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Non-specific effects of measles, mumps, and rubella (MMR) vaccination in high income setting: population based cohort study in the Netherlands

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ABSTRACT OBJECTIVES

To investigate whether measles, mumps, and rubella (MMR) vaccine has positive non-specific effects in a high income setting and to compare rates of hospital admissions for infections between children aged ≤ 2 years who received live MMR vaccine and those who received an inactivated vaccine against diphtheria, tetanus, pertussis, polio, and *Haemophilus influenzae* type b (DTaP-IPV-Hib) as their most recent vaccination.

DESIGN

Nationwide population based cohort study.

SETTING

In the Netherlands, DTaP-IPV-Hib+pneumococcal vaccination (PCV) is recommended at ages 2, 3, 4, and 11 months and MMR + meningococcal C (MenC) vaccination at age 14 months. Data from the national vaccine register were linked to hospital admission data.

PARTICIPANTS

1 096 594 children born in 2005-11 who received the first four DTaP-IPV-Hib+PCV vaccines.

MAIN OUTCOME MEASURES

Hazard ratio for admission to hospital for infection in children with MMR+MenC compared with the fourth DTaP-IPV-Hib+PCV as their most recent vaccination. Cox regression was performed with most recent vaccination as time dependent variable, adjusted for potential confounders. Analyses were repeated with admission for injuries or poisoning as a negative control outcome. In addition, rate of admission for infection was compared between the fourth and third DTaP-IPV-Hib+PCV as most recent vaccination.

RESULTS

Having had MMR+MenC as the most recent vaccination was associated with a hazard ratio of 0.62 (95% confidence interval 0.57 to 0.67) for admission to hospital for infection and 0.84 (0.73 to 0.96) for injuries or poisoning, compared with the fourth DTaP-IPV-Hib+PCV as most recent vaccination. The fourth DTaP-IPV-Hib+PCV as most recent vaccination was associated with a hazard ratio of 0.69 (0.63 to 0.76) for admission to hospital for infection, compared with the third DTaP-IPV-Hib+PCV as most recent vaccination.

CONCLUSIONS

Healthy vaccinee bias could at least partly explain the observed lower rate of admission to hospital with infection after MMR vaccination. The lower rate is associated with receipt of any additional vaccine, not specifically MMR vaccine. This emphasises the caution required in the interpretation of findings from observational studies on non-specific effects of vaccination.

Introduction

Vaccines against measles; diphtheria, pertussis, and tetanus (DTP); and polio have led to large declines in morbidity and mortality from the targeted diseases.^{1,2} It has been suggested that these vaccines could also affect morbidity and mortality from infections other than those targeted by the vaccines—that is, that they have non-specific effects.³ Several studies observed beneficial non-specific effects of live attenuated vaccines (such as measles and BCG) and deleterious non-specific effects of inactivated vaccines (such as DTP vaccine), with, in general, stronger effects in girls than in boys.^{4, 5, 6, 7, 8, 9, 10} Moreover, the sequence of vaccination could be important. Receipt of a live attenuated measles vaccine after an inactivated DTP vaccine might be associated with lower morbidity and mortality, compared with receipt of a DTP vaccine after or at the same time as a measles vaccine.^{11, 12, 13, 14, 15, 16} Which immunological mechanisms could underlie these potential non-specific effects of vaccination on susceptibility to infectious disease is currently unknown. Trained innate immunity, which depends on epigenetic reprogramming of innate immune cells, could explain some of the non-specific effects.^{17, 18} Another potential mechanism is through T cell mediated cross reactivity.¹⁸

Few studies on non-specific effects have been performed in high income countries, which have low rates of infant mortality from infectious diseases.

WHAT IS ALREADY KNOWN ON THIS TOPIC

Live attenuated vaccines (such as measles) could have beneficial non-specific effects and inactivated vaccines (such as DTP) could have deleterious non-specific effects

The evidence for non-specific effects of vaccines remains weak as most evidence comes from observational studies that are vulnerable to bias

WHAT THIS STUDY ADDS

Healthy vaccinee bias at least partly explains the observed lower rate of hospital admission for infection after MMR vaccination; this lower rate is associated with receipt of any additional vaccine and not specifically with MMR

The presence of non-specific effects of MMR vaccination cannot be excluded, but the possible non-specific effects cannot be distinguished from bias

The findings emphasise the importance of caution in the interpretation of findings from observational studies on non-specific effects of vaccination

The public health relevance of non-specific effects of vaccines in high income countries is largely unknown. Two nationwide Danish studies reported measles, mumps, and rubella (MMR) vaccine to be associated with a 16% lower rate of hospital admissions for infectious disease¹⁰ and a 22% lower rate of hospital contacts for respiratory syncytial virus¹⁹ compared with DTP-IPV-Hib as most recent vaccine. No differences were observed between boys and girls. The Danish studies used timing of MMR vaccination as the (time varying) exposure.

