



Diphtheria tetanus pertussis poliomyelitis vaccination and reported atopic disorders in 8–12-year-old children

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Abstract

Background: Evidence for the relationship between the diphtheria tetanus pertussis (DTP) vaccination and atopic disorders is inconclusive, because the available studies that constitute the evidence are liable to confounding by indication.

Study objective: To assess the relationship between diphtheria–tetanus–pertussis-(inactivated) poliomyelitis vaccination (DTP-IPV) in the first year of life and reported atopic disorders at primary school age.

Method: 1875 children attending Orthodox Reformed (Protestant) primary schools in the Netherlands returned questionnaires with data on vaccination status, atopic symptoms and lifetime atopic disorders (asthma, hay fever, eczema and food allergy), and possible confounders.

Results: The adjusted odds ratio of any atopic disorder (vaccinated/unvaccinated) was 1.00 (CI_{95%}: 0.80–1.24). For asthma, hay fever, eczema and food allergy the results were respectively: 1.04 (CI_{95%}: 0.76–1.42), 0.79 (CI_{95%}: 0.55–1.12), 0.87 (CI_{95%}: 0.66–1.14) and 1.13 (CI_{95%}: 0.71–1.81).

Conclusion: The DTP-IPV vaccination was not related to reported atopic disorders at primary school age.

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Keyword: DTP vaccination atopy asthma

1. Introduction

The prevalence of allergic disorders has increased during the past decades and much effort has been put into identifying risk factors for these disorders. These include elements of western lifestyle such as increased hygiene and subsequent decreased exposure to pathogens and number of infectious diseases. The potential influence of childhood vaccinations is often the subject of heated debate, both in the lay press and in the scientific literature. It is therefore important to com-

municate results of studies in a subtle way in order to avoid biased interpretations by the lay press which could unnecessarily reduce adherence of the general public to vaccination programmes.

Many studies have investigated the relationship between childhood vaccinations and allergic disorders. A recent systematic review concluded that these vaccinations do not cause allergic diseases [1]. However, concerning the diphtheria–tetanus–pertussis (DTP) vaccination, this conclusion is controversial [2], mainly because of the possibility of bias in these studies. For in all these (observational) studies the reason for not vaccinating may well have been health-related and thus outcome-related, which is a classic example of confounding by indication. A randomised controlled trial

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would avoid this bias, but may not be feasible for ethical reasons.

In the Netherlands about 6% of the population adhere to the Orthodox Reformed (Protestant) religion and a part of this group refrains from vaccinations for religious reasons, others make their own appraisal and decide that it is justified to have their children vaccinated, often for fear of the consequences of an epidemic.

We conducted an observational cross-sectional study within this religious group to study the relationship between the DTP-(inactivated) poliomyelitis vaccination (DTP-IPV) and atopic disorders, at the same time minimizing the problem of confounding by indication.

1.1. Aim of the study

To assess the relationship between receiving the DTP-IPV in the first year of life and reported atopic disorders at primary school age.

2. Methods

2.1. Childhood vaccinations in the Netherlands

According to the National Vaccination Programme, infants get four vaccinations of DTP-IPV and Haemophilus influenzae type B (Hib) in their first year of life. At 14 months children are immunized with the measles mumps rubella vaccine (MMR) and meningococcal c vaccine (Neis Vax). At 4 years children receive a vaccination for diphtheria–tetanus–(inactivated) poliomyelitis (DT-IPV) and acellular pertussis (aP), and at 9 years DT-IPV and MMR. Because the Hib vaccination was introduced in 1993 and the Neis Vax in 2003, part of the population under study did not regularly receive the Hib vaccination and the whole population received the Neis Vax (if accepted) at age 7 years or older. Parents receive a summary of their child's vaccinations which they have to keep for registration of future vaccinations.

2.2. Study area, population and design

In the Netherlands the Orthodox Reformed religion is a subdivision of the Protestant Church. Children from this religious group attend Orthodox Reformed schools which are clustered in an area which stretches from the south-west of the Netherlands to the east. Many of these people refuse vaccinations for religious reasons.

