Allergy EUROPEAN JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY

EAACI

Allergy

ORIGINAL ARTICLE EPIDEMIOLOGY AND GENETICS

Timing of routine infant vaccinations and risk of food allergy and eczema at one year of age

N. Kiraly[1,2], J. J. Koplin[1,3], N. W. Crawford[2,4,5], S. Bannister[2], K. L. Flanagan[6], P. G. Holt[7,8], L. C. Gurrin[1,3], A. J. Lowe[1,3], M. L. K. Tang[5,9,10], M. Wake[5,11,12], A.-L. Ponsonby[5,13], S. C. Dharmage[1,3] & K. J. Allen[1,5,10,14]

[1]Gastro & Food Allergy, Murdoch Childrens Research Institute, Parkville, VIC, Australia; [2]Department of General Medicine, The Royal Children's Hospital, Parkville, VIC, Australia; [3]Melbourne School of Population and Global Health, The University of Melbourne, Carlton, VIC, Australia; [4]SAEFVIC, Murdoch Childrens Research Institute; [5]Department of Paediatrics, The University of Melbourne, Parkville, VIC, Australia; [6]Department of Immunology, Monash University, Prahran, VIC, Australia; [7]Telethon Institute for Child Health Research, University of Western Australia, Perth, WA, Australia; [8]Queensland Children's Medical Research Institute, University of Queensland, Brisbane, QLD, Australia; [9]Allergy & Immune Disorders, Murdoch Childrens Research Institute; [10]Department of Allergy and Immunology, The Royal Children's Hospital; [11]Community Health Services Research, Murdoch Childrens Research Institute; [12]The Centre for Community Child Health, The Royal Children's Hospital; [13]Environmental & Genetic Epidemiology Research, Murdoch Childrens Research Institute; [14]Institute of Inflammation and Repair, University of Manchester, Manchester, UK

To cite this article: Kiraly N, Koplin JJ, Crawford NW, Bannister S, Flanagan KL, Holt PG, Gurrin LC, Lowe AJ, Tang MLK, Wake M, Ponsonby A-L, Dharmage SC, Allen KJ. Timing of routine infant vaccinations and risk of food allergy and eczema at one year of age. *Allergy* 2016; **71**:541–549

Keywords

Atopic hypersensitivity; food allergy; eczema; DTaP vaccine; infant.

Correspondence

Prof. Katrina J. Allen, Gastro & Food Allergy, Murdoch Childrens Research Institute, Royal Children's Hospital, 50 Flemington Rd Parkville, Vic. 3052, Australia.
Tel.: +61 3 9936 6752
Fax: +61 3 9348 1391
E-mail: katrina.allen@mcri.edu.au

Study registration: This observational study was registered with ANZCTR, trial ID ACTRN12614001193662.

Accepted for publication 20 December 2015

DOI:10.1111/all.12830

Edited by: Stephan Weidinger

Abstract

Background: Epidemiological evidence suggests that routine vaccinations can have nontargeted effects on susceptibility to infections and allergic disease. Such effects may depend on age at vaccination, and a delay in pertussis vaccination has been linked to reduced risk of allergic disease. We aimed to test the hypothesis that delay in vaccines containing diphtheria–tetanus–acellular pertussis (DTaP) is associated with reduced risk of food allergy and other allergic diseases.

Methods: HealthNuts is a population-based cohort in Melbourne, Australia. Twelve-month-old infants were skin prick-tested to common food allergens, and sensitized infants were offered oral food challenges to determine food allergy status. In this data linkage study, vaccination data for children in the HealthNuts cohort were obtained from the Australian Childhood Immunisation Register. Associations were examined between age at the first dose of DTaP and allergic disease.

Results: Of 4433 children, 109 (2.5%) received the first dose of DTaP one month late (delayed DTaP). Overall, delayed DTaP was not associated with primary outcomes of food allergy (adjusted odds ratio (aOR) 0.77; 95% CI: 0.36–1.62, $P = 0.49$) or atopic sensitization (aOR: 0.66; 95% CI: 0.35–1.24, $P = 0.19$). Amongst secondary outcomes, delayed DTaP was associated with reduced eczema (aOR: 0.57; 95% CI: 0.34–0.97, $P = 0.04$) and reduced use of eczema medication (aOR: 0.45; 95% CI: 0.24–0.83, $P = 0.01$).

Conclusions: There was no overall association between delayed DTaP and food allergy; however, children with delayed DTaP had less eczema and less use of eczema medication. Timing of routine infant immunizations may affect susceptibility to allergic disease.

The prevalence of food allergy is rising in industrialized countries, and Melbourne, Australia, has the highest reported prevalence of childhood food allergy in the world (1). While some environmental factors have been clearly associated with protection against food allergy, including older siblings, pet ownership, timing of introduction of allergenic food (2) and vitamin D sufficiency (3), the causes for the increasing prevalence of food allergy are largely unknown.

There is evidence that immunizations given early in life have the potential to deviate the immune system towards a more, or less, allergic phenotype. Bacille Calmette–Guérin (BCG), the live-attenuated tuberculosis vaccine, has been associated with protection against allergic disease (4), and randomized trials are ongoing to test this association (5). In contrast, studies of the associations between inactivated pertussis vaccines and allergic disease have shown conflicting results with statistical heterogeneity (6–9). The only randomized trial of pertussis vaccine and allergy compared both whole-cell and acellular pertussis vaccines against a diphtheria–tetanus control vaccine and found no large differences in eczema or other allergic diseases at the age of 2 ½ years (10). However, this study did not include an unvaccinated control group. If other components (diphtheria or tetanus toxoids or adjuvants) contribute a biological effect on allergic disease, the ability of the randomized trial to detect differences between vaccine groups may have been limited (11).