Most of the evidence on non-specific effects of vaccines originates from observational studies, which are prone to bias.²⁰ An example is healthy vaccinee bias (also known as healthy user bias or frailty bias), which occurs when children who are more susceptible to illness are vaccinated later or not at all, resulting in an overestimation of the beneficial effect of the next vaccination. Recently, two systematic reviews of the potential non-specific effects of DTP and measles vaccines, which were commissioned by the World Health Organization strategic advisory group of experts (SAGE), were published.²¹ Findings suggest that receipt of measles vaccine reduces overall mortality more than would be expected through the effects on the targeted disease, while DTP vaccination might be associated with an increase in all cause mortality.²¹ As most evidence came from observational studies (10 observational studies for DTP^{11 12 22 23 24 25 26 27 28}; four trials^{6 29 30 31} and 17 observational studies^{11 12 13 22 28 32 33 34 35 36 37 38 39 40 41 42} for measles), however, the conclusion was that these findings should be interpreted with caution.

We set out to reproduce the findings of the Danish studies on non-specific effects of MMR vaccination in a population based nationwide cohort study of more than a million Dutch children using similar methods. We investigated the rate of hospital admissions related to infectious disease after receipt of the live attenuated MMR vaccine (given at the same time as vaccination against meningococcal disease group C, MenC) versus inactivated DTP containing vaccine (also includes vaccinations against polio (inactivated polio vaccine, IPV) and *Haemophilus influenzae* type b and given at the same time as a vaccination against pneumococcal disease) as most recent vaccine. In addition, we explored whether healthy vaccinee bias was present.

Methods

Study design

The Dutch national immunisation programme was implemented in 1957 and is coordinated and evaluated by the Dutch National Institute for Public Health and the Environment. Table 1 shows the recommended vaccines included in the programme in the first 24 months of life, and table A in appendix 1 shows the years of introduction into the programme. The DTaP-IPV-Hib vaccine, recommended at ages 2, 3, 4, and 11 months, consists of vaccinations against diphtheria, tetanus, pertussis (acellular), polio, and conjugate vaccine against *H influenzae* type b (Hib) and is administered simultaneously with a multivalent

Table 1 | Vaccines recommended in first 24 months of life according to Dutch national immunisation programme

Age (months)	Vaccinations
2	DTaP-IPV-Hib(-HepB since August 2011)+PCV
3	DTaP-IPV-Hib(-HepB since August 2011)+PCV
4	DTaP-IPV-Hib(-HepB since August 2011)+PCV
11	DTaP-IPV-Hib(-HepB since August 2011)+PCV
14	MMR + MenC

DTaP=diphtheria, tetanus, pertussis (acellular); HepB=hepatitis B; Hib=*H influenzae* type b; IPV=inactivated polio vaccine; MenC=meningococcal disease group C; MMR=measles, mumps, rubella; PCV=pneumococcal conjugate vaccine.

conjugate vaccination against pneumococcal disease (PCV). The vaccination against MMR is recommended at age 14 months and administered simultaneously with vaccination against meningococcal disease serogroup C (MenC). Only the MMR vaccine is a live vaccine; the other vaccines are inactivated/non-live vaccines.

Vaccination data

In 2005, an electronic national immunisation register “Præventis” was implemented in the Netherlands. The register is linked to the population register. This means that all children and young people aged under 19 who are officially registered in the Netherlands are included in the immunisation register. Præventis does not include undocumented children living in the Netherlands. Parents receive invitation letters automatically created by Præventis to get their child(ren) vaccinated at a specific date and time at a healthy children clinic near their homes according to the immunisation programme. Data on administered vaccinations (that is, vaccine characteristics, dose, date of administration) are entered in Præventis.⁴³

Hospital admission data

The national medical register administered by Dutch hospital data provided data on hospital admissions from 1 January 2005 to 31 December 2012. Dutch hospital data requests hospitals and university medical centres in the Netherlands to voluntarily supply data on admissions. The coverage of all hospital admissions decreased with time, with 3% missing in 2005 to 25% in 2012.⁴⁴

Primary and secondary discharge diagnoses and dates of admissions were available from the register. Diagnoses were coded according to the ICD-9-CM (international classification of diseases, ninth revision, clinical modification). We included upper respiratory infections, lower respiratory infections, gastrointestinal infections, and other infections (table B in appendix 1). In the main analysis we included only hospital admissions that lasted more than one day (and thus included an overnight stay) to exclude day admissions related to planned examinations and surgeries. In a sensitivity analysis, we included all admissions.

