time Susceptible-Exposed-Infected-Removed model (Figure 2), with time steps of 1 week, separately in each region North, Middle, and South. In our model, we tracked four unobserved variables in each region: the numbers of susceptibles *X*, new infections *I*, latently infected individuals *E*, and symptomatic and infectious measles cases *Y*. We assumed an incubation period of 11.7 days[24] (1.67 weeks) and an infectious period of 1 week.[25,26] Our data consisted of the numbers of notified cases *C* and the numbers of hospitalized cases *H*, which were both subsets of the measles cases *Y*. Hospitalization was modeled with a fixed probability *p*hosp and notification with a random probability *pl t* notify( ) for region *l* in week *t*. Our model parameter of primary interest was φ, which is the proportional reduction in *R* during the school vacation. Because the model does not include a total population size with individuals immune by vaccination or previous infection, we could not estimate the basic reproduction number *R*0 (*R* in a susceptible population); instead, we estimated the effective reproduction number *R*eff at the start of the epidemic, which is the number of secondary cases infected per primary case in a partially immune population. In a second step, independent of the Susceptible-Exposed-Infected-Removed model, we used *R*eff to estimate *R*0 by estimating the proportion of the population that was susceptible at the start of the epidemic from seroprevalence data (see below). We also estimated *p*local, which is the proportion of secondary cases that were infected exclusively locally (within the region). The parameters *R*eff, φ, and *p*local together determine the local and global (across all regions) reproduction numbers during school term as well as during the vacation: *R*L school, *R*G school, *R*Lholiday, and *R*Gholiday .To calculate our second main outcome, the number of cases averted as a result of the vacation,

we used the estimated number of unobserved measles cases and compared it to a counterfactual epidemic over the same period in the absence of school vacations. The counterfactual was obtained by simulating the epidemic with our model but without vacation effect, once for each posterior sample from the Markov Chain Monte Carlo (MCMC) chain (see below), starting with the situation in week 27 (start of the first vacation).

Data Analysis and Model Comparison

Full details of the data analysis, alternative models, and model comparison are given in eAppendix; http://links.lww.com/EDE/B343. The model was fitted by Bayesian MCMC in JAGS software,[27] called from statistical software R[28] by use of the runjags package.[29] We used uninformative Jeffrey's priors for proportions (with the exception of *p*notify) and mildly informative priors for other parameters to improve MCMC mixing. For *p*notify, we used an informative prior based on a survey after the 1999–2000 measles epidemic in the same region, which identified *X*survey = 15 officially notified measles cases among *N*survey = 164 self-reported cases.[30]

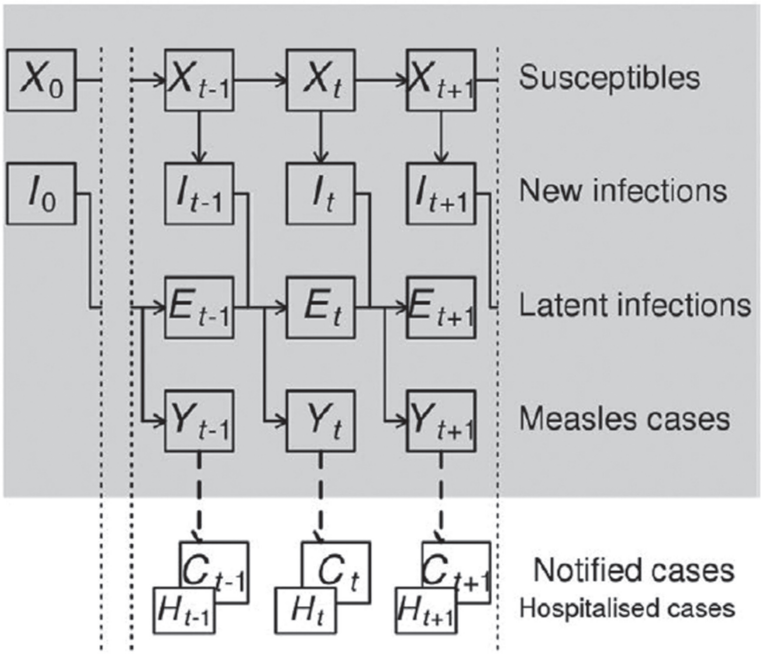

Figure 2. Flow diagram of the transmission and notification models. Time *t* is discrete with time steps of 1 week. Solid arrows indicate the flow of individuals between compartments and in time (infectious cases *Y* do not move to the next week: they recover). Dashed arrows indicate observation of part of the measles cases by notification and/or hospitalization. Compartments in the gray box are not observed.

Both to address sensitivity of our estimates to model assumptions and to explore additional effects of the school vacations, we fitted a set of alternative models to the data. These models included a change in the proportion of local transmission during the school vacation, different local reproduction numbers in the three regions, and a systematic time trend and vacation effect on case notification rates, in all combinations. We also tested for sensitivity to the notification rate by fixing *p*notify to the mean of the informative prior (0.091), and we tested if the reduction in transmission was a true vacation effect or a more general seasonal effect. Predictive performance of models was compared by means of the Watanabe–Akaike Information Criterion (WAIC)[31]: the lower the criterion, the better the model predicts the data. As a rule of thumb, models with <2 points difference in WAIC are considered equivalent; a >5 points difference is strong evidence in favor of the lower-criterion model.

The Number and Proportion of Susceptibles

The above analysis results in an estimate for the initial number of susceptibles. To validate this estimate and to translate the estimated *R*eff to an estimate for *R*0, we used seroprevalence data and OPI population data to estimate proportions and numbers of susceptibles. Because of the uncertainty in how to define the affected population and number of susceptibles, we took three approaches (full details in eAppendix; http://links.lww.com/EDE/B343):

- All OPIs that were not maternally immune, not vaccinated, and not seropositive due to natural infection.
- All OPIs that were seronegative
- All people in OPI municipalities that were seronegative (sample population of municipalities with low vaccination coverage in the serosurveillance dataset21).

Results

Figure 1 shows the measles notification and hospitalization data and the model fit: for each week, the predicted notifications (±95% prediction interval), based on the posterior distribution of parameters and unobserved variables. The median predictions describe the epidemic curve in the three regions well. The epidemic in the South is interrupted by the vacation and does not really take off anymore at the

end of the summer. In the Middle region, the vacation leads to a large decrease in incidence, followed by a second peak in the autumn, after the vacation. In the North, the epidemic seems to start in the summer vacation, but at a slow pace, followed by an acceleration when the schools start again. The hospitalization data (right) seem to fit better than the notification data (left), at least in the North just after the summer vacation and in the Middle during the summer vacation. Apparently, the incidence patterns in the two time series do not completely match. The fact that the model predicts the (less-biased) hospitalizations better than the notifications is reflected by the wide prediction intervals with the latter.

Parameter estimates (with 95% credible intervals [CI]) are shown in Table 1. The total reproduction number (local plus global) decreased from 1.96 (95% CI: 1.82, 2.11) to 0.92 (95% CI: 0.82, 1.02) during school vacations, a 53% (95% CI: 45%, 60%) lower contact rate. Most contacts, 83% (95% CI: 76%, 89%), were exclusively local, that is, within the region. The mean notification probability for the whole epidemic is estimated to be 16% (95% CI: 11%, 22%) but with large variation between weeks and across regions (standard deviation 6.8%). Furthermore, it was estimated that 1.1% (95% CI: 0.8%, 1.5%) of all measles cases were hospitalized.

TABLE 1. Parameter Estimates of Default Model

Parameter	Description	Posterior Median	95% Credible Interval
Transmission			
R_{eff}	Effective reproduction number during school term	1.96	1.82, 2.11
$(1-\varphi)R_{\text{eff}}$	Effective reproduction number during vacation	0.92	0.82, 1.02
p_{local}	Proportion of transmission exclusively within own region	83%	76%, 89%
φ	Overall reduction in reproduction number during vacation	53%	45%, 60%
Notification			
p_{notify}	Mean notification probability	16%	11%, 22%
$p_{\text{hospitalize}}$	Hospitalization probability	1.1%	0.8%, 1.5%
$\text{sd}(p_{\text{notify}})$	Standard deviation of notification probability	6.8%	4.6%, 9.9%

Figure 3 explicitly shows the week-by-week posterior notification rates with median ranging from 8% to 27% (andan outlier of 43%), indicating that indeed, especially in the North and Middle, there are extended periods of several weeks with consistently lower or higher proportions of cases notified. In all regions, the notification is higher when the outbreak is in its growth phase: first in the South with about 43% notified in a single week, then in the Middle just before the summer vacation, and finally in the North after the vacation.

The impact of the school holiday on epidemic size in the three regions is shown in Table 2, where the estimated numbers of susceptibles and total measles cases are compared to the counterfactual simulated number of cases. A total of about

4900 (3400, 7100) cases has been averted, which would have meant 30% more cases than currently observed. With the same notification rate, this would have resulted in about 3400 notified cases, about the same as in the previous epidemic in 1999–2000.[32] The vacations had the largest impact in the Middle, where a disproportionate part of these averted cases would have occurred if the summer vacation had not interrupted transmission just before incidence would have peaked.

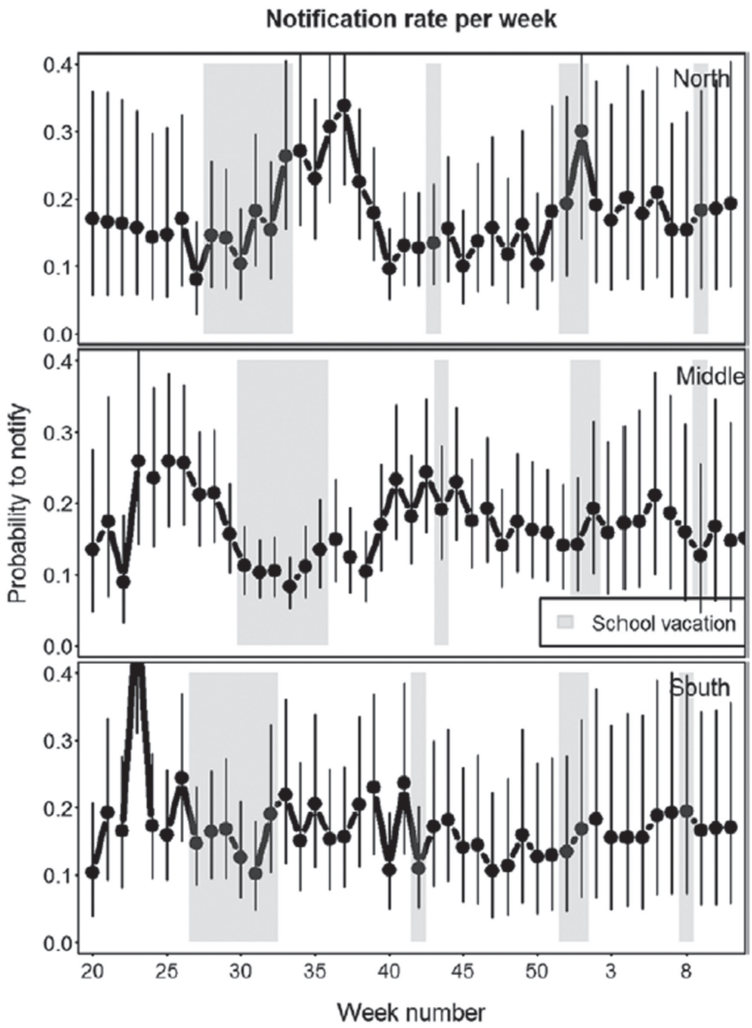

Figure 3. Weekly posterior notification rates (median and 95% credible intervals) in the three regions.