In a cross-sectional design we assessed by means of questionnaires the relationship between receiving the DTP-IPV in the first year of life and atopic symptoms or lifetime disease at age 8–12 years.

Permission was requested from the Boards of the Orthodox Reformed primary schools to distribute letters of invitation, information and questionnaires directed to the parents of children aged 8–12 years. In most cases these letters were

also signed by a staff member of the coordinating District Health Service in the region at issue.

The study was approved by the Ethics Committee of Erasmus MC and all participants (parents) gave informed consent for participation in the study.

The study was financed by the Department of General Practice of Erasmus MC. AstraZeneca, who provided the anaesthetic patches, had no role in the design, collection, analysis and interpretation of the data, writing of the report or the decision to submit the paper for publication.

2.3. Sample size calculation

We assumed a significance level of 5%, a power of 80%, and a prevalence of allergic disorders of 10% in the lowest risk group. Our aim was to detect an absolute risk difference of at least 5%. It was unknown beforehand what the distribution of the risk factor under study would be in our population. However, we assumed a distribution that would not be skewed more than 3:7, i.e. 30% vaccinated children and 70% unvaccinated children (or vice versa). In the statistically most unfavourable situation, 10% atopic disorders in the smallest group (30%) and 15% atopic disorders in the other group (70%), we would need a total of 1769 children to detect this difference (corresponding to an odds ratio of 1.6) with a two-sided test.

2.4. Participating schools and children

Relevant schools were approached by mail (and subsequently by telephone) requesting permission to distribute packages containing a letter of invitation, information about the study, informed consent form and a questionnaire. We continued approaching schools until we judged that the required number of questionnaires would be returned. Of the 55 schools that were asked for permission 17 (31%) refused, mainly because the Boards expected the topic of vaccinations to be too sensitive. In total 4480 packages were distributed in the period autumn 2003–spring 2004 in classes 5–8 (corresponding to age 8–12 years) of the 38 participating schools. In total 1875 questionnaires (42%) were returned of which 1872 were suitable for analysis.

2.5. Data collection

One of the parents, preferably the mother, was asked to fill out the questionnaire. Parents were asked to have the summary of their child's vaccinations available when answering the questionnaire. Informed consent contained questions on consent to participate in the study, consent for the investigator to obtain officially registered vaccination data, blood sampling (for validation purposes in a subset of the participants), and storage of the data. The questionnaire asked for symptoms of (and physician diagnosis of) asthma, hay fever and eczema (Dutch translation of the ISAAC questionnaire [3,4]), physician-diagnosed food allergy ever, childhood

vaccinations, BCG vaccinations and influenza vaccinations, gender, passive smoking (prenatally, during the first year of life and currently), date of birth, perinatal data, birth order, ZIP code, year of birth of the mother, any atopic disorders of parents or siblings, family composition, education, income, living conditions (current and during the first year of life), day care attendance, breast feeding, nutrition during the past month, frequency of exercise, body length and weight, and having had common infectious diseases preventable by vaccinations (measles, mumps, rubella and pertussis).

2.6. Statistical analysis

The relationship between the DTP-IPV in the first year of life and (current symptoms or “ever had”) asthma, hay fever, eczema, food allergy, and “any atopic disorder” (at least one of these diseases) was evaluated by means of logistic regression. In the main analyses we ignored the questions on physician diagnosis because the more conservative religious adherents are possibly less inclined to visit their general practitioner thus introducing bias. However, we repeated all analyses with physician-diagnosed disorders as outcomes. Analyses were performed both in univariate and multivariate models. Potential confounders are shown in Table 1. A variable was included in the multivariate model if it changed the univariate point estimate by at least 10% [5].

The question on income is often a sensitive one which people tend to skip. Therefore, for those participants who did not answer this question we ranked them according to income data linked to their ZIP code [6] and imputed a code for income such that the total distribution of income remained the same as it was for the participants who did answer this question.

The main analyses were repeated taking the multilevel structure (level 1: children/level 2: families/level 3: schools) into account.