Covariates

Præventis provided data on sex, parents' country of birth, and postcode, and Statistics Netherlands

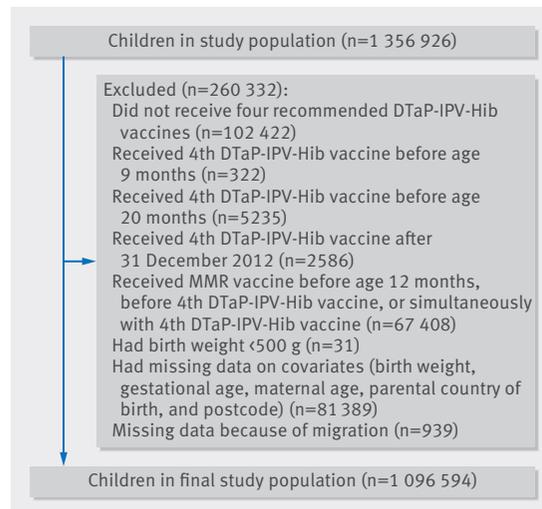


Fig 1 | Flow chart of children included in study population according to compliance with vaccination schedule

provided data on death, migration, and parental educational level. Parental educational level was classified as low (elementary education or pre-vocational education), medium (senior general secondary education, pre-university education, or vocational education), or high (college or university). The Netherlands perinatal registry provided data on birth weight, gestational age, maternal age, and parity (as a proxy for number of siblings).

Linkage of data sources

The population register (the municipal personal records database) was the main database to which all databases were linked to get a unique anonymised number with which the different databases could be linked. Of all 1 357 461 children that were included in Præventis, 1 356 926 (>99%) were successfully linked with the population register with a unique personal identifier, the citizen service number. Data from the national medical register, Statistics Netherlands, and the Netherlands perinatal registry were linked to the population register by using probability matching, based on sex, date of birth, and postcode. For the national medical register, unique records were successfully linked, ranging from 97.3% in 2005 to 98.7% in 2012.⁴⁵

Population for analysis

For the present study, vaccination data from 1 356 926 Dutch children born from 1 January 2005 to 31 December 2011 who were all eligible for the routine immunisation programme were available from the electronic national immunisation register Præventis (fig 1). Of these children, 93% received the first four recommended DTaP-IPV-Hib+PCV vaccines and were eligible for inclusion in this study. We excluded children who did not receive these DTP containing vaccinations (n=102 422) to limit the possibility of bias attributable to factors related to low vaccination coverage (such as refusal based on

religion). We also excluded children who received the fourth DTaP-IPV-Hib+PCV vaccine either before age 9 months (n=322), after age 20 months (n=5235), after 31 December 2012 (n=2586), after the MMR vaccine (n=18 110), or simultaneously with the MMR vaccine (n=4333). In addition, we excluded children who received an MMR vaccine before age 12 months (n=44 965) because these children are recommended to receive another MMR vaccine at 14 months and therefore follow a different vaccination schedule than the other children in the study population. After exclusion of children with a birth weight <500 g (n=31), missing data on covariates (birth weight, gestational age, maternal age, parental country of birth, and postal code) (n=81 389), or missing data because of migration (n=939), 1 096 594 children (81%) remained for analysis.

Statistical analysis

Main analysis

Cox proportional hazards models were used to estimate hazard ratios for hospital admissions related to infectious disease according to the most recent vaccination (MMR+MenC versus fourth DTaP-IPV-Hib+PCV), with last received vaccination included as a time varying variable changing at the age of receipt of the MMR+MenC vaccine (statistical code provided in appendix 2). Children entered the model at the age of receipt of the fourth DTaP-IPV-Hib+PCV and were followed until the age of hospital admission for infection (in case of an event) or were censored at death, age 24 months, age of emigration, or age on 31 December 2012, whichever came first. Age was used as timescale for the Cox regression. We included age at MMR+MenC vaccination as a time variable exposure and used age as a timescale in the Cox regression; this effectively means that at each age (in days) we compared children who had already received the MMR+MenC vaccine with children who had not yet received the MMR+MenC vaccine, thereby adjusting for age.

Analyses were stratified by date of birth to fully control for age, season, and calendar year. Associations were adjusted for sex, chronic diseases (Y/N) before baseline (age 9 months; list of ICD-9 codes, shown in table C in appendix 1), admission to hospital for any reason in the month before baseline (the fourth DTaP-IPV-Hib+PCV is received from age 9 months onwards, a fixed month of 8 months of age was chosen for this variable) (Y/N), birth weight, gestational age, maternal age and parity (1, 2, 3, and ≥ 4), parents' country of birth (two Dutch parents, one Dutch and one non-Dutch parent, and two non-Dutch parents), and postcode in three digits. Analyses were stratified by type of infection—for instance, upper respiratory, lower respiratory, gastrointestinal, and other infections. Sex and birth year were examined as potential effect modifiers by an interaction test and stratified analysis. Because a lot of data were missing for level of parental educational, we additionally adjusted associations for level (low, medium, or

high) in a subsample of 79% of eligible children with complete information.