TABLE 2. Estimations of Epidemic Size: All Values Are Posterior Medians With 95% Credible Intervals and Projections are Based on Model Simulations

				Projection (Without Vacation)	
Region	Susceptible at Onset	Estimated Cases	Observed Notifications	Projected Cases	Averted Cases
North	3900 (2800, 5600)	2600 (1900, 3800)	474	3200 (2300, 4700)	600 (300, 900)
Middle	18,600 (13,900, 26,100)	11,600 (8600, 16,300)	1810	15,400 (11,500, 21,600)	3800 (2600, 5400)
South	3600 (2600, 5100)	2400 (1700, 3300)	416	2900 (2100, 4200)	600 (300, 900)
All	26,000 (19,600, 36,100)	16,600 (12,600, 23,200)	2700	21,500 (16,200, 29,900)	4900 (3400, 7100)

By using cross-sectional serology data, we took three approaches to approximate the number of susceptibles at the start of the epidemic and used this to calculate *R*0, assuming *R*eff = 1.96. In two approaches, we assumed the susceptibles to be a proportion of OPIs only; then, the number of susceptibles was estimated at 28,000 unvaccinated or immune from natural infection or 32,000 seronegative. Both these estimates are in line with those from our transmission model (Table 2). These numbers represent 11.2% or 12.8% of the OPI population, resulting in *R*0 estimates of 18 or 15, respectively. In the third approach, we assumed the susceptibles to be the seronegatives among all people in OPI municipalities. That resulted in an estimated 45,000 susceptible individuals, which is 7.3% of that population and corresponds to an *R*0 estimate of 27.

Three sets of alternative models were fitted to the data to address some of the model assumptions. Table 3 shows ΔWAIC values for all fitted models, with the default as reference. First, more complex models were fitted to explore additional vacation effects and robustness of our main results. Figure 4 shows that none of the main results (φ and number of averted cases) change with more complex models. Table 3 does not indicate a systematic time trend or vacation effect on notification, but models allowing for a change in the proportion of local transmission during the vacation do fit the data better (5-point decrease in WAIC), suggesting a shift from 88% (95% CI: 81%, 94%) to 25% (95% CI: 0.5%, 51%) local transmission in the vacation period. This is equivalent to an 86% decrease in *R*L from 1.67 to 0.23 and a threefold increase in *R*G from 0.23 to 0.69. Assuming different local reproduction numbers in the three regions further improves the fit (3–5 WAIC points), suggesting lower transmissibility in the North and therefore a smaller effect of the vacation in this region (Figure 4).

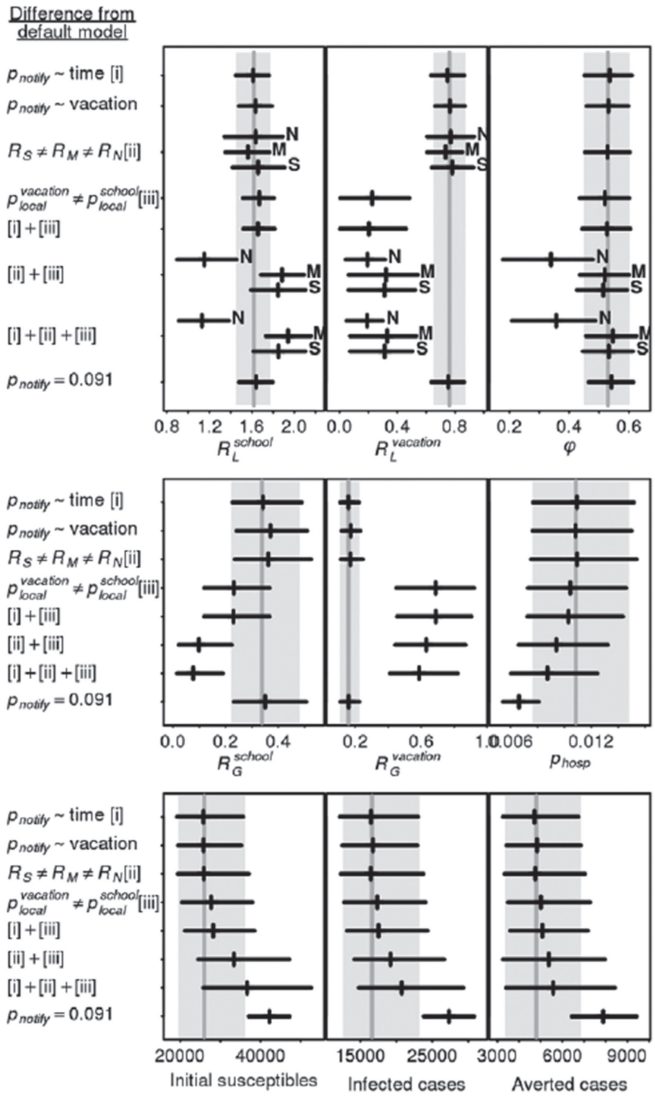

Figure 4. Sensitivity of parameter estimates to model choice. For each of the parameters, the gray line (area) indicates the posterior median (95% credible interval, CI) from the default model. The black crosses (lines) indicate the posterior medians (95% CIs) from the alternative models, from top to bottom: including a time trend in the notification rate (i); including a summer vacation effect in the notification rate; different local reproduction numbers in the three regions (ii); change in proportion of local contacts during the school vacation (iii); three combinations of (i), (ii), and (iii); fixing the mean notification rate at 0.091. Models with (ii) have separate estimates of local reproduction numbers for the three regions; models with (ii) + (iii) also for the reduction in transmission.

TABLE 3. Model Comparison by WAIC (Watanabe–Akaike Information Criterion)

Timing of Holidays	Change in p_{local} During Holiday	Different R_L Between Regions	Notification Model			Change in WAIC
			Time Trend	Holiday Effect	Fixed at 0.091	
Original data						0
Original data				Yes		8.58
Original data			Yes			0.14
Original data			Yes	Yes		11.31
Original data		Yes				0.99
Original data		Yes		Yes		12.63
Original data		Yes	Yes			4.07
Original data		Yes	Yes	Yes		13.61
Original data	Yes					−4.76
Original data	Yes			Yes		2.20
Original data	Yes		Yes			−1.02
Original data	Yes		Yes	Yes		5.64
Original data	Yes	Yes				−8.48
Original data	Yes	Yes		Yes		1.66
Original data	Yes	Yes	Yes			−10.62
Original data	Yes	Yes	Yes	Yes		1.18
Original data					Yes	13.06
Start in week 26						40.58
Start in week 27						30.25
Start in week 28						18.46
Start in week 29						16.03
Start in week 30						20.42
Start in week 31						31.72

Second, the notification probability was fixed at 9.1%, which was the mean of the prior distribution, because the mean posterior was so much higher. This does result in a higher WAIC (Table 3), and in a different scaling of observed incidence versus estimated epidemic size, but not in different estimates for reproduction numbers and the reduction in transmission φ (Figure 4). This model results in an estimated susceptible population before the epidemic of 42,000, which matches the above estimate of 45,000 from seroprevalence data assuming the affected population to have consisted of all individuals in the OPI municipalities.

Third, various alternative vacation schemes were used to test for a more general seasonal effect instead of a specific vacation effect. We did this by changing the vacation data $Vi(t)$ such that they were the same for the three regions, starting in either of the weeks 26 through 31 (originally they started in week 27, 28, and 30). Fitting these alternative models resulted in ΔWAICs > 15, a strong indication that the reduction in transmission was best explained by the true (regionally specific) vacations (Table 3).

Discussion

We estimated the effect of school vacations on measles transmission by fitting a transmission model to data of the Dutch measles epidemic of 2013–2014. By estimating the reproduction number during school term and during the vacation, we found a contact rate reduction of 53% (95% CI: 45%, 60%) during the school vacation. We estimate that about 16,600 people were infected during the entire epidemic and that 4900 were averted as a result from the vacation. Better fitting and more complex models (as measured by WAIC) suggest that the within-region and across-region effects on transmission were different: transmission within regions had decreased by about 86%, partially compensated by a threefold increase in global transmission. All estimates were insensitive to model assumptions on the case notification process, such as a time trend in notification or reduced notification during the vacation.

The 53% contact rate reduction during the vacation is similar to estimates from the prevaccination era, when comparing school-term and summer vacation transmission rates,[2,33] though direct comparison is difficult because in those studies vacation reduction was not estimated as a parameter and transmission rates were not translated to a standard susceptible population as we do with *R*eff. Our analysis shows that it is likely that the reduction can largely be attributed to a lower contact rate among susceptible children at school, for two reasons. First, the different vacation schemes in the three regions provided the opportunity to test if the reduction in transmission was associated with the school vacations. Indeed, alternative models where we assumed synchronized 6-week periods with a change in reproduction number all fitted worse (WAIC more than 15 points higher). Second, in more complex models, we estimated that in addition to an overall decrease in transmission, the proportion of exclusively local transmission decreased from 88% to 25% during the vacation, indicating fewer contacts in a less constrained setting, which was also observed in movement patterns in UK school children.[34] A large part of influenza transmission also takes place at schools, with explicit estimates for seasonal influenza up to 20% with 20 years of surveillance data in France[12] and 26% in a closely monitored outbreak in Mexico City[13]; for pandemic influenza: 50% in Alberta, Canada,[14] and 54% in The Netherlands.[15]

The shift from mainly local to more global transmission is an important result that supports the role of schools, but it must be realized that the estimates themselves are specific for the particular population and regionalization. Looking closely at the

epidemic curves in the three regions, we see that the incidences in the South and the Middle region decrease during the vacation and rebound thereafter but that the epidemic in the North had a slow start with an increase in incidence during the vacation. The more complex models may explain this by a lower local reproduction number in the North, followed by more cross-regional transmission during the vacation. Our main quantitative estimates, the reduction in transmission during the vacation and the number of averted cases, were insensitive to the complexity of the transmission model.