For the imputation procedure (see “Objective measurement of allergy”) we used a multiple imputation technique with five imputed data sets [7]. Analyses were carried out on each of these five sets and results were pooled [7]. Imputation was done with IVEware version 2.0.

SPSS version 11.0 and ML-win version 1.1 were used for all other computations. A two-sided *p*-value of 0.05 or less was considered significant in all tests.

2.7. Objective measurement of allergy

In total 72% of the participants gave informed consent for a blood sample (73.6% of the vaccinated, and 70.3% of the unvaccinated children). From these children we selected a random stratified sample of 100 children. Stratification was performed such that there was a 1:4 distribution of DTP-IPV (yes/no) and an equal distribution of atopic symptoms (yes/no) and primary school class (5/6/7/8). Two research assistants visited the participating schools to take the blood samples. Emla[®] anaesthetic patches (AstraZeneca, Zoeter-

Table 1

Potential confounders in the relationship between the DTP-IPV and atopic disorders

Season of birth
Birth order
Gender
Gestational age
Birth weight
Age of the mother at the time of delivery
Exposure to smoking (prenatally, during the first year of life and currently)
Breast feeding for four months or more (yes/no)
Housing in the first year of life (rural and living on a farm with livestock/rural other/city)
Pet keeping (furry pets or birds yes/no) during the first year of life and currently
Day care starting at age 6 months or less (yes/no)
Current age
Asthma and/or allergy of the parents and/or siblings
Highest educational level of the parents
Family income
Current level of urbanization (five levels)
Living on a farm with livestock (yes/no)
Sibship size
Mould in the child's bedroom in the past year
Frequent (more than 5 days/week) consumption of fruit (yes/no)
(raw or cooked) vegetables (yes/no)
anti-oxidants (yes/no)
unskimmed dairy products (yes/no)
wholemeal bread (yes/no)
Frequent (at least 1 day/week) consumption of fish
Frequent exercise (school gym at least once a week and playing games with physical activity for at least half an hour a day and either being a member of a sporting club or walking or cycling from home to school vice versa for at least 1 h a day)
Body mass index
Hib vaccination

meer, The Netherlands) were used to minimize needle pain. We performed RAST tests using the Pharmacia[®] RIA method to determine specific IgE to five of the most common aero allergens in the Netherlands (house dust mite (d1), cocksfoot pollen (g3), common silver birch pollen (t3), cat epithelium and dander (e1) and dog dander (e5)) in the sera of these 100 children to obtain an objective measurement of allergy.

Allergy was defined as at least one RAST class 2 or higher (i.e. IgE $\geq$ 0.7 IU/ml); we linked this objective variable to reported asthma or hay fever (current symptoms or “ever had”). Moreover, on the basis of these objective allergy data of 100 children and other relevant variables, we imputed the objective allergy for the remaining 1773 children.

2.8. Validation of the risk factor

To validate our question on the DTP-IPV we performed tests for tetanus toxoid IgG and diphtheria IgG antibodies in the sera of the 80 children (from the sample of 100 mentioned under “Validation of the outcome”) that according to their parents were DTP-IPV unvaccinated. All sera were stored at minus 20 degree Celsius until being tested in one run. The following EIA kits were used to test for tetanus toxoid

IgG and diphtheria IgG antibodies: RIDASCREEN® Tetanus test and RIDASCREEN® Diphtheria test (both R-Biopharm, Germany). Children with titres of at least 0.6 IU/ml were considered as being vaccinated for the pathogen concerned.

In addition we randomly selected a group of 120 children [of those 1761 (94.1%, 99.0% of the vaccinated and 85.2% of the unvaccinated children) who gave informed consent to obtain officially registered vaccination data] and compared the official registrations of the vaccinations in the first year of life with those reported by the parents.

2.9. Assessment of selection bias

In case of a low response rate the possibility of selection bias had to be evaluated.