The same analysis was performed with admission for injury or poisoning (ICD-9-CM codes 800-999) as a negative control outcome.⁴⁶

Analyses were performed with SAS version 9.2 (SAS Institute, Cary, NC) and Stata 14 (StataCorp, College Station, TX).

Sensitivity analyses

Firstly, we performed the same analysis as described above with all hospital admissions for infections taken into account, thus also including day admissions without an overnight stay.

Secondly, we performed another analysis taking into account repeated hospital admissions with the Andersen-Gill model⁴⁷ as an extension of the Cox model. We excluded all hospital admissions for infection that occurred less than 14 days after a previous discharge because multiple admissions within a short period could be attributable to the same infection. In cases of a previous hospital admission, children re-entered the study 14 days after discharge and were followed until a next admission or censored at death, age 24 months, or age at 31 December 2012, whichever came first.

In a third analysis, we compared the fourth with the third DTaP-IPV-Hib+PCV vaccination. Vaccination was included as a time varying variable changing at the age of the fourth vaccination. Children entered the model from the age of receipt of the third vaccination and were followed until the age of admission for infection or were censored at death, age of MMR+MenC vaccination, age 14 months, or age on 31 December 2012, whichever came first.

Patient involvement

No patients were involved in setting the research question or the outcome measures, nor were they involved in developing plans for recruitment, design, or implementation of the study. No patients were asked to advise on interpretation or writing up of results. There are no plans to disseminate the results of the research to study participants or the relevant patient community.

Results

Study population

Table 2 shows characteristics of the 1 096 594 children included in the analyses. Children received the fourth DTaP-IPV-Hib+PCV vaccination at a median age of 11.2 months and the MMR+MenC vaccination at a median age of 14.3 months. Almost all children received the MMR vaccination (99.6%) (fig 2), and about 99% of all children received PCV with the DTaP-IPV-Hib vaccines and the MenC vaccine with the MMR vaccine. Most children had at least one Dutch parent (89.1%), and about half of the children had at least one parent with higher education (51.1% of all children with data on parental educational level). About 1% of all children were admitted to hospital for any reason at age 8 months, and 2.5% had previously had a diagnosis of a chronic disease before receipt of the fourth DTaP-IPV-Hib+PCV vaccine.

MMR+MenC v fourth DTaP-IPV-Hib+PCV in relation to admission for infection (main analysis)

During 1 061 242 person years of follow-up, 10 961 children were admitted to hospital for more than one day (this was 26% of all admissions for infection, meaning that 74% of all admissions for infection were day admissions) for an infection (admission rate 10/1000 person years). Admission rates declined with age, from 15/1000 person years at age 12 months to 7/1000 person years at age 24 months (fig 3). Similarly, admission rates declined with age in those who received MMR+MenC as their most recent vaccination (average admission rate of 9/1000 person years). In children whose most recent vaccination was the fourth DTaP-IPV-Hib+PCV, admission rates slightly declined

Table 2 | Characteristics of 1 096 594 Dutch children included in study of infections after vaccination. Figures are numbers (percentage) unless stated otherwise

	Data
Median (IQR) age at fourth DTaP-IPV-Hib vaccination (months)	11.2 (11.0-11.6)
Median (IQR) age at MMR vaccination (months)*	14.3 (14.0-14.8)
Boys	561 407 (51.2)
Median (IQR) birth weight (g)	3460 (3100-3810)
Median (IQR) gestational age (weeks)	39.9 (38.7-40.7)
Median (IQR) maternal age at birth of child (years)	31 (27-34)
Maternal parity†:	
One	505 851 (46.1)
Two	400 703 (36.5)
Three	138 565 (12.6)
Four or more	51 473 (4.7)
Highest parental educational level‡:	
Low	115 205 (13.2)
Medium	310 445 (35.6)
High	445 544 (51.1)
Parental country of birth:	
Netherlands	834 580 (76.1)
Netherlands and foreign	142 555 (13.0)
Foreign	119 457 (10.9)
Chronic disease of child	27 430 (2.5)
Hospital admission for any reason at age 8 months	11 706 (1.1)

IQR=interquartile range.

* Data available for 1 092 625 children.

† Represents number of childbirths of mother.

‡ Highest educational level of household (either parent). Data available for 871 194 children.