Comparison of the different notification models by WAIC did not discern a systematic time trend (increase or decrease) nor a vacation effect on notification of infections but did show a large variation in notification rates across regions and weeks. The week-by-week posterior notification probabilities in the three regions (Figure 3) do, however, suggest some patterns. First, notification seems to have decreased during the vacation in the Middle region, where most of the epidemic took place and where the vacation had the greatest impact. Second, notification rates seem higher in the initial phase of the local epidemics, which was only after the summer vacation in the North. These patterns could have been true but unidentifiable with our dataset because they occurred in different weeks in the three regions and could therefore better be explained by the random notification term. Better understanding the variation in notification probably requires more detailed data on possible covariates, such as differences in notification policies between local health services and changes therein through time, and external factors, such as media attention,[35] but that was not in the scope of our current analysis.

A difficulty in the current transmission model fit is that we use notification and hospitalization data but no data on the underlying epidemic size or susceptible population. In the model, these observations are scaled by means of a weakly informative prior on the notification rate (mean 9.1%), based on a survey from the previous 1999–2000 epidemic. The posterior notification rate turned out much higher (mean 16%), and although this did not affect the estimated reproduction numbers and reduction in transmission, it does cast doubt on the accuracy of the estimated true epidemic size and cases averted due to the vacation (Figure 4). To put these estimates in perspective, we also estimated the size of the susceptible population by using cross-sectional seroprevalence data, assuming that the epidemic spread only in the OPI population or in the municipalities with a large proportion of OPIs. In the OPI population alone, we estimated that 28,000–32,000

people were susceptible before the epidemic, in line with the estimated notification rate of 16%, substantially more than estimated for 1999–2000.[30] In the more extended population of OPI municipalities, we arrived at 45,000 susceptibles, more in line with a notification rate as in 1999–2000. The corresponding estimates for *R*0 were all in the range of earlier estimates,[36,37] albeit on the lower end (15 or 18) when concentrating on OPIs only and on the higher end (27) assuming a larger population including non-OPIs.

School closure is occasionally used as a control measure for measles outbreaks.[16,17] Our analysis shows that this could indeed be very effective in reducing local transmission. However, our results also show that measles transmission is not completely eliminated, so to stop a local outbreak, schools should be closed long enough to prevent resurgence once they reopen. That will only be useful if there are not yet many cases and if the local community is well protected by vaccination. Otherwise, schools should be closed for a long time with major economic and societal impact,[18,19] which in addition increases the risk for measles introduction into other areas by an increase in transmission across regions.

Our analysis was motivated by the fact that since the start of mass vaccination programs, our understanding of measles dynamics in current populations is decreasing because of the effective vaccination program. This is especially true for transmission in relation to school vacations. The Dutch 2013–2014 epidemic provided excellent data for an improved estimate. Our main results, which are not specific to this epidemic, are a large reduction in contact rates during the vacation (53% in our case) and the observation that there was a shift from local to cross-regional transmission. Scaling of the observed notifications to the underlying epidemic curve proved more difficult, requiring more detailed data on the notification process. That also affects our estimates for the basic reproduction number *R*0, though we can confidently put lower and upper bounds at 15 and 27. These results show that there have been no fundamental changes in measles transmission dynamics since introduction of mass vaccination.

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Chapter 12

General Discussion

The objective of this thesis was to study the large measles outbreak that took place in the Netherlands in 2013/14. We aimed to obtain new evidence on measles virus infection, its epidemiology and the effects of early measles vaccination, in order to inform measles control in the Netherlands and beyond.

After a general introduction in **Chapter 1**, we described in **Chapter 2** the measles outbreak of 2013/14 that occurred in The Netherlands. The outbreak took mainly place within the orthodox Protestant community. We described the epidemiology of the outbreak by analysing measles notifications and comparing key characteristics with the previous (1999/2000) outbreak in the orthodox Protestant community. In total, 2700 measles cases, 181 hospitalisations and 1 death were reported. The median age was ten years, four years older than in the previous epidemic in 1999/2000. We found that the longer inter-epidemic periods nowadays shift the age distribution of reported measles cases to higher age groups. As the risk of complications and hospitalisations increases with higher age, this implies that higher risks of complications and hospitalisations will characterise future outbreaks.

In **Chapter 3,** we assessed the actual burden of the 2013/14 measles outbreak in a first study that used two different approaches that assess the completeness of reporting. Both approaches estimated the number of reported cases to be around 9% of all measles case (8.8% and 9.1%), which lends support to the credibility and validity of both approaches. Given that 9% of cases were reported, the estimated total size of the 2013/14 outbreak approximated 31,400 measles cases.

In **Chapter 4,** we assessed the severity and contagiousness of 2539 unvaccinated (94%), 121 once vaccinated (5%) and 16 (1%) at least twice-vaccinated measles cases. Compared with unvaccinated and once-vaccinated cases, twice-vaccinated cases were less severe and less infectious. None of the twice-vaccinated cases reported complications or required hospitalisation, and none was reported to be the source of other measles cases. In contrast, 15% of the unvaccinated and once-vaccinated patients reported either complications or hospitalisation and 8% of unvaccinated and 6% of once-vaccinated patients were reported to be the source of other measles cases. Our findings support the recommendation of the WHO of a two-dose MMR vaccination schedule [8].

In **Chapters 5, 6, and 7**, we discussed the uptake, effectiveness, and tolerability of an early MMR vaccination offered to infants between 6 and 14 months of age.

The national vaccination register is individual-based and is therefore suitable for tailored interventions such as the administration of an early MMR to infants between 6-14 months of age who live in municipalities with low vaccination coverage (<90%). The vaccine uptake of invited infants was 57% (5800 out of 10,097 infants). Infants at highest risk of measles exposure (i.e. those without prior DTP-IPV vaccination and from families who refuse vaccination) had an intriguing lower uptake (1%) than infants with lower exposure to measles (with prior DTP-IPV vaccination (70%). Our study was the first to assess the vaccine effectiveness of an MMR vaccination administered to infants 6-14-months-old against *laboratory-confirmed* measles in an observational setting. It was also first to assess the tolerability of the vaccine at age six months in a high-income country. The crude estimate of the early MMR vaccine effectiveness was estimated to be 94% (95%CI 79%-98%). However, when we, in contrary to previous studies, took into account of the different levels in exposure between the vaccinated and unvaccinated infants, the effectiveness decreased to 71% (95%CI -72%-95%). Administering the vaccine to infants of six months old was considered well-tolerated. Adverse events were similar or fewer than observed among 14 month-olds infants.

In **Chapters 8 and 9**, we determined the protection against measles at the individual and population level. We assessed the correlate of protection against measles and subclinical (asymptomatic) measles among once vaccinated children. We used paired blood samples, of which the first sample was taken after the start of the outbreak. The correlate of protection against measles was estimated to be below 0.345 IU/ml, and the correlate against subclinical measles was found to be 2.1 IU/ml. Correlates of protection guide seroprevalence studies in distinguishing those immune from those susceptible. In a seroprevalence study in The Netherlands, participants born around the introduction of measles vaccination (1972 - 1990) appeared to have a relatively high proportion of susceptible individuals. Using a bead-based multiplex immunoassay (MIA), more than 5% of individuals had antibody levels below the assumed level of protection. In **Chapter 9**, we reassessed the seroprevalence of cohort 1972-1990 using the plaque reduction neutralisation test (PRNT). The PRNT tests for measles-specific antibodies that can neutralise measles virus, in contrast to the MIA, which tests for the presence of measles-specific antibodies. Based on PRNT and MIA results, protective antibody levels of birth cohort 1972-1990 were 99% and 94%, respectively. We found that thirty years after vaccination, the protective level of the oldest birth cohort that was vaccinated was still high. Furthermore, we revealed that the vast majority of the remaining susceptible individuals was unvaccinated.

In **Chapter 10**, we assessed the economic burden of the measles epidemic with a societal perspective. We estimated that the measles outbreak caused an economic burden of ≈€3.9 million. The 2700 reported cases resulted in an estimated €1,443 per reported case. The main cost was the deployment of employees at local health authorities.

During the epidemic, the number of reported cases decreased during school vacations. We hypothesised that the reduced number of reported cases was either due to reduced reporting or to reduced transmission. In **Chapter 11**, we estimated a reduction of 53% (95% credible interval: 45%, 60%) of the contact rate during school vacation using a transmission model. There was a shift from mainly local transmission during school term to mainly cross-regional transmission during school vacations. A vacation effect on the notification rate was not observed. Despite a reduced contact rate during the school vacation, measles transmission was not stopped, which makes it unlikely that school closure is a possible effective control measure.

In this final chapter, **Chapter 12**, we now discuss all studies included in this thesis by describing what was known before the study, our findings and the added value. Based on this, we make recommendations, discuss implications of the findings and provide direction for areas of future research.

Chapter 2: Large measles epidemic in The Netherlands, May 2013 to March 2014: changing epidemiology

Since the introduction of measles vaccination in 1976, The Netherlands has experienced several large measles epidemics, in for example 1988, 1992–94, and 1999–2000 [1,2]. These outbreaks mainly affected orthodox Protestants, a geographically clustered population with overall lower measles-mumps-rubella first dose (MMR-1) vaccination coverage (60%) than the rest of the country (~ 95%) [3]. A serological survey carried out in 2006–2007 confirmed a high risk of a large measles epidemic among orthodox Protestants [4]. The seroprevalence of anti-measles antibodies was 36% in 1-4-year-old orthodox Protestant children, and 63% in 5-9 year-olds. Another outbreak was therefore expected among the Dutch orthodox Protestants.

The expected outbreak started in May 2013. This epidemic was large with 2,700 reported cases, including 181 hospitalisations, and one death [5]. The risk of complications was highest in cases below four years or above 40 years of age (both 16%). The median age in the epidemic was ten years, four years older than in the previous epidemic in 1999–2000. The higher median age was consistent with a longer inter-epidemic interval before the epidemic of 2013/14 (13 years) compared with the inter-epidemic before the epidemic of 1999-2000 (6 years). Another remarkable finding was the difference in measles incidence below the age of eight years. The measles incidence observed in 2013/2014 was half the incidence in 1999-2000. A plausible explanation is the increasing vaccination coverage among orthodox Protestants. A survey among 18-30-year-old orthodox Protestants found that this group was more likely to be vaccinated than their parents were and that their children were more likely to be vaccinated [6].

An improvement in vaccination coverage will affect the epidemiology of future epidemics: longer inter-epidemic periods, a shift in age-distribution to older ages, and associated higher complication and hospitalisation rates are to be expected. Complication and hospitalisation rates are highest among very young children and adults [7]. On the other hand, an increased vaccination coverage will lead to a decreased incidence. While the vaccination coverage over the last decades increased among orthodox Protestants, the vaccination coverage in the general population of The Netherlands decreased from 96.0% in birth cohort 2011 to 92.9% in birth cohort 2016. This decline seems to have halted, as the vaccination coverage remained 92.9 % in birth cohort 2015 and 2016. A decreased vaccination coverage in the general population, however, might lead to a spillover of measles virus transmission during future outbreaks from the orthodox Protestant into the general population of The Netherlands.