If selection bias would be present, at least one of the four cells (no atopy/no DTP-IPV, atopy/no DTP-IPV, no atopy/DTP-IPV, atopy/DTP-IPV) would be over- or under-represented, thus causing a spurious relationship. Selection bias is unlikely if no selection on DTP-IPV status has occurred. In order to verify this we asked the District Health Services covering the participating schools to estimate from their records the vaccination coverage per school. Thereafter, we compared the vaccination coverage of the responders for each school separately, and the total in all schools, with the vaccination coverage reported by the District Health Services.

Finally, we assessed the odds ratios (ORs) pertaining to the relationship under study and the response rate for each school separately and checked by means of linear regression, weighted with the inverse of the squared standard error of the log(OR), whether there was a relationship between the log(OR) and the response rate. In case of selection bias one would expect a (negative or positive) relationship between ORs and response rate.

3. Results

3.1. Relationship between DTP-IPV and atopy

A total of 1875 children returned the questionnaire (a response rate of 42%), of which three questionnaires were excluded because of missing vaccination status. Of the remaining 1872 children, 671 (35.8%) were not vaccinated for DTP-IPV in the first year of life. Of the 1201 vaccinated children, 21 (1.7%) were incompletely vaccinated for DTP-IPV (i.e. they did not receive all four vaccinations scheduled in the first year of life).

Of all children, 873 (46.6%) reported any form of lifetime atopic disorder. Characteristics of the children, by DTP-IPV status, are presented in Table 2.

Table 3 shows prevalences and both univariate and multivariate ORs with 95% confidence intervals (CI_{95%}) for the relationships between DTP-IPV and atopic disorders. Atopy appeared to be slightly more prevalent in vaccinated children

Table 2

Characteristics of the participating children by DTP-IPV status

	Unvaccinated (%) (N = 671 ^a)	Vaccinated (%) (N = 1201 ^a)
Gender		
Male	52.5	50.3
Female	47.5	49.7
Age (years)		
<10	44.0	42.2
≥10	55.7	57.4
Unknown	0.3	0.4
Atopic disorders in parents and/or siblings		
Yes	61.3	65.5
No	38.6	34.0
Unknown	0.1	0.5
Highest education of parents		
Primary or less	0.4	0.4
Lower professional and lower secondary	34.1	23.8
Medium professional	28.5	29.2
Higher secondary	10.1	15.0
High professional and university	26.5	31.1
Unknown	0.3	0.4
Number of children in the family		
1	0.7	1.2
2	1.8	15.2
3	9.2	30.1
4	22.5	22.6
≥5	65.6	30.8
Unknown	0.1	0.2
Pertussis infection ^b		
Yes	42.2	8.9
No	57.8	91.1
Mumps infection ^b		
Yes	7.3	2.9
No	92.7	97.1
Measles infection ^b		
Yes	86.1	18.4
No	13.9	81.6
Rubella infection ^b		
Yes	22.2	6.5
No	77.8	93.5
Hib vaccination 1st year		
Yes	0.0	54.3
No	99.6	34.8
Unknown	0.4	10.9
Pertussis part of DTP-IPV 1st year (vaccinated only)		
Yes		90.1
No		3.8
Unknown		6.1
MMR at 14 months		
Yes	0.1	89.7
No	99.6	8.4
Unknown	0.3	1.9

^a Unvaccinated means: no DTP-IPV in the first year of life; vaccinated means: at least one DTP-IPV in the first year of life. For three children this characteristic was unknown.

^b Reported infection.

Table 3

Atopic disorders for (diphtheria tetanus pertussis poliomyelitis) vaccinated and unvaccinated children: prevalences (%) and crude and adjusted (adj) odds ratios (OR) with 95% confidence intervals (CI_{95%})

	Percent	OR	AdjOR	CI _{95%}
Asthma ^a				
Vaccinated	15.2	1.28	1.04	0.76–1.42
Unvaccinated	12.4			
Hay fever ^a				
Vaccinated	17.9	1.14	0.79	0.55–1.12
Unvaccinated	16.2			
Eczema ^a				
Vaccinated	34.2	1.14	0.87	0.66–1.14
Unvaccinated	31.1			
Food allergy ^a				
Vaccinated	7.9	1.29	1.13	0.71–1.81
Unvaccinated	6.3			
Any atopic disorder ^a				
Vaccinated	48.7	1.27	1.00	0.80–1.24
Unvaccinated	42.9			

^a Ever had the disease or having symptoms at present.