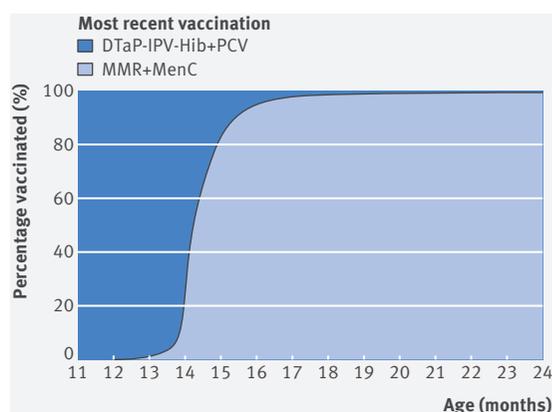


Fig 2 | Proportion of children according to most recent vaccination with DTaP-IPV-Hib+PCV and MMR+MenC, based on 1 096 594 children included in present study

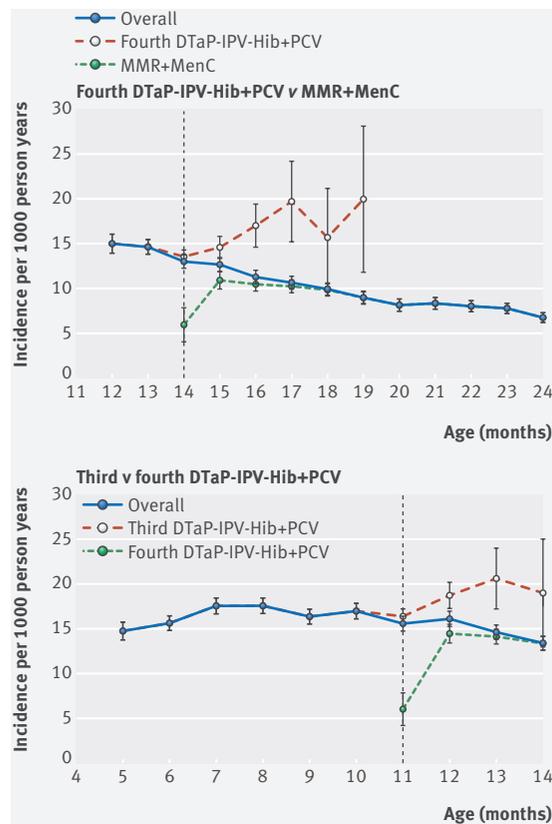


Fig 3 | Hospital admissions rates for infection with 95% confidence intervals for fourth DTaP-IPV-Hib+PCV v MMR+MenC as most recently received vaccination (in top panel line for fourth DTaP-IPV-Hib+PCV represents admission rate in children who had not yet received MMR+MenC vaccination) and for third v fourth DTaP-IPV-Hib+PCV (bottom panel). Dashed vertical lines represent recommended age of vaccination (top: MMR+MenC at age 14 months; bottom: fourth DTaP-IPV-Hib+PCV at age 11 months). Each estimate represents incidence of month before that month—for example, estimate at age 12 months comprises incidence for age 335–364 days. Note that incidence of admissions for MMR vaccinated individuals aged 12–14 months was not reported because too few children in study population received MMR at age 12–14 months (see fig 2), and, of these, <10 were admitted to hospital for infection. Because of privacy reasons, we cannot report data that apply to <10 individuals

until 14 months, which is the median age of receipt of MMR+MenC, and increased thereafter until age 17 months. Admission rates at age 17 months were 1.8 times higher in those whose most recent vaccination was the fourth DTaP-IPV-Hib+PCV, compared with the overall admission rates (20/1000 v 11/1000 person years). The average admission rate in those whose most recent vaccination was the fourth DTaP-IPV-Hib+PCV was 14/1000 person years.

Compared with the fourth DTaP-IPV-Hib+PCV as most recent vaccination, receipt of MMR+MenC as the most recent vaccination was associated with a age adjusted hazard ratio of 0.60 (95% confidence interval 0.55 to 0.65) for hospital admissions related to infectious disease (table 3). After additional adjustment for sex, chronic disease, admissions at age 8 months, birth weight, gestational age, maternal age and parity, parents' country of birth, and postcode, the hazard ratio was 0.62 (0.57 to 0.67). No effect modification was observed for sex ($P=0.55$ for interaction) and birth cohort ($P=0.29$ for interaction) (table D in appendix 1). Additional adjustment for level of parents' educational in the subsample of children with available data changed the results marginally (0.64, 0.58 to 0.70). When we took repeated hospital admissions for infections into account (event rate 11/1000 person years), the adjusted hazard ratio (0.72, 0.66 to 0.79) was slightly increased.