During the 2013-2014 outbreak, we observed 61 reported cases of measles infection in children aged between 14 months and eight years who, according to the national immunisation programme, had received a single MMR vaccination. The expected number of measles breakthrough cases following a single vaccination dose is relatively high because approximately 2-5% fail to develop an adequate immune response upon the first vaccination [8,9]. Out of those who do not respond to the first dose, more than 80% develop an adequate immune response after a second MMR vaccination [8]. Whereas in The Netherlands, children receive their 2nd MMR vaccination at age nine years, most other European countries administer the second MMR at a younger age [10]. An earlier second MMR vaccination has

the potential to prevent several cases of primary vaccine failure during future outbreaks. In choosing a new timing schedule for a second MMR vaccination, the immunity on the long term should be monitored, as well as the consequences for the immunity of the other two components (mumps and rubella) combined in the MMR vaccine.

Chapter 3. The tip of the iceberg: incompleteness of measles reporting during a large outbreak in The Netherlands in 2013–2014.

Measles is a notifiable disease, but not all measles patients seek health care consultations, nor do physicians report every consultation for measles. The number of reported cases was therefore an underestimation of the total number of measles virus infections. So far, two approaches have been used to assess the completeness of measles reporting. A first approach uses community-based surveys to identify measles cases, and then assesses how many of them are reported in a register. This survey approach has been used as early as 1926 in the USA [11]. Since then, a few other community-based surveys from all over the world have been published, reporting that notified measles cases range from 3% up to 64% of total infections [12,13]. These surveys, however, all originate from the 1900s and lack laboratory confirmation of cases [11,14-17]. Another approach assesses the completeness of measles reporting by comparing the number of reported cases with the number of people projected as susceptible and assuming that almost all of these are infected [18]. This approach resulted in estimated completeness of reporting ranging from 7% for a measles outbreak in 1999–2000 in The Netherlands [19], up to 63% for endemic measles in England and Wales in 1946–1979 [18].

In Chapter 3, we assessed the number of measles cases in a municipality in the Bible belt with a community-based survey and the number of susceptible children prior to the outbreak in The Netherlands. We compared both with the number of reported cases and concluded that the completeness of reporting was approximately 9% with both approaches. The community-based survey revealed the determinants for measles cases to be reported (older age at infection, being infected at the start of an outbreak, having complications, seeking health care). The proximity of both estimates lends support to the credibility and validity of both approaches. Adjusting for the incompleteness of reporting, the size of the 2013–2014 outbreak approximated 31 400 measles virus infections.

In our survey, contrary to previous completeness of reporting studies, self-reported measles cases were tested for measles-specific IgG antibodies to confirm infection with the virus. Our study suggests that estimates based on calculating the expected number of infections only can come surprisingly close to empirical estimates obtained in community-based surveys. For confirmation, similar studies should be carried out in other populations. We estimated the number of susceptibles based on vaccination coverage and population data. Another option of estimating the number of susceptibles is by conducting seroprevalence studies, as conducted recently in The Netherlands [20]. Estimating the completeness of reporting allows assessing the total number of measles virus infections. Having estimates of the number of measles virus infections makes it possible to calculate the risk of complications upon infection with measles virus and assess the disease burden of measles.

Chapter 4. Severity and infectiousness of measles vaccine failures in a large epidemic, the Netherlands, 2013-2014

Protection against measles on the population level is becoming largely dependent on vaccine-induced immunity. In The Netherlands, people born since 1975 are offered measles vaccination. To evaluate the measles vaccination programme in The Netherlands, monitoring of breakthrough infections is indispensable. Important parameters to measure are severity of infection and, in particular, contagiousness of vaccinated measles cases to assess a possible role of vaccine failures in the transmission of measles virus.

We found that the severity and infectiousness of once-vaccinated cases (respectively, 15% and 8%) were similar to unvaccinated cases (respectively, 15% and 6%), whereas symptoms in twice-vaccinated cases were milder and twice-vaccinated measles cases were less infectious than unvaccinated cases. None of the twice-vaccinated cases was hospitalised, nor reported complications, nor transmitted measles to another reported case. We found that twice-vaccinated individuals had a reduced risk of 99% (95% CI 11-100) of severe measles and a reduced risk of 98% CI -203-100) on being the source of infection for other measles cases compared with unvaccinated individuals. These reduced risks were estimated by assuming a vaccine effectiveness of 94% of a two-dose vaccination against measles [21]. These findings support the current vaccination schedule of two doses. This also suggests that the majority of once-vaccinated measles cases

concerned primary vaccine failures and twice-vaccinated cases concerned primarily secondary vaccine failures.

During a more closely surveyed outbreak in The Netherlands among health care personnel, no transmission was observed by twice-vaccinated measles cases either [22]. Documentation on the transmission of measles virus by twice-vaccinated individuals is scarce [23]. Out of 26 breakthrough measles virus infections in twice-vaccinated individuals in the US, three measles cases were the source of infection in others. The high proportion of secondary vaccine failures in this group explains the rare observation of transmission from twice-vaccinated cases [24]. Secondary vaccine failures are due to the waning of antibodies or incomplete immunity and can be distinguished from a regular infection by the early on presence of high-avidity measles IgG antibodies and very high neutralising antibody concentrations [25]. Because of the accelerated immune response, clinical severity and viral load are lower [26]. Decreased symptoms, such as coughing, and a reduced viral load, seem a plausible explanation for the absence of transmission of measles virus by twice-vaccinated cases during the epidemic of 2013/14. Given that an increasing percentage of the population depends on vaccine-induced immunity and that this immunity might wane with an ageing vaccinated population, we might see an increase in primary and, in particular, secondary vaccine failures in future outbreaks. Hence, enhanced surveillance of measles vaccine failures, including laboratory characterisation to differentiate primary and secondary vaccine failure and follow-up of contacts to assess infectiousness, is warranted.

Chapter 5. A novel measles outbreak control strategy in The Netherlands in 2013-2014 using a national electronic immunization uptake register: A study of early MMR uptake and its determinants.

In The Netherlands, the first dose of MMR is scheduled at 14 months of age and the second dose at nine years of age. Most countries in Europe have the first vaccination scheduled around 12 months of age [10], as recommended by World Health Organization (WHO) for countries without endemic transmission of measles [27]. WHO advises countries with ongoing transmission to vaccinate infants at nine months of age. On-time delivery of the first dose of measles-containing-vaccine (MCV) is regarded as essential to ensure optimal protection during the susceptible period in infancy. Most six-month-old infants have lost detectable maternal anti-measles antibodies, as observed by our group and others [28,29], and are at a higher

risk of developing measles as well as complications, such as pneumonia and otitis media [5]. The Dutch national immunisation programme, therefore, recommends children aged between 6 and 14 months to be vaccinated with the MMR vaccine before travelling to countries with endemic or epidemic measles transmission.

Following the first cases of the measles epidemic in May 2013, an outbreak management team advised the Ministry of Health, Welfare and Sports to offer an early MMR to infants between 6 and 14 months of age. In Chapter 5, we assessed the uptake of this vaccination campaign. Out of 10,097 invited infants, we observed that 5,800 infants (57%) received an early vaccination [30]. Of note was that of infants with prior DTP-IPV vaccinations, 70% received an early MMR, whereas only 1% was vaccinated of the infants who did not receive any dose of DTP-IPV. These infants were unfortunately at the highest risk of exposure to measles, as these infants had a higher probability of having unvaccinated siblings, who had a probability of transmitting measles.

This questions the impact of this intervention for those at the highest risk, because exposure to measles virus of young infants is more likely in families who refuse to immunise. The incidence observed among the age of 6-13 months during the outbreak of 2013-2014 was half the incidence of the outbreak of 1999-2000 [5]. The incidence among children between 14 months and three years of age was, however, also half the incidence of 1999-2000. Further analyses are required in addition to descriptive epidemiology to assess the impact of the early MMR on the occurrence of measles in the targeted group.

Exposure to measles seems however very likely for a large proportion of the early MMR vaccinated children, and impact of early MMR vaccination is therefore likely to be expected. Measles virus transmission took place mainly within the orthodox Protestant community because 40% refrain from vaccinating. Sixty per cent, however, do vaccinate [3]. The 40% that refrains from vaccinating comprises the infants without prior DTP-IPV vaccination, very few of whom received an early MMR vaccination (1%). These infants had the highest risk of exposure, due to potential measles within the family assuming siblings were also unvaccinated. The infants with prior DTP-IPV vaccination of whom 70% received an early MMR, however, partly fall under the 60% who do vaccinate in the orthodox Protestant community. These infants probably had no exposure to measles virus within the family but most likely elsewhere. Contact data from England showed that more than half of the weekly social contacts of children under one year of age are outside the

family, such as in nurseries/childcare (8%) and during leisure activities 24% [31]. Moreover, the majority of contacts of infants under one year are with children up to 10 years [32], which is precisely the age-group that was affected the most during the outbreak [5].

Having social contact data of infants might contribute to an actual estimation of the impact of future implementations of the early vaccination. Beyond assessing the impact of an early MMR, having data on social contacts of infants also provides quantitative data to evaluate other interventions to control other childhood diseases. It has to be taken into account that social contact data may be specific to the specific group affected by the outbreak. Regardless of the order of magnitude of the number of measles cases that were prevented, measles is dangerous, especially in children under the age of one [7], and can be prevented with a safe vaccine [33], so from a precautionary principle, offering early MMR is warranted.

The existence of a national electronic immunisation register such as Præventis allowed this targeted outbreak intervention, whereby infants were invited individually for an early MMR based on their risk (living in a municipality with MMR-1 coverage <90% and age between 6–14 months). The intervention was uniquely tailored. Feasible in the Netherlands but hard to achieve in many other countries due to the absence of vaccination registers. In Germany, for example, the vaccination coverage is determined based on surveys and insurance refund claim data [34], because the individual vaccination status is not centrally registered. This speaks for having the vaccination status for every individual registered centrally to be able to implement tailored vaccination campaigns.

Chapter 6: Tolerability of Early Measles-Mumps-Rubella Vaccination in Infants Aged 6-14 Months During a Measles Outbreak in The Netherlands in 2013-2014.

The current MMR vaccine used in The Netherlands is licensed at the European Medicines Agency for use in individuals aged 12 months and older. MMR vaccination at the age of six months is regarded as safe [35], based on studies mainly performed during vaccination campaigns in developing countries. The tolerability of MMR vaccination at six months of age had not been assessed in a developed country, in contrary to abundance in literature of the tolerability at the age of 14 months [8].