[univariate OR = 1.27, CI_{95%} = 1.05–1.59], but this difference disappeared when we adjusted for confounders [OR = 1.00, CI_{95%} = 0.80–1.24]. The differences for the separate disorders were small and not significant (Table 3). In all models family size, education and atopic history of parents or siblings proved to be confounders. When we analysed the models with physician-diagnosed diseases as outcome variables the adjusted ORs tended to be somewhat larger, although they were never statistically significant: for any atopic disorder the adjusted OR was 1.04 [CI_{95%} = 0.82–1.31] (Table 4).

Multilevel analysis of the multivariate models presented in Table 2 yielded similar results (data not shown).

Table 4

Physician diagnosed atopic disorders for (diphtheria tetanus pertussis poliomyelitis) vaccinated and unvaccinated children: prevalences (%) and crude and adjusted (adj) odds ratios (OR) with 95% confidence intervals (CI_{95%})

	Percent	OR	AdjOR	CI _{95%}
Asthma				
Vaccinated	11.4	1.23	1.03	0.72–1.46
Unvaccinated	9.5			
Hay fever				
Vaccinated	5.5	1.30	1.06	0.59–1.90
Unvaccinated	4.3			
Eczema				
Vaccinated	22.6	1.19	0.96	0.73–1.25
Unvaccinated	19.7			
Food allergy				
Vaccinated	7.9	1.29	1.13	0.71–1.81
Unvaccinated	6.3			
Any atopic disorder				
Vaccinated	33.2	1.27	1.04	0.82–1.31
Unvaccinated	28.3			

Table 5

Reported asthma and/or hay fever vs. objective allergy in a subset of participants (*n* = 100)

Reported symptoms ^a	Objective allergy ^b		Total <i>n</i> (%)
	No <i>n</i> (%)	Yes <i>n</i> (%)	
No			
DTP-IPV no	33 (73)	12 (27)	45 (100)
DTP-IPV yes	7 (78)	2 (22)	9 (100)
Total	40 (74)	14 (26)	54 (100)
Yes			
DTP-IPV no	12 (34)	23 (66)	35 (100)
DTP-IPV yes	8 (73)	3 (27)	11 (100)
Total	20 (44)	26 (56)	46 (100)

^a Symptoms or ever had asthma and/or hay fever.

^b At least one RAST class 2 or higher (i.e. IgE ≥ 0.7 IU/ml) (out of five RASTs for: house dust mite, grass pollen, birch pollen, cat dander and dog dander).

3.2. Objective measurement of allergy

Of the 100 children who donated blood, 46 (46.0%) reported symptoms of (or ever had) asthma or hay fever. Of these, 26 (56.5%) were allergic according to the RAST results. Of the remaining 54, who reported neither asthma nor hay fever, 40 (74.1%) were not allergic according to the RAST results. Of the remaining 14 (who had at least one RAST class 2 or higher and did not report asthma or hay fever), 7 (50%) reported symptoms of eczema. Table 5 shows these data, also broken down by vaccination status. It seems that, although the numbers are small, vaccinated children who reported asthma or hay fever, have less allergy according to our objective measurements than unvaccinated children (27% and 66%, respectively).

Analysis of the imputed datasets yielded an adjusted OR for objective allergy (DTP-IPV yes/DTP-IPV no) of 0.38 [CI_{95%}: 0.18–0.82], suggesting a protective effect of vaccinations.