Type of infection

Of all 10 961 hospital admissions related to infection, 43% were for gastrointestinal infections, 40% for upper respiratory infections, 31% for lower respiratory infections, and 17% for other infections. The hazard ratio for admission according to most recent vaccination ranged between 0.54 (95% confidence interval 0.48 to 0.62) for upper respiratory infections to 0.70 (95% 0.61 to 0.80) for gastrointestinal infections (table E in appendix 1).

All admissions (including day admissions)

When we took into account all hospital admissions for infections, including day admissions without an overnight stay, 41 976 children were admitted to hospital for infection during 1 047 465 person

Table 3 | Hazard ratio (95% CI) for hospital admissions (>1 day) for infections (outcome of interest) and for injuries or poisoning (negative control outcome) according to most recent vaccination

Outcome	Most recent vaccination	Events/person years	HR (95% CI)*	
			Age adjusted	Fully adjusted†
Infections	Fourth DTaP-IPV-Hib+PCV	4111/284 786	1.00	1.00
	MMR+MenC	6850/776 456	0.60 (0.55 to 0.65)	0.62 (0.57 to 0.67)
Injuries or poisoning	Fourth DTaP-IPV-Hib+PCV	3605/285 676	1.00	1.00
	MMR+MenC	5150/782 738	0.81 (0.71 to 0.93)	0.84 (0.73 to 0.96)
Infections	Third DTaP-IPV-Hib+PCV	10 654/639 484	1.00	1.00
	Fourth DTaP-IPV-Hib+PCV	3185/231 001	0.66 (0.60 to 0.72)	0.69 (0.63 to 0.76)‡

* Hazard ratios from Cox regression with age as underlying time scale and stratified for date of birth.

† Adjusted for sex, chronic disease, admission for any reason at age 8 months, birth weight, gestational age, maternal age and parity, parental country of birth, and postcode.

‡ Adjusted for sex, chronic disease, birth weight, gestational age, maternal age and parity, parental country of birth, and postcode.

years of follow-up. Of these admissions, 83% were for upper respiratory infections, 9% for lower respiratory infections, 12% for gastrointestinal infections, and 6% for other infections. Receipt of MMR+MenC as the most recent vaccination instead of the fourth DTaP-IPV-Hib+PCV was associated with a fully adjusted hazard ratio of 0.40 (95% confidence interval 0.38 to 0.41) for hospital admissions related to infectious disease, which was driven mainly by upper respiratory infections (table F in appendix 2).

Negative control outcome

During 1 068 414 person years of follow-up, 5150 children were admitted to hospital for more than one day because of injury or poisoning (negative control outcome). After full adjustment, we observed the hazard ratio was 0.84 (95% confidence interval 0.73 to 0.96) for admission for injuries or poisoning with MMR+MenC as the most recent vaccination compared with the fourth DTaP-IPV-Hib+PCV as most recent vaccination (table 3). When we also considered admissions without an overnight stay, the fully adjusted hazard ratio was 0.80 (0.71 to 0.89) (table F in appendix 1).

Fourth v third DTaP-IPV-Hib+PCV in relation to hospital admission for infection

During 870 485 person years of follow-up, 13 839 children were admitted for more than one day for infection (admission rate 16/1000 person years). The average admission rate in those with the fourth DTaP-IPV-Hib+PCV as their most recent vaccination was 14/1000 person years. In those with the third DTaP-IPV-Hib+PCV as their most recent vaccination, admission rates were quite stable until age 11 months, which is the median age of receipt of the fourth vaccination, and increased thereafter until age 14 months (fig 3). Admission rates at age 14 months were 1.5 times higher in those with the third DTaP-IPV-Hib+PCV as their most recent vaccination compared with the overall admission rates (19/1000 v 13/1000 person years). The average admission rate in those with third vaccination as their most recent was 17/1000 person years.

Receipt of the fourth DTaP-IPV-Hib+PCV as the most recent vaccination was associated with a age adjusted hazard ratio of 0.66 (95% confidence interval 0.60 to 0.72) for hospital admissions for infectious disease compared with the third DTaP-IPV-Hib+PCV vaccination as the most recent (table 3). After additional adjustment for sex, chronic disease, birth weight, gestational age, maternal age, parity, parents' country of birth, and postcode, the hazard ratio was 0.69 (0.63 to 0.76). When we took into account admissions without an overnight stay, the fully adjusted hazard ratio was 0.48 (0.46 to 0.51) (table F in appendix 1). The adjusted hazard ratios by type of infection ranged from 0.59 (0.47 to 0.74) for other infections to 0.79 (0.67 to 0.92) for lower respiratory infections (table E in appendix 1).