In Chapter 6, we described the adverse events (AE) in 962 MMR vaccinated infants. Parents of infants vaccinated at 6–8 months of age reported less systemic AEs (32%) than parents of children vaccinated at 9–11 months (45%) and 12–14 months (43%) of age ($p < .01$). For local AEs, there were no significant differences (5%, 7%, and 10%, respectively; $p = .08$). A possible explanation for the lower frequencies of AEs in younger infants is the presence of measles-specific maternal antibodies that impede replication of vaccine virus and thereby prevent the occurrence of systemic AEs.

The sample size (n = 962) was large enough to test the occurrences of adverse effects up to one in every 100 vaccinated infants. To detect rarer adverse events, a larger observational study was required, which is very challenging given the current response rates in observational studies. Lareb is the Dutch national pharmacovigilance centre, which includes passive surveillance of adverse events of vaccinations. Anyone with adverse events following vaccination is recommended to notify Lareb. However, the surveillance organised by Lareb is passive, resulting in an incomplete registration of AE and no control group. One way to circumvent the small sample size, lack of control group in a passive reporting system, and lack of long term follow-up is by linking the Praeventis database (the vaccination register of The Netherlands) with health care use registries, as has been done in Denmark. Of 657,461 children, health-related information was linked with the vaccination register through a unique identifier that is used in all national registries to assess possible associations with the MMR vaccination [36]. Given that Denmark also belongs to the European Union, and is therefore covered by the General Data Protection Regulation, it may be feasible to introduce a similar system in The Netherlands to optimise surveillance activities of the national immunisation programme including the monitoring of adverse events of vaccinations.

Chapter 7. Effectiveness of Early Measles, Mumps, and Rubella Vaccination Among 6-14-Month-Old Infants During an Epidemic in the Netherlands: An Observational Cohort Study.

In a systematic review of case-control and cohort studies, the effectiveness against laboratory-confirmed measles of a 1-dose measles-containing vaccine (MCV) administered at the age of 9–11 months was estimated to be 84%, while the vaccine effectiveness (VE) for infants who were vaccinated at the age of ≥12 months was 93% [21]. VE estimates for infants vaccinated below nine months of

age are scarce. Before our study, no VE estimates against laboratory-confirmed measles had been reported in observational studies among infants vaccinated at nine months or younger.

We showed that infants vaccinated between 6 and 14 months of age had a reduced risk, compared with unvaccinated infants, of laboratory-confirmed measles during an epidemic in The Netherlands, with a crude VE estimate of 94%. However, this changed when we took account of different levels of exposure between the vaccinated and unvaccinated group. The parents' choice to vaccinate usually aligns with the choices of their social network [37], and this can result in clusters of unvaccinated children [38], in for example day-care centres and elementary schools, resulting in different levels of measles exposure. Our VE estimate, adjusted for proxies of exposure to measles, against laboratory-confirmed measles declined to 71%. Due to the low numbers in our study, this estimate was no longer statistically significant. We concluded that early-vaccinated infants were at lower risk of measles than unvaccinated infants were, partly due to the herd protection provided by the regular national immunisation programme in The Netherlands.

The presence of maternal anti-measles antibodies that eliminate the vaccine virus and immaturity of the immune system causes a reduced effectiveness of an MMR when vaccinating below one year of age [39]. Part of the reduced effectiveness can be overcome by a second and third vaccination that ensures that most children, who did not develop an adequate immune response upon the first vaccination, become protected at revaccination [8,40]. However, in children who did develop an immune response at the first vaccination, revaccination only provides a temporary elevation of antibody levels. Six months after a secondary or tertiary vaccination, antibody concentrations are as high as before revaccination [40-42]. A reduced immune response following an early MMR vaccination compared with a vaccination 14 months of age therefore persists despite revaccinations [9,43]. Hence, the immunological basis for providing a second opportunity for measles vaccination is mainly to immunise those children who fail to respond to the first dose [8].

A reduced immunological response in early-vaccinated infants was reflected by a decreased protection against clinical measles in Canada. The attack rate was higher among children who received their first vaccination at 12 months (4%) compared to children who received their first vaccination at 15 months (2%) [44]. Recently, a small number of children to whom an early MMR was administered during the

outbreak in The Netherlands in 2013/14 had mild measles during a local outbreak in Urk (Hahné, personal communication).

Thus, administering MMR vaccine to 6-14 month-old infants provides immediate protection in the majority of infants although some of these infants continue with a reduced immune response, which leaves them more susceptible to a mild infection on the long-term, compared with infants vaccinated at a later age.

Future early MMR?

Based on the findings in Chapter 5, 6, and 7, we advise implementing a future early MMR during outbreaks between 6 and 14 months of age. The direct risk of measles at the age of 6–14 months outweighs the reduced immunogenicity in the long term. An important argument supporting this recommendation is that measles cases observed among infants who received an early vaccination are mild and considered secondary vaccine failures. Future implementation of the early MMR should be questioned when cases who were vaccinated early, experience full-blown measles, despite revaccination. Here, we discuss an optional change in the early MMR campaign to optimise the protection against measles on the short and long term.

The lower antibody levels in the long term that gave rise to the cases in Urk might be overcome by offering a different booster vaccination. In The Netherlands, MMR vaccinations are given subcutaneously. Although the first dose seems to give highest antibody responses when given subcutaneously, aerosol inhalation of attenuated measles virus gave equivalent or superior immune responses when used as a booster vaccine [45,46]. These results are most likely due to the different route of entrance giving better immune responses in areas where a natural infection route can be expected, as was found in macaques' models [47]. The implementation of aerosol vaccination is mostly a point of discussion in developing countries, where this vaccination offers logistical advantages over the classical needle vaccine. Public health authorities are probably not very tempted to replace a very effective and successful vaccine [48]. Nevertheless, the possible improvements offered by vaccines that are administered elsewhere, possibly resembling a natural infection route contrary to the current subcutaneous vaccine, merits further research.

The discussion point above concerns a possibility to improve an intervention, which, with the described tolerability and effectiveness [33,49], is already convincing enough to be implemented elsewhere with high measles incidence. In Europe,

incidence rates are highest among infants below one year [50]. From July 1, 2016 until June 30, 2017 the highest age-specific notification rate was observed in infants under one year of age (254.8 cases per million population), followed by children aged 1–4 years (124.8 cases per million population). During these 12 months, four deaths of unvaccinated infants below one year were reported. Especially Rumania suffers mortality due to measles. From 2013 until 2016, 45 fatal cases of measles were reported in unvaccinated infants below one year [51].

The disease burden prevented by early MMR vaccination campaigns consists of not only measles cases, but also potential cases of subacute sclerosing panencephalitis (SSPE) in the following years. SSPE is a rare, late-onset, neurological complication of natural measles virus infection. It may be a very rare complication of measles, but there is no proven treatment of SSPE, and SSPE leads to death. Reported SSPE incidence was estimated to be 4 -11 cases of SSPE per 100,000 cases of measles in the US [52]. Among reported measles cases in children below five years of age, one out of every 1700 to 3300 developed SSPE in Germany [53]. The risk of SSPE is estimated to be higher among young children according to data from the UK [54]. In the UK, infants under one year of age had a risk of 18 out of 100,000 compared with 1.1 per 100,000 among children infected with measles at five years of age or older [54]. Since a large part of the measles mortality is caused by SSPE in the Netherlands, a mandatory reporting of SSPE would be desirable. The only estimate in the Netherlands was between 1976 and 1986, when 77 cases were recorded, of which the majority (80%) had measles before the age of five [55].

Chapter 8. Additional evidence on serological correlates of protection against measles

The individual protection against measles is defined by the correlate of protection. The correlate of protection is a level of immune response correlated with protection against disease [56]. Correlates of protection guide the development of vaccines and the interpretation of seroprevalence studies. So far, only two studies contributed to the assessment of the correlate of protection of measles and found that antibody levels of 0.12 IU/ml were protective against measles [57,58]. Given the scarcity of estimates and that the neutralisation assay is subject to variation [59], more estimates are warranted. The anticipated epidemic in The Netherlands provided a unique opportunity to estimate the correlate of protection against measles as well as subclinical measles virus infection.

Paired blood samples of once vaccinated children were collected shortly after onset of the 2013-2014 measles outbreak and after the outbreak. Correlates for protection were assessed by considering the lowest neutralising antibody levels in children without MV infection. The correlate of protection against clinical measles symptoms was lower than 0.345 IU/ml. We observed a decreasing attack rate of subclinical MV infection with increasing levels of specific antibodies up to 2.1 IU/ml, above which no subclinical MV infections were detected.

Given the lack of estimates for the correlate of protection, our estimates are a useful complementation of current literature. Based on the evidence we observed, we could not report more than that the correlate of protection against measles is below 0.345 because of the limited range of antibody levels in our study group. Besides three seronegative once-vaccinated children, all other antibody levels started at 0.345 IU/ml. The correlate of protection against subclinical measles was observed within the range of antibodies within our study group and is therefore more robust.

As the enrolment of children in our study started after onset of the outbreak, children had the probability of being infected before the first measurement. What seemed to be a limitation of the study turned out to be an exciting finding. The use of four different tests enabled us to define the serological profiles per individual. Out of this data, we distinguished three groups. A group that experienced measles before inclusion in the study, a group that was not exposed to measles, and a group that was infected during the study period. With these defined groups, we were able to estimate the correlates of protection by excluding those that were exposed before enrolment. As we have shown that the first blood sample of participants do not necessarily need to be taken prior to an outbreak, future studies do not necessarily need to start prior to an outbreak.

The correlate of protection is currently exclusively based on humoral immunity only, while the humoral immunity is complemented by cellular immunity in the protection against measles [60]. Future studies should consider including the measurement of cellular immunity to assess its role in protection against measles.

Chapter 9. Seroprevalence of measles in The Netherlands: reassessed using a focus reduction neutralisation tests

Seroprevalence studies assess the protection against measles on the population level. Enzyme immunoassays (EIA) have been the preferred type of assay to study large number of samples in population-based seroprevalence studies [61] because antibodies of several pathogens can be measured at the same time, and EIAs are cheaper and easier to perform than the plaque reduction neutralisation test (PRNT). The PRNT, however, is considered the gold standard for measurement of neutralising antibodies, because it is aligned with the correlate of protection [57], and measures virus neutralising antibodies, rather than EIAs, which measures all measles-specific antibodies [59,62]. Although validated EIAs may show a good correlation with the PRN assay, EIAs have been shown repeatedly to display suboptimal sensitivity for detection of measles IgG in cohorts with vaccine-acquired immunity [62-64]. Using an EIA could therefore lead to overestimating the percentage of susceptibles in a population where the majority of individuals have vaccine-induced measles immunity [65,66]. In The Netherlands, a population-based cross-sectional seroprevalence study was conducted in 2006-2007 (n = 7900) (14). Serum samples were analysed by a multiplex immunoassay (MIA), a bead-based EIA, which revealed that immunity levels of birth cohort 1972 – 1986 were below the target immunity levels of the WHO of 95%.