Two other important issues might impact on these previous observational studies of pertussis vaccines. First, confounding may occur due to the factors associated with receipt or refusal of vaccination. Second, heterogeneity of vaccination timing may lead to heterogeneity in study findings, as the age of exposure to the immune-modulating effects of vaccines may be important for the resulting immune phenotype (12). Five studies have investigated age of pertussis vaccination and allergic disease (9, 13–16), with three suggesting that delayed vaccination is protective against asthma (15), hay fever (13) and atopic sensitization (14) and two studies showing no association between timing of pertussis vaccination and asthma (9, 16) or eczema (16). Four of these studies investigated the whole-cell pertussis vaccine no longer used in most industrialized countries (13–16), only one investigated eczema (16) and none investigated food allergy.

The HealthNuts cohort is a population sample of one-year-old infants recruited to study prevalence and risk factors for food allergy (17). By linking with vaccination data from the Australian Childhood Immunisation Register (ACIR), the HealthNuts cohort was utilized to test the hypothesis that a delay in the first dose of an acellular pertussis-containing vaccine is associated with reduced prevalence of food allergy, eczema, wheeze or bronchiolitis in the first year of life.

Methods

The HealthNuts cohort

The HealthNuts cohort is comprised of 5276 infants who were recruited at immunization clinics across Melbourne, Australia, between 2007 and 2011 (17, 18). Parents provided written informed consent and completed an extensive survey of demography and history of allergic diseases, and their infants were examined for eczema and underwent a skin prick test (SPT) to common childhood food allergens (whole hen's egg, peanut, sesame and shellfish or cow's milk) (1). Children with a SPT reaction to any allergen (SPT wheal ≥1 mm after subtracting the negative control) were invited to attend the oral food challenge clinic (928 of 1089 (85%) sensitized children attended) along with a random sample of SPT-negative controls (n = 218, approximately 19% of the cohort who received oral food challenges). We used 1 mm as the criterion for invitation to the oral food challenge clinic to ensure that no children with potential food allergy were missed. In the oral food challenge, clinic SPTs were repeated, blood samples were taken for specific IgE and infants were given oral food challenges with each food to which they were sensitized (18).

Immunization exposures and data linkage

Over the birth years of the cohort, the Australian National Immunisation Program Schedule included a birth dose of hepatitis B vaccine followed by diphtheria–tetanus–acellular pertussis vaccine (DTaP) at two, four and six months of age, usually as part of Infanrix Hexa® (GSK, Boronia, Vic., Australia) also containing inactivated polio vaccine (IPV), hepatitis B vaccine and *Haemophilus influenzae* type b vaccine (Hib) (Fig. S1). A 13-valent pneumococcal conjugate vaccine (PCV) and oral rotavirus vaccination was also usually co-administered. In Australia, all childhood immunizations are recorded in the ACIR.

Children were eligible for the present study if they remained in the HealthNuts cohort with up-to-date contact details in May 2014. A letter was sent to the parents of all eligible children outlining the details of the study. Unless parents opted out, data on routine vaccinations were sought from ACIR between October 2014 and March 2015. Children were matched on first name, surname and date of birth with or without postcode; only definite matches were included. All children who received acellular pertussis vaccine also received diphtheria and tetanus components; thus, the age at first dose of DTaP was considered the primary exposure irrespective of other vaccines co-administered. Delayed DTaP was defined as the first dose given after 90 days of age (one month late) as per the Australian National Immunisation Program. Children with missing data for the first dose of DTaP but with data for subsequent doses (n = 46) were excluded from the primary analyses, as it was likely these children received prior doses of DTaP at an unknown time. Vaccination data were available from personal records for eight of these 46 children as part of HealthNuts age six follow-up (currently underway), all of whom received a dose of DTaP prior to the first dose recorded on ACIR (seven were vaccinated on time and one was delayed).

Allergic disease outcomes

Primary outcomes

Food allergy. Children were classified as food allergic if they had a SPT wheal ≥2 mm (after subtracting the negative control) or specific IgE > 0.35 kU_A/L at the oral food challenge clinic visit and any of the following within 2 h of oral food challenge: ≥3 concurrent noncontact urticaria lasting 5 min or more; perioral or periorbital angioedema; vomiting; or circulatory or respiratory compromise. Only children with reactions to egg, peanut and sesame were considered food allergic for this analysis because challenges were not performed for cow's milk and shellfish. Children were also deemed food allergic (without performing oral food challenges) if they had a positive SPT and a confirmed reaction to egg within the past one month, or to peanut or sesame within the past two months (1). Children with a positive food challenge but negative SPT and specific IgE < 0.35 kU_A/L (Fig. 1) were excluded from the food allergy analyses because it was unclear if they had IgE-mediated food allergy.

Atopic sensitization. All children with a SPT wheal ≥2 mm (after subtracting the negative control) to egg, peanut or sesame at the community clinic were classified as having atopic sensitization.