Discussion

In more than a million Dutch children aged 11-24 months, there was a 38% lower rate of hospital admissions related to infectious disease in those who had MMR+MenC as their most recent vaccination, compared with those who had DTaP-IPV-Hib+PCV as their most recent vaccination. We also observed a 16% lower rate of admission for injuries or poisoning (negative control outcome) in children with MMR+MenC as their most recent vaccination. Moreover, there was a 31% lower rate of admissions related to infectious disease for children with the fourth DTaP-IPV-Hib+PCV as their most recent vaccination, compared with the third as their most recent vaccine. These findings suggest that a lower rate of admission is associated with adherence to the routinely recommended schedule. It is likely that healthy vaccinee bias was present and (at least partly) explains the lower rate of infections that we observed for receipt of an additional vaccine, rather than being an effect of specifically receiving MMR. The findings of this large scale observational study on non-specific effects emphasise the extreme difficulty in interpreting such results given the likely presence of healthy vaccinee bias.

We observed that after the median age of receipt of the next vaccine, which was MMR+MenC at 14 months in the MMR+MenC versus DTaP-IPV-Hib+PCV analysis and the fourth DTaP-IPV-Hib+PCV at 11 months in the analysis of fourth versus third DTaP-IPV-Hib+PCV, admission rates among those who deviated from the recommended schedule suddenly increased compared with the overall admissions rates. This suggests that vaccination is postponed in children who are more prone to admission. The confounders that we included in the analysis did not considerably change the hazard ratio, so apparently these confounders do not explain the increased risk of admission in children in whom vaccination is postponed. An unmeasured confounder could be acute illness, which might be associated with timing of vaccination and rate of admission. The lower rate of admission for infection for the fourth DTaP-IPV-Hib+PCV as most recent vaccination compared with the third also suggests that receipt of an additional vaccination (and therefore adherence to the routinely recommended schedule) is followed by a lower rate of admission, and this is thus not a finding that can be attributed to MMR specifically. This raises concerns for past and future observational studies on non-specific effects and emphasises that evidence from randomised trials that also investigate different sequences of vaccines is needed to draw conclusions on this matter, as was concluded by the recently published WHO-SAGE review.²¹

In a nationwide population based cohort of about 500 000 Danish children,¹⁰ the rate of hospital admissions related to infectious disease was 14% lower in those with MMR as their most recent vaccination compared with DTaP-IPV-Hib as their most recent vaccination. Their report of non-specific effects of MMR vaccination was strengthened by the fact that they found a 62% higher rate of hospital admissions

related to infectious disease in children who received DTaP-IPV-Hib after MMR vaccination, and they did not find any effect of MMR vaccination on emergency department visits after unintentional injury. A difference between the Danish study and our study is that there was more variation in the Danish study in the age at MMR vaccination (median 15.8 (interquartile range 15.2-17.0) v 14.3 (14.0-14.8)), which could be attributed to a different vaccination system. In the Netherlands, appointments for each vaccination are made in child health clinics in advance. In Denmark, parents have to make an appointment with the GP themselves for their children to receive vaccinations. This might have led to more random variation in the age at MMR vaccination and therefore proportionally less variation from factors related to the child's health in Denmark than in the Netherlands. The system in the Netherlands, with a more fixed schedule as result of prescheduled appointments, more clearly shows the existence of healthy vaccinee bias. This could explain the discrepancy between findings. It seems likely, however, that healthy vaccinee bias is also present in the Danish study. We therefore consider it likely that the lower rate of infection as estimated in the Danish setting overestimates any non-specific effects.

Several randomised controlled trials have been performed in low income countries to assess non-specific effects of vaccines containing measles on all cause mortality. The pooled relative risk from four randomised controlled trials was 0.74 (95% confidence interval 0.51 to 1.07), pointing towards beneficial effects of receipt of such vaccines.²¹ Like these vaccines, BCG vaccination, another live attenuated vaccine, has also been associated with non-specific effects in low income countries.²¹ A recently published randomised controlled study on BCG vaccination at birth in Denmark, however, did not find an effect on all cause hospital admissions⁴⁸ or childhood infections reported by parents.⁴⁹