We re-estimated the seroprevalence in The Netherlands for birth cohort 1972-1990 with sera from a population-based seroprevalence study, which was conducted in 2006 in The Netherlands. In addition, we assessed the performance of the MIA. We tested all samples with measles-specific antibody concentrations around the cut-off (the group below 0.3) and a representative sample of all samples with antibody concentrations far above the cut-off; we guaranteed a valid assessment of the performance of the MIA compared with the PRNT but limited workload and costs. Based on PRNT and MIA results, protective antibody levels of birth cohort 1972-1990 were 99% and 93%, respectively. Whereas the seroprevalence assessed with the MIA showed a diverse group of both vaccinated and unvaccinated persons susceptible to measles, the assessment with the PRNT, almost exclusively identified unvaccinated persons to be susceptible. We concluded that the oldest vaccinated birth cohort against measles are excellently protected against measles and that remaining gaps of immunity can be found in unvaccinated individuals born around the introduction of measles (1976).

These susceptible unvaccinated individuals comprise 2% of their birth cohorts. Herd protection prevents these individuals from measles. However, they are not the only susceptible group in The Netherlands, and they too are at risk during outbreaks among risk groups such as orthodox Protestants and anthroposophists[67]. These individuals are at high risk of developing complications (given their age) and are at risk while travelling to areas with measles transmission. Two per cent of cohort 1972-1981 signifies approximately 80.000 susceptible individuals. A group of at least 80.000 individuals is therefore at risk of measles, and can subsequently introduce measles virus in The Netherlands.

Contrary to other risk groups who refuse vaccination, these individuals born around the time of introduction of measles vaccination might be more willing to vaccinate. Vaccination is currently recommended for unvaccinated individuals born since 1965 when travelling to areas with measles transmission [68]. People are, however, not inclined to attend a travelling clinic when travelling within Europe, despite the high measles incidence in recent years. Perhaps a more active vaccination campaign is warranted for this particular group. Health care workers born since 1965 are also recommended to be vaccinated when immunity is lacking [69]. A stricter regulation concerning immunity against measles at the start of a function in healthcare settings, where regular contact with patients is present, needs to be considered given the risk of nosocomial transmission [70].

Last, it is noteworthy that the oldest cohort was vaccinated in 1976, and their blood sample was taken in 2006, after 30 years. Whether their antibodies are still sufficient in the decades to come remains to be seen and must be further investigated, legitimating new seroprevalence studies in The Netherlands [71,72], of which one is recently conducted in 2016/17 [20].

Chapter 10. The economic burden of the measles outbreak in The Netherlands, 2013-2014

In addition to the effects of disease on society, measles outbreaks have economic consequences. A measles outbreak demands a range of responses from the National Institute for Public Health and the Environment (RIVM), municipal public health services (MHS), and health care. Assessing outbreak costs, including costs of response activities by public health authorities, can help in preparing for future outbreaks and in optimising the allocation of public resources. In Australia, the

public health unit cost for responding to a single case of measles was $1,701 [73], a similar amount to our results. In the United States, the costs of containing an outbreak were much higher, estimated at $6,180 per case [74,75].

Using a societal perspective, we estimated that the measles outbreak led to substantial costs of ≈€3.9 million. These costs divided by the total number of reported cases result in an estimated €1,443 per case. Outbreak management costs were the primary cost comprising €1,540,000, with activities such as response to extensive media attention, registration of notified cases, and more surveillance activities than usual.

Compared with the overall health care costs that are spent yearly in The Netherlands (≈€94 billion in 2013), the costs that were caused by this outbreak can be considered as very little, only 0.0042%. However, when we take different perspectives, the costs of the outbreak can be considered high. The costs of giving one MMR vaccination are approximately €16 [76] (expenditure on the MMR-vaccine was € 2.6 million in 2013, number of births 171,341, and vaccination coverage of 93%). If all 30,000 infected people had been given a catch-up vaccination prior to the outbreak, it would have cost €480,000, a bit more than 10% of the outbreak cost. This is, of course, a hypothetical situation; the majority of the 30,000 cases do not want to be vaccinated. In short, from an economic perspective, the prevention of this outbreak by MMR vaccination would have been cost saving.

Chapter 11. The reduction of measles transmission during school vacations

During the 2013/14 measles outbreak in The Netherlands, a decrease in the number of reported cases during the summer holidays was observed. We set out to assess whether this was caused by a decrease in measles virus transmission and/or a decrease in the completeness of reporting. We also evaluated whether closing schools might be an effective measure to stop measles virus transmission during outbreaks.

In Chapter 11, we estimated a reduction of 53% in the contact rate (95% credible interval: 45%, 60%) during school vacations. The reduced contact rate caused a reduction in measles virus transmission during the summer vacation and led to an estimated 4900 (3400, 7100) averted cases. There was a shift from mainly

local transmission during school term to mainly cross-regional transmission during school vacations. The reduced transmission estimate was insensitive to model assumptions on the case notification process, such as a time trend in notification or reduced notification during the vacation.

We concluded that school vacations are associated with greatly reduced overall measles virus transmission. However, transmission is not eliminated, and increased long-distance transmission is likely to promote spread to other areas. Therefore, we expect that school closure is unlikely to prevent measles epidemics unless the outbreak is still limited to a few cases. A confirmation of the 4900 averted cases may be found by identifying pockets of susceptible individuals born before 2013/14 in the Bible belt in the recently conducted seroprevalence study in The Netherlands [20]

General recommendations:

Early MMR vaccination

- Based on our findings that the early MMR vaccination offered during the 2013/2014 measles outbreak in the Netherlands was obtained by the majority of parents of invited infants 6-14-month-old (58%), was well-tolerated (adverse events were equal or lower compared with older infants), and effective (71%), we advise to implement a similar early MMR vaccination campaign during future measles outbreaks in The Netherlands (Chapters 5, 6, and 7).

- Long terms effects of early measles vaccination at 6-14 months of age should be further assessed by future research, including immunological and vaccine effectiveness studies. One way could be by monitoring the age of vaccination in cases of measles vaccine failures, which will provide insight into the effectiveness of early MMR vaccination. Long-term effects of early MMR vaccination can also be studied by linking the national vaccination register with health-related registers to study the long-term vaccine effectiveness and further confirm the tolerability with an increased sample size (Chapters 6 and 7).

- Given that the highest measles disease and mortality burden and risk for complications including SSPE in Europe are observed among infants below one year of age, early MMR campaigns should be implemented in Europe in areas with high incidence of measles virus infection (Chapter 7).

Monitoring measles susceptibility

- Based on the seroprevalence reassessed with plaque reduction neutralisation assay, we concluded that the national immunisation programme is very effective in providing immunological protection against measles (Chapter 9). Vaccinated individuals of the first birth cohorts who were vaccinated are still protected after 30 years. Seroprevalence studies are warranted in the future to monitor the protection against measles among the Dutch population and especially the first birth cohorts who were offered MMR vaccination.

- In view of the small number of studies that estimated the correlate of protection, we recommend setting up new correlates of protection studies in case of future outbreaks, even if the outbreak is already in progress, since we have shown that already infected individuals can be identified and subsequently excluded when estimating the correlate of protection (Chapter 8).

Addressing immunity gaps

- Unvaccinated individuals born around 1976, when measles vaccination was included in the Dutch national immunisation programme, are relatively often susceptible (Chapter 9). Two per cent of the birth cohort 1972 – 1981 is susceptible, comprising roughly 80,000 individuals. This group has a relatively high risk of complications after measles due to their age. Currently, unvaccinated individuals born around the time of measles vaccine introduction are recommended to be vaccinated when travelling to measles endemic countries. However, a travel consultation is rare if one travels within Europe. Hence, other opportunities to reach these cohorts need to be explored. Health care workers are of particular relevance among susceptible groups since they are at increased risk of exposure and of infecting others. For this group, enhanced measures, such as an obligatory MMR immunisation when unvaccinated before starting a new job in health care settings, should be considered.

- During the 2013-2014 measles outbreak, 61 cases were reported in once vaccinated individuals between 4 and 9 years of age (Chapter 2). Other European countries usually provide the second dose of measles vaccine between 1 and 4 years of age. A similar measles vaccination schedule in The Netherlands may have prevented a large part of these 61 vaccinated cases. Hence, the optimal age of the second MMR should be reconsidered in the Netherlands, taking into account consequences for measles immunity on

the long-term as well as the consequences for immunity against rubella and mumps.

Measles surveillance

- We used two approaches to estimate the completeness of measles reporting. (Chapter 3). One approach concerned a community-based survey, and the other a comparison between the number of susceptible individuals prior to the outbreak and the number of reported cases. The close similarity between both estimates suggests that an assessment using only the latter method would be sufficient to estimate the total burden of large measles outbreaks that affect the entire Bible belt.

- SSPE causes a large proportion of measles mortality in the Netherlands. To date, however, SSPE has not been a reportable disease. Making SSPE a notifiable disease would allow a better assessment of its disease and mortality burden in The Netherlands (Chapter 2).

- The Dutch population is becoming more and more dependent on vaccine-induced immunity, whereby waning immunity may lead to susceptibility in the long run. Hence, we might see an increase in primary and, in particular, secondary vaccine failures in future outbreaks. Enhanced surveillance of measles vaccine failures, including laboratory characterisation to differentiate primary and secondary vaccine failure and follow-up of contacts to assess infectiousness is warranted (Chapter 4).

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Appendix

Nederlandse samenvatting

Mazelen wordt veroorzaakt door het mazelenvirus. Het mazelenvirus is een van de meest besmettelijke ziekteverwekkers die we kennen. De symptomen bestaan uit hoesten, koorts, rode ogen, neusverkoudheid en een uitslag met rode vlekjes die 2-4 dagen na de eerste symptomen optreedt. In de meeste gevallen herstellen patiënten binnen 7 - 10 dagen na het begin van de infectie. Veel voorkomende complicaties zijn oorontsteking (4% van de gerapporteerde gevallen) en longontsteking (6%). Ontsteking van de hersenen (encefalitis) komt voor in ongeveer 1-4 per 1000-2000 gerapporteerde gevallen. Complicaties treden het vaakst op bij kinderen onder de 5 jaar en volwassenen ouder dan 20 jaar. De sterfte aan mazelen is ongeveer 0,05% in de rijke landen, maar wel 5% in sommige Afrikaanse landen. Geschat wordt dat dagelijks ongeveer 365 mensen, vooral kinderen in ontwikkelingslanden, sterven aan de gevolgen van de mazelen.