3.3. Validation of the risk factor

Of the 80 allegedly DTP-IPV unvaccinated (in the first year of life) children tested for tetanus toxoid IgG and diphtheria IgG antibodies, six proved to have titres of tetanus toxoid IgG of at least 0.6 IU/ml. However, four of these indicated in the questionnaire that they had a tetanus vaccination after an accident. Two of the remaining children also had titres of diphtheria IgG of at least 0.6 IU/ml. A third child had a diphtheria IgG titre over 0.6 IU/ml, but its tetanus titre was only 0.32 IU/ml. These results raise doubt about the vaccination status of three out of 80 allegedly unvaccinated children (3.8%).

We requested official vaccination registration data for 120 children, of whom 3 turned out to be untraceable. Comparison of reported infant vaccinations with the official registration data in the remaining 117 participants yielded a perfect match in 107 children (91.5%); in four cases there was disagreement about the pertussis component and in five cases about the Hib

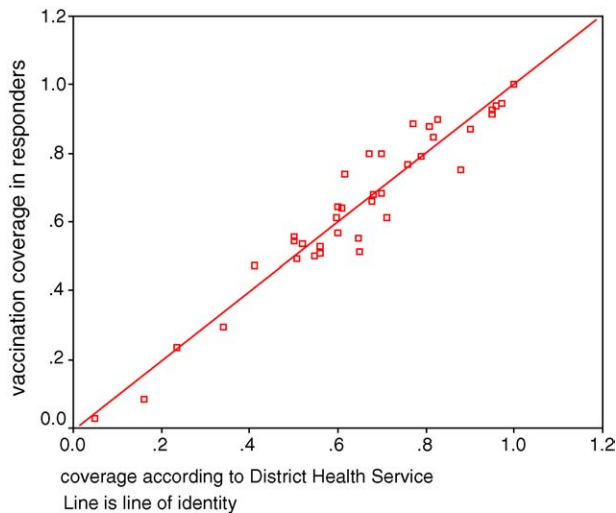


Fig. 1. Vaccination coverage in the 38 schools.

vaccination. On the DTP-IPV there was disagreement in only one case.

3.4. Selection bias

For each school we compared the vaccination coverage according to the records of the District Health Service with the vaccination coverage in our responders. Results for all 38 schools are shown in Fig. 1. The mean difference is 0.1% (S.D. 6.4%). The vaccination coverage for all schools, estimated with the help of the records of the District Health Service, was 63.8%, whereas the vaccination coverage of the responders was 63.1%.

Fig. 2 shows for 37 schools (for one school which had 100% vaccination coverage, no OR was computed) the relationship between the $\ln(\text{OR})$ of atopy (DTP-IPV yes/DTP-IPV no) and the response rate. The weighted linear regression line seems to be descending slightly, indicating a possible small decrease of the OR with increasing response rate.

4. Discussion

The present study showed no relationship between the DTP-IPV in the first year of life and reported atopic disorders in primary school children. This is the first study to compare vaccinated and unvaccinated children in a population where refraining from vaccinations is unlikely to be health-related. Moreover, the two groups had the same religious background, lived in the same area and attended the same schools. Possible differences were extensively surveyed and controlled for in the analyses. This situation is second best after a randomized controlled trial, which would not be feasible for ethical reasons.

Koppen et al. [1] concluded in their review, that the DTP vaccination does not cause allergic disease. This conclusion