Although we show that healthy vaccinee bias is probably present in our analysis, the impact of this bias is hard to quantify, and we cannot exclude that non-specific effects are still present in our study. If we assume that the lower rate of infection after the fourth versus the third DTaP-IPV-Hib+PCV vaccination is due to healthy vaccinee bias, the difference in hazard ratios between the MMR+MenC versus DTaP-IPV-Hib+PCV (hazard ratio of 0.62, 95% confidence interval 0.57 to 0.67) and the fourth versus the third DTaP-IPV-Hib+PCV (0.69, 0.63 to 0.76) could be ascribed to non-specific effects. Furthermore, in the MMR+MenC versus DTaP-IPV-Hib+PCV analysis the effect was stronger for respiratory infections than for other infections, but this was not the case for the fourth versus the third DTaP-IPV-Hib+PCV analysis. As non-specific effects have been found to be stronger for respiratory infections,^{10 19} these findings could indicate the presence of non-specific effects of MMR. This is rather indirect evidence, comparing different populations of children, however, and therefore not robust. These findings emphasise the difficulty of investigating non-

specific effects of vaccination in observational studies. This is also confirmed by our findings for the negative control outcome. We observed a 14% (95% confidence interval 4% to 27%) lower rate of admission for injuries or poisoning, which can obviously not be explained by non-specific effects. This finding could also be explained by healthy vaccinee bias but to a lesser extent.

It should be noted that in the Netherlands, the DTaP-IPV-Hib vaccine is administered with a multivalent conjugate vaccination against pneumococcal disease and the MMR vaccine is administered with vaccination against MenC. We cannot exclude the possibility that beneficial non-specific effects of the live MMR vaccine were masked by the simultaneous co-administration of the attenuated MenC vaccine. The Danish study that reported non-specific effects of MMR vaccination studied only MMR vaccination and not the combination of MMR and MenC, which could also explain the different findings with our study. Other European countries might also have the opportunity to study non-specific effects of vaccination and the potential for healthy vaccinee bias. As in the Netherlands, many European countries first give a vaccine containing DTP, mostly around age 12 months, and thereafter a MMR vaccine at age 15-18 months (ECDC: <http://vaccine-schedule.ecdc.europa.eu/>). There are also some countries, however, that first give the MMR vaccine and then the DTP vaccine, although in most of these countries the MMR vaccine is given together with PCV or MenC vaccination. Nevertheless, it would be interesting to see the results of a similar analysis from countries with a "reversed" schedule.

Strengths and weaknesses of study

A major strength of our study is that it is nationwide population based and included more than a million children. Because nearly all children received both the DTaP-IPV-Hib+PCV and MMR+MenC vaccinations, children acted as their own controls. Analyses were stratified by date of birth to fully control for age, year, and season. The large sample size enabled us to perform multiple stratified analyses. Moreover, the Dutch vaccination system, which is characterised by its fixed schedule as a result of prescheduled appointments, provided the opportunity to explore and illustrate the presence of healthy vaccinee bias. A limitation of the study is that we did not have information on the reason for delayed vaccination. Moreover, it should be noted that not all hospital admissions were captured in the national medication registration and that completeness decreased with time (97% in 2005 to 75% in 2012).⁴⁴ We assumed that the completeness was not associated with the timing of vaccination in our study and therefore would not confound the results. The interaction test confirmed this; we observed no effect modification for birth cohort. As these findings were based on admission data, we took only severely affected children into account. Therefore, it would be of interest to investigate the association between most recent vaccination and GP consultations for infections.

As this would enable us to take into account acute illness (for example, a GP visit for fever, as vaccination should be avoided if a child's temperature is $\geq 38.5^{\circ}\text{C}^{50}$) as a time varying variable, it could provide more insight in the effect of acute illness as part of healthy vaccinee bias.

In conclusion, throughout the world vaccination has contributed to a decline in mortality and morbidity as result of specific vaccine effects. Evidence for non-specific effects, however, particularly in high income countries, remains uncertain. In our observational study on non-specific effects of vaccination in more than a million Dutch children, a lower rate of hospital admission for infection followed receipt of an additional vaccination (and therefore adherence to the routinely recommended schedule) and could not be attributed to MMR specifically. Receipt of MMR+MenC as most recent vaccination was associated with a 38% lower rate of admission related to infectious disease, compared with DTaP-IPV-Hib+PCV as most recent vaccination. We also observed a 31% lower rate of admission for infection with receipt of the fourth DTaP-IPV-Hib+PCV as most recent vaccination, compared with the third as most recent vaccination. These findings, together with those for the negative control outcome, suggest that the delay of a vaccination (be it DTaP-IPV-Hib+PCV or MMR+MenC) might depend on the health status of a child, rather than the other way around. We cannot exclude the presence of non-specific effects of MMR vaccination, but we are unable to disentangle the possible non-specific effects from bias. Our findings emphasise the importance of interpreting findings from observational studies on non-specific effects of vaccination with great caution.

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Ethical approval: Not required.

Transparency: The corresponding author (MK) affirms that the manuscript is an honest, accurate, and transparent account of the study being reported, no important aspects of the study have been omitted, and any discrepancies from the study as planned have been explained.

Data sharing: The statistical code is available in appendix 2. Individual data are not available because of privacy reasons.

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Appendix 1: Supplementary tables
Appendix 2: Supplementary information