In Nederland is het mazelenvaccin in 1976 aan het rijksvaccinatieprogramma toegevoegd. In de eerste drie jaar bestond het mazelenvaccinatieprogramma uit een eenmalige injectie. Daarna zijn er een of twee extra injecties aan toegevoegd. Sinds 1987 wordt een programma met twee doses bof-mazelen-rode hond (BMR) aangeboden met de eerste vaccinatie op de leeftijd van 14 maanden en de tweede op negen jaar. Sinds het begin van de jaren ,90 lag de vaccinatiegraad voor de eerste BMR-vaccinatie boven de 95%. Later is de vaccinatiegraad van zowel de eerste BMR-vaccinatie als die van twee doses afgenomen tot ongeveer 93% en 90%. Op dit moment lijkt de vaccinatiegraad van de eerste BMR-vaccinatie zich te stabiliseren rond 93%. De vaccinatiegraad is echter niet homogeen verdeeld over Nederland. Een regio die zich uitstrekt van het zuidwesten tot het noordoosten van Nederland (de bijbelgordel) wordt gekenmerkt door een lagere vaccinatiegraad. In sommige gemeenten ligt de vaccinatiegraad zelfs onder de 70%. Deze lage vaccinatiegraad wordt voornamelijk veroorzaakt door personen van de bevindelijk gereformeerde gemeenschap die afzien van vaccinatie.

Sinds de invoering van de mazelenvaccinatie in het rijksvaccinatieprogramma is het rapporteren van een mazelenvirusinfectie wettelijk verplicht. De jaarlijkse incidentie is laag op een aantal jaren na, toen epidemieën plaatsvonden in de bevindelijk gereformeerde gemeenschap. Sinds de laatste mazelen uitbraak van 1999-2000, is de incidentie van mazelen in Nederland onder de 5 personen per miljoen inwoners gebleven, behalve in 2008 en 2013 ten gevolge van uitbraken. Een wereldwijde incidentie van minder dan 5 gevallen per miljoen inwoners is

een van de mijlpalen in de wereldwijde eliminatie van de mazelen opgesteld door de Wereldgezondheidsorganisatie (WHO). Ondanks de epidemieën is het aantal sterfgevallen door mazelen in Nederland heel laag. Tijdens de uitbraak van 1999-2000 stierven 3 personen aan de gevolgen van het mazelenvirus. Naast de sterfte van mazelen tijdens een epidemie leidt mazelen ook tot sterftegevallen nog jaren na het doormaken van mazelen. Dit betreft gevallen van subacute scleroserende panencefalitis (SSPE). SSPE manifesteert zich gemiddeld zes jaar na de eerste besmetting met het mazelenvirus. SSPE komt niet voor bij gevaccineerde personen. Het is gelukkig een zeer zeldzame complicatie van mazelen, maar wel bijna altijd met een fatale afloop; slechts 5% van de mensen met SSPE ondergaat spontane remissie, terwijl de resterende 95% binnen vijf jaar na de diagnose sterft. In totaal worden 4 tot 11 gevallen van SSPE verwacht bij elke 100.000 gevallen van mazelen.

Op basis van de resultaten van een seroprevalentiestudie en wiskundige modellering liep Nederland een hoog risico op een volgende grote mazelenuitbraak in gebieden met een lage vaccinatiegraad. De uitbraak beschreven in dit proefschrift begon in mei 2013 en eindigde in maart 2014. Het doel van dit proefschrift was het bestuderen van een grote mazelenepidemie in Nederland om meer inzicht te krijgen in de huidige epidemiologie en methoden van bestrijding van mazelen.

In **hoofdstuk 2** startten wij met een uitvoerige beschrijving van de epidemie in de jaren 2013/14. Verdiepend onderzoek is beschreven in volgende hoofdstukken met verschillende thema's : de onderrapportage (**hoofdstuk 3**), de ernst en besmettelijkheid van gevaccineerde gevallen (**hoofdstuk 4**), de economische kosten (**hoofdstuk 10**) en ten slotte onderzoek naar de daling van gerapporteerde gevallen tijdens de zomer schoolvakantie (**hoofdstuk 11**). Als reactie op de uitbraak werd besloten een vervroegde BMR-vaccinatie aan te bieden aan kinderen tussen 6 en 14 maanden oud. De reden voor deze controlemaatregel was de afwezigheid van mazelenimmuniteit bij deze kinderen en de grotere kans op complicatie bij deze allerjongste kinderen. Om deze controlemaatregel nader te evalueren en om te beoordelen of een soortgelijke controlemaatregel tijdens toekomstige uitbraken moet worden toegepast, werden verschillende studies uitgevoerd om de acceptatie (**hoofdstuk 5**), de bijwerkingen (**hoofdstuk 7**) en de effectiviteit van het vaccin (**hoofdstuk 6**) te beoordelen. In **hoofdstukken 8 en 9** hebben wij onderzoek gedaan naar de immuniteit tegen mazelen op individueel- en populatieniveau. In het resterende deel van dit hoofdstuk bespreken we de bevindingen van onze studies afzonderlijk. We sluiten af met een aantal aanbevelingen voortkomend uit deze studies.

In **hoofdstuk 2** beschrijven we de mazelenepidemie van 2013/14 in detail. De epidemie vond voornamelijk plaats binnen de bevindelijke gereformeerde gemeenschap. We hebben de epidemiologie van de uitbraak beschreven door de meldingen van mazelen te analyseren en de belangrijkste kenmerken te vergelijken met de vorige epidemie uit 1999-2000. In totaal werden 2700 gevallen van mazelen, 181 ziekenhuisopnames en één sterfgeval gemeld. Opvallend was de hogere leeftijd tijdens de epidemie van 2013-14, de mediaan was rond 10 jaar en daarmee 4 jaar hoger dan in de epidemie van 1999-2000. De hogere leeftijd ging gepaard met een langere periode tussen de epidemieën. Uitgaande van een toenemende vaccinatiegraad in de bevindelijke gereformeerde gemeente verwacht men dat het aantal mazelen gevallen binnen deze groep zal dalen tijdens toekomstige epidemieën, maar dat de gemiddelde leeftijd zal toenemen. Aangezien het risico op complicaties en ziekenhuisopnames ook toeneemt met de leeftijd, betekent dit dat toekomstige epidemieën gekenmerkt zullen worden door een hoger percentage aan complicaties en ook ziekenhuisopnames.

In **hoofdstuk 3** hebben we berekend hoeveel gevallen van mazelen er werkelijk waren gedurende epidemie, omdat het rapporteren van mazelen onderhevig is aan onderrapportage. In onze studie hebben wij twee verschillende benaderingen toegepast om de volledigheid van de rapportage te beoordelen. Beide benaderingen schatten het aantal gemelde gevallen op ongeveer 9% van alle gevallen van mazelen (8,8% en 9,1%). De overeenkomst tussen beide uitkomsten komt de geloofwaardigheid en validiteit van beide benaderingen ten goede. Aangezien 9% van de gevallen werd gemeld, werd de totale omvang van de uitbraak van 2013-2014 geraamd op ongeveer 31.400 gevallen van mazelen.

In **hoofdstuk 4** hebben we de ernst en besmettelijkheid beoordeeld van 2539 ongevaccineerde gevallen van mazelen (94%), 121 eenmaal gevaccineerde gevallen (5%) en 16 personen (1%) kregen symptomen van mazelen ondanks twee BMR-vaccinaties (1%). Vergeleken met ongevaccineerde personen en eenmaal gevaccineerde personen gevallen waren personen die twee keer waren gevaccineerd en mazelen kregen veel minder ernstig ziek en ook minder besmettelijk voor anderen. Geen van de tweemaal gevaccineerde gevallen meldde complicaties of vereiste ziekenhuisopname en geen van de tweemaal gevaccineerde gevallen was de bron van andere gevallen van mazelen. Daarentegen meldde 15% van de ongevaccineerde en eenmaal gevaccineerde patiënten complicaties of ziekenhuisopname, 8% van de ongevaccineerde en 6% van de eenmaal gevaccineerde patiënten werden aangeduid als de bron van andere gerapporteerde

gevallen van mazelen. Onze bevindingen ondersteunen de aanbeveling van de WHO van een mazelenvaccinatieschema met twee doses.

In de **hoofdstukken 5, 6 en 7**, bespraken we de acceptatie, effectiviteit en bijwerkingen van een vervroegde BMR-vaccinatie op de leeftijd van 6 tot 14 maanden. Het nationale vaccinatieregister van Nederland (Praeventis) heeft de vaccinatiestatus op individueel niveau gedocumenteerd, en kan daarom gebruikt worden voor interventies op maat, zoals het aanbieden van een vervroegde BMR-vaccinatie aan kinderen tussen 6 en 14 maanden die afkomstig zijn uit gemeenten met een lage vaccinatiegraad (<90%). De acceptatie van de vervroegde BMR-vaccinatie was 57% (5.800 van de 10.097 zuigelingen). Teleurstellend was echter het grote onderscheid in acceptatie tussen ouders die hun baby reeds hadden laten vaccineren tegen difterie, kinkhoest, tetanus en polio (DKTP), namelijk 70% en ouders die hun baby niet hadden laten vaccineren, 1 procent. Naast de acceptatie evalueerden wij ook de effectiviteit van de vervroegde BMR-vaccinatie. Onze studie was de eerste die de effectiviteit meette aan de hand van laboratoriumbevestigde gevallen van mazelen in een observationele context en de eerste studie die de bijwerkingen op 6 maanden evalueerde in een hoog inkomensland. De eerste effectiviteitsschatting van de vervroegde BMR-vaccinatie was 94% (95%CI 79%-98%). Wanneer we echter, in tegenstelling tot eerdere studies, rekening hielden met de verschillende blootstellingsniveaus tussen de gevaccineerde en ongevaccineerde zuigelingen, daalde de effectiviteit tot 71% (95%CI -72%-95%). In andere woorden, gevaccineerde zuigelingen hadden een 94% lagere kans op mazelen vergeleken met ongevaccineerde kinderen, maar een deel van het effect werd veroorzaakt door de groepsbescherming doordat mensen in de omgeving wel waren gevaccineerd. Tenslotte, concludeerden wij dat de BMR-vaccinatie zelf goed werd verdragen door kinderen van nog maar 6 tot 14 maanden oud. Het aantal bijwerkingen was gelijk of minder dan zuigelingen die worden gevaccineerd met 14 maanden.