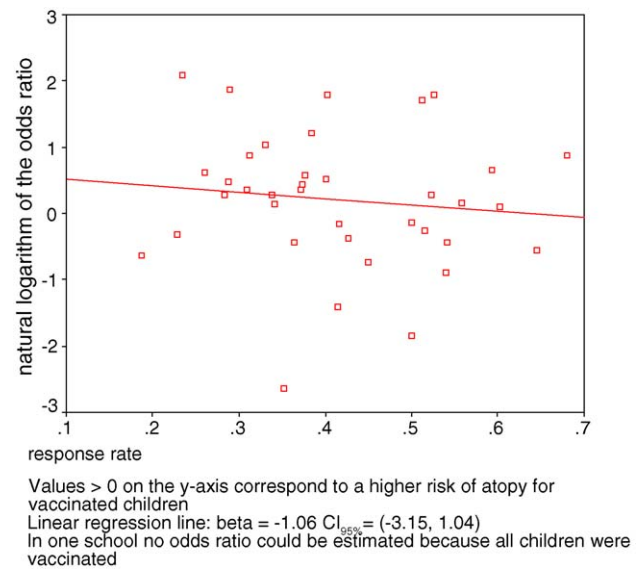


Fig. 2. Relationship between the logarithm of the odds ratio (any atopy) (DTP-IPV yes/DTP-IPV no) and response rate to the questionnaire. Values >0 on the y-axis correspond to a higher risk of atopy for vaccinated children. Linear regression line: $\beta = -1.06$ $CI_{95\%} = (-3.15, 1.04)$. In one school no odds ratio could be estimated because all children were vaccinated.

was not entirely justified, because confounding by indication could have caused biased results in all studies included in their review and, moreover, the conclusions of these studies were not unequivocal [2]. However, the results of the present study support the conclusion that the DTP-IPV indeed is not associated with allergic disease.

Atopic disorders of family members could be viewed as intermediate variables in the relationship between vaccinations and atopy, because the vaccination status of family members is likely to be highly correlated. However, we considered this predictor to be too important to omit from the models.

Vaccinations other than the DTP-IPV were not included in the model, because having had other vaccinations was highly correlated to acceptance of the DTP-IPV. An exception was the HiB vaccination; this was introduced in 1993 and, consequently, a major part of the otherwise vaccinated children did not get the HiB vaccination.

Another consequence of the fact that almost all DTP-IPV unvaccinated children were completely unvaccinated is that our conclusion, that the two groups do not differ in prevalence of atopic disorders, applies to childhood vaccinations in general. In this respect also concerns about additives (such as the aluminium-containing adjuvans), are not supported by the present study.

We did not include histories of infections of pertussis, mumps, measles and rubella in the multivariate model, as these are obviously intermediate variables in the relationship between vaccinations and atopy. Moreover, also reversed causation could play a role because children predisposed to allergic disease have a different antiviral immunity [8]. Finally, it

is plausible that these diseases were more often diagnosed in the unvaccinated group, also because they probably result in more symptoms. Children with a reported history of pertussis, mumps and measles reported more atopy, but for children who allegedly had had rubella less atopy was reported.

Physician-diagnosed atopy showed similar results as self-reported atopy, but indicating slightly more atopy in the vaccinated group. This difference may be explained by the possibility that unvaccinated (probably more traditional) people tend to be less inclined to visit their general practitioner. Possible health care utilization bias was the reason why we took reported symptoms and disease as the main outcome variable.

In a previous study (not included in the review mentioned above) we concluded that pertussis vaccinated children had a lower risk of atopic disorders [9]. However, this conclusion was based on only 44 possibly genetically related, unvaccinated children who attended the same school. Also, our analysis of objective allergy with imputed data showed an almost identical result. However, these estimates were based on a subsample of only 100 children, 20 vaccinated and 80 unvaccinated. Moreover, the fraction of children that consented to blood donation was 72% which may have led to selection bias. For these reasons these results should be viewed with some caution.

A possible explanation for the relationship observed (i.e. unvaccinated children have more often increased specific IgE than vaccinated children) could be that parents' reports of their child's symptoms are possibly influenced by the knowledge that they are participants in a study, whereas objective measurements are not.

4.1. Limitations of the study

4.1.1. Design

The design was cross-sectional, implying that some variables were assessed retrospectively. This applies mainly to confounders concerning pregnancy and the first year of life, but not to vaccinations, as these were registered at the time they were administered. Parents receive a list with their child's vaccinations.

4.1.2. Reliability of the outcome

The outcomes analysed in the present study are symptoms of atopic disease reported by the parents. Total agreement with our objective definition of allergy was 66%: just over half of the children reporting symptoms showed allergic sensitization and about one quarter of the children without symptoms were sensitized. Similar figures are also found in the literature [10].