In de **hoofdstukken 8 en 9** hebben we de bescherming tegen mazelen op individueel en bevolkingsniveau nauwkeuriger vastgesteld. We beoordeelden de samenhang tussen concentraties aan antilichamen en het voorkomen van mazelen en subklinische (asymptomatische) mazelen gedurende de epidemie bij eenmalig gevaccineerde kinderen. Van elk kind, hadden we gepaarde bloedmonsters, waarvan het eerste monster werd genomen snel na het begin van de mazelenuitbraak en de tweede na uitdoving van de epidemie. De minimale concentratie aan antilichamen die bescherming bood tegen mazelen was 0,345 IU/ml. De concentratie die tegen subklinisch mazelen beschermt was 2,1 IU/ml. Deze beschermende

concentraties werden geschat met de virus neutralisatietest. Het schatten van deze beschermende concentraties is belangrijk als leidraad voor nieuwe vaccin ontwikkelingsstudies, maar ook essentieel is in seroprevalentiestudies. Seroprevalentiestudies bestuderen de immuniteit van een populatie tegen een bepaald pathogeen door het meten van antilichaamconcentraties van vele personen. In een seroprevalentiestudie in Nederland is gebleken dat personen geboren tussen 1972 en 1990 relatief het minst beschermd zijn. Met behulp van een multiplex immunoassay (MIA) werd geconcludeerd dat minder dan 95% van dit geboortecohort een antilichaamconcentratie boven het veronderstelde beschermingsconcentratie had. In **hoofdstuk 9** hebben we de seroprevalentie van dit geboortecohort opnieuw beoordeeld met behulp van een virus neutralisatietest. Deze test toetst de aanwezigheid van mazelen-specifieke antilichamen die in staat zijn om het mazelenvirus te neutraliseren, terwijl de MIA enkel de aanwezigheid meet van een breder palet aan. Gebaseerd op afzonderlijke resultaten van de neutralisatietest en de MIA, waren de beschermende antilichamenniveaus van geboortecohort 1972-1990 hoger en respectievelijk 99% en 94%. Hieruit concludeerden we dat de eerste gevaccineerde individuen bijna allemaal nog beschermd waren tegen mazelen. Van de kleine groep die nog vatbaar was, bleek de overgrote meerderheid ongevaccineerd.

In **hoofdstuk 10** hebben we de economische last van de mazelenepidemie geschat vanuit een maatschappelijk perspectief. We schatten dat de mazelenuitbraak een economische last van ≈€ 3,9 miljoen veroorzaakte. De 2700 gemelde gevallen resulteerden in een geschatte 1.443 euro per gemeld geval. De belangrijkste kosten waren de inzet van werknemers bij de GGD'en.

Tijdens de epidemie daalde het aantal gemelde gevallen van mazelen tijdens de schoolvakanties. We stelden de hypothese op dat het afgenomen aantal gemelde gevallen kwam door of een verminderde rapportage van meldingen in de zomer of een vermindering van mazelenvirus transmissie. Met behulp van een wiskundig transmissiemodel schatten we in **hoofdstuk 11** dat gedurende de zomervakantie het aantal contacten verminderde met de helft. Er was een verschuiving van hoofdzakelijk lokale transmissie van mazelen tijdens het schooljaar naar hoofdzakelijk interregionale transmissie tijdens de schoolvakanties. Een vakantie-effect op de compleetheid van meldingen werd niet waargenomen. Ondanks een vermindering van het aantal contacten tijdens de schoolvakantie werd de mazelenvirus transmissie dus niet gestopt, waardoor het onwaarschijnlijk is dat

de sluiting van een school een hele effectieve controlemaatregel is, behalve als de uitbraak nogbeperkt is tot een klein aantal gevallen van mazelen.

In het laatste hoofdstuk, **hoofdstuk 12**, bespreken we alle studies die in dit proefschrift zijn opgenomen. We beschrijven wat er vóór de studie bekend was, onze bevindingen en de toegevoegde waarde van onze studie. Op basis hiervan doen we aanbevelingen, bespreken we de implicaties van de bevindingen en geven we richting aan toekomstig onderzoek. Hieronder bespreken we de algemene aanbevelingen voortkomend uit dit proefschrift. Deze aanbevelingen zijn op te delen in vier categorieën namelijk: de vervroegde BMR-vaccinatie, identificatie van vatbare groepen, aanpak bij gebrekkige immuniteit, en de surveillance van mazelen.

Vervroegde BMR-vaccinatie
Wij concludeerden dat de vervroegde BMR-vaccinatie effectief was (71%), goed verdragen werd en dat de opname redelijk was (57%). Derhalve adviseren wij om een soortgelijke vervroegde BMR-vaccinatie uit te voeren bij toekomstige epidemieën in Nederland (hoofdstukken 5, 6 en 7).

De langetermijneffecten van de vervroegde BMR-vaccinatie moeten verder worden beoordeeld door middel van toekomstig onderzoek, wat in ieder geval immunologische en effectiviteitsstudies zou moeten omvatten. Het registreren van de leeftijd tijdens vaccinatie van gevaccineerde gevallen zal inzicht geven in de effectiviteit van de vervroegde BMR-vaccinatie. De langetermijneffecten van de vervroegde BMR-vaccinatie kunnen ook worden bestudeerd door het nationale vaccinatieregister te koppelen aan gezondheidsgerelateerde registers alsmede ook de verdraagzaamheid met een grotere steekproefomvang te bevestigen (hoofdstukken 6 en 7).

Aangezien in Europa de grootste last van de mazelenziekte en -sterfte en het grootste risico op complicaties, waaronder SSPE, wordt waargenomen bij zuigelingen jonger dan één jaar, zouden vervroegde BMR-vaccinatie campagnes uitgevoerd moeten worden in gebieden met een hoge incidentie van mazelen in Europa (hoofdstuk 7).

Identificatie van vatbare groepen

- Op basis van de seroprevalentie, beoordeeld met de virus neutralisatietest, concludeerden wij dat het rijksvaccinatieprogramma zeer effectief is in het bieden van immunologische bescherming tegen mazelen (hoofdstuk 9). De gevaccineerde personen van de eerste geboortecohorten die gevaccineerd werden, zijn 30 jaar na dato nog steeds beschermd. Of deze bescherming zo blijft in de komende decennia, noodzaakt het uitvoeren van toekomstige seroprevalentiestudies zodat een eventuele afnemende immuniteit waargenomen kan worden in de Nederlandse bevolking en in het bijzonder bij de eerste gevaccineerde geboortecohorten uit de jaren 70.

- Gezien slechts een klein aantal schattingen bekend zijn die geschat hebben hoe hoog de concentratie aan antilichamen nodig is ter bescherming tegen mazelen, adviseren wij om nieuwe studies op te zetten in geval van toekomstige uitbraken. Mocht de uitbraak al aan de gang zijn, dan staat dit een adequate schatting niet in de weg, aangezien we hebben aangetoond dat reeds geïnfecteerde personen geïdentificeerd kunnen worden en vervolgens uitgesloten kunnen worden bij het schatten van de beschermende antilichaamconcentratie (hoofdstuk 8).

Aanpak bij gebrekkige immuniteit

- Ongevaccineerde personen die geboren zijn rond 1976, toen de vaccinatie tegen mazelen werd opgenomen in het Nederlandse rijksvaccinatieprogramma, zijn relatief vaak vatbaar (hoofdstuk 9). Twee procent van geboortecohort 1972-1981 is vatbaar, wat gelijk staat aan ongeveer 80.000 personen. Deze groep heeft een relatief hoog risico op complicaties na mazelen vanwege hun leeftijd. Momenteel wordt aanbevolen om ongevaccineerde personen geboren na 1965 zich te laten vaccineren bij reizen naar landen waar mazelen endemisch is. Echter, wordt bij een reis binnen Europa slechts zelden een reizigerskliniek geraadpleegd. Daarom moeten andere mogelijkheden worden onderzocht om deze vatbare individuen te bereiken. Professionals in de gezondheidszorg zijn van bijzonder belang, aangezien zij een verhoogd risico lopen om blootgesteld te worden aan mazelen, maar ook kwetsbare patiënten kunnen besmetten. Strengere maatregelen zouden moeten worden overwogen, zoals een verplichte BMR-vaccinatie in geval van een ontoereikende immuniteit alvorens men werkzaam is in de gezondheidszorg.

- Tijdens de uitbraak van mazelen in 2013-2014 werden 61 eenmaal gevaccineerde gevallen gemeld tussen 4 en 9 jaar oud (hoofdstuk 2). Andere Europese landen bieden gewoonlijk de tweede BMR-vaccinatie aan kinderen van 1 tot 4 jaar oud. Een soortgelijk vaccinatieschema had tijdens de epidemie in Nederland een groot deel van deze 61 gevaccineerde gevallen hebben kunnen voorkomen. Een heroverweging van de optimale leeftijd van de tweede BMR-vaccinatie is daarom gerechtvaardigd waarbij rekening gehouden dient te worden met de gevolgen voor de immuniteit tegen mazelen op de lange termijn en de gevolgen voor de immuniteit tegen rodehond en bof.

Surveillance van mazelen

- We hebben twee benaderingen gebruikt om de volledigheid van de rapportage van mazelen te schatten (hoofdstuk 3). De ene benadering betrof een vragenlijstonderzoek in een gemeente uit de bijbelgordel en de andere een vergelijking tussen het aantal vatbare personen vóór de uitbraak en het aantal gerapporteerde gevallen. De overeenstemming tussen beide schattingen suggereert dat een schatting met alleen de laatste methode voldoende is om de totale ziektelast van een grote uitbraak in de bijbelgordel uit te rekenen.

- SSPE veroorzaakt een groot deel van de mazelensterfte in Nederland. Tot op heden is SSPE echter niet meldingsplichtig. Door van SSPE een meldingsplichtige ziekte te maken, zou een betere beoordeling van de ziektelast en sterfte in Nederland mogelijk zijn (hoofdstuk 2).

- De Nederlandse bevolking wordt steeds afhankelijker van immuniteit door vaccinatie. Een mogelijk afnemende immuniteit op de lange termijn kan leiden tot individuen met matige bescherming tegen mazelen. Dit leidt mogelijkerwijs tot een toename in het aantal, voornamelijk tweemaal, gevaccineerde gevallen. Een verbeterde surveillance van gevaccineerde gevallen bestaande uit diagnostiek dat in staat is de oorzaak van het falende vaccin te vinden en contactonderzoek om de besmettelijkheid te beoordelen, is gerechtvaardigd (hoofdstuk 4).

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About the author

Tom Woudenberg was born on the 16th of August, 1986 in Amersfoort (The Netherlands). In 2008, he started a Bachelor of Health sciences at the VU University in Amsterdam and upon completion; he started a master's degree specializing in infectious diseases. During his master's thesis, he studied in Brazil at Fiocruz, researching the burden of dengue in the state of Rio de Janeiro. In 2014, he commenced researching a PhD at the National Institute of Public Health and the Environment (RIVM) in The Netherlands, resulting in this thesis. As of 2018, he continued to study infectious diseases as an EPIET-fellow at the Landesamt für Gesundheit und Lebensmittelsicherheit (LGL) in Munich. The EPIET-fellowship is a European training programme organized by the European Centre for Disease prevention and Control.