4.1.3. Reliability of the risk factor

There was an almost 100% agreement between reported DTP-IPV and officially registered DTP-IPV. In sera of a random stratified sample of 80 children who were not DTP-IPV vaccinated in the first year, we checked whether tetanus tox-

oid IgG or diphtheria IgG antibodies above a relevant level could be detected. Moreover, in a random sample of 100 children, we compared the reported DTP-IPV with the officially registered vaccination data. Based on the results of these checks, we conclude that the main risk factor was fairly reliably reported, although some misclassification was detected. Moreover, only 85% of the unvaccinated children (versus 99% of the vaccinated children) gave informed consent to obtain official registration data; for blood samples this was 70% and 74%, respectively. These figures could raise some concern about the reliability of the reported risk factor, because parents may have reasons to conceal the true vaccination status of their children. On the other hand, it would be more likely that these people would refuse to participate in the study at all.

4.1.4. Selection bias

4.1.4.1. Schools. Seventeen out of 55 schools (31%) refused collaboration. Reasons mentioned by the directors were: "topic of vaccinations too sensitive among the parents", "we have no unvaccinated pupils", "too busy". We believe that these reasons are not related to the relationship under study and that therefore selection bias due to non-response of schools is unlikely.

4.1.4.2. Children. Because only 42% of the approached children responded, the possibility of selection bias had to be evaluated. We checked this in two ways: first we compared the vaccination coverage of the responders with the vaccination coverage of the target group. The difference was less than 2%, implying that no selection on vaccination status had occurred; this made selection bias unlikely. Secondly, for the 38 participating schools we investigated the relationship between the response rate and the relationship between DTP-IPV and atopic disorder. There appeared to be a slightly negative trend, i.e. if there would be selection bias, the "true" OR would be smaller (i.e. a relatively smaller risk of atopy in vaccinated children) than the OR in our results.

4.1.5. External validity

The study was conducted in a relatively homogeneous group of people, all from a common cultural background. Although this is a strong aspect of the study design, it could also raise questions about the generalizability of the conclusions. However, there is no reason to assume that the group of people under study differ biologically from the general Dutch population such that childhood vaccinations would have a different impact on atopy.

5. Conclusion

In the Dutch population the DTP-IPV is unlikely to be associated with a higher risk of reported atopic disorders at primary school age.

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References

- [1] Koppen S, De Groot R, Neijens HJ, Nagelkerke N, Van Eden W, Rumke HC. No epidemiological evidence for infant vaccinations to cause allergic disease. *Vaccine* 2004;22:3375–85.
- [2] Bernsen RM, van der Wouden JC. Re: No epidemiological evidence for infant vaccinations to cause allergic disease. Letter to the Editor. *Vaccine* 2005;23:1427.
- [3] Asher MI, Keil U, Anderson HR, et al. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. *Eur Respir J* 1995;8:483–91.
- [4] Anyo G, Brunekreef B, de Meer G, Aarts F, Janssen NA, van Vliet P. Early, current and past pet ownership: associations with sensitization, bronchial responsiveness and allergic symptoms in school children. *Clin Exp Allergy* 2002;32:361–6.
- [5] Rothman K. *Modern epidemiology*. Philadelphia: Lippincott-Raven; 1998. pp. 256–257.
- [6] Kerncijfers vierposities postcodegebieden. Voorburg/Heerlen: Centraal Bureau voor de Statistiek; 1995.
- [7] Rubin DB, Schenker N. Multiple imputation in health care databases: an overview and some applications. *Stat Med* 1991;10:585–98.
- [8] Copenhaver CC, Gern JE, Li Z, Shult PA, Rosenthal LA, Mikus LD, et al. Cytokine response patterns, exposure to viruses, and respiratory infections in the first year of life. *Am J Respir Crit Care Med* 2004;170:175–80.
- [9] Bernsen RM, de Jongste JC, van der Wouden JC. Lower risk of atopic disorders in whole cell pertussis-vaccinated children. *Eur Respir J* 2003;22:962–4.
- [10] Kurukulaaratchy RJ, Fenn M, Matthews S, Arshad SH. Characterisation of atopic and non-atopic wheeze in 10 year old children. *Thorax* 2004;59:563–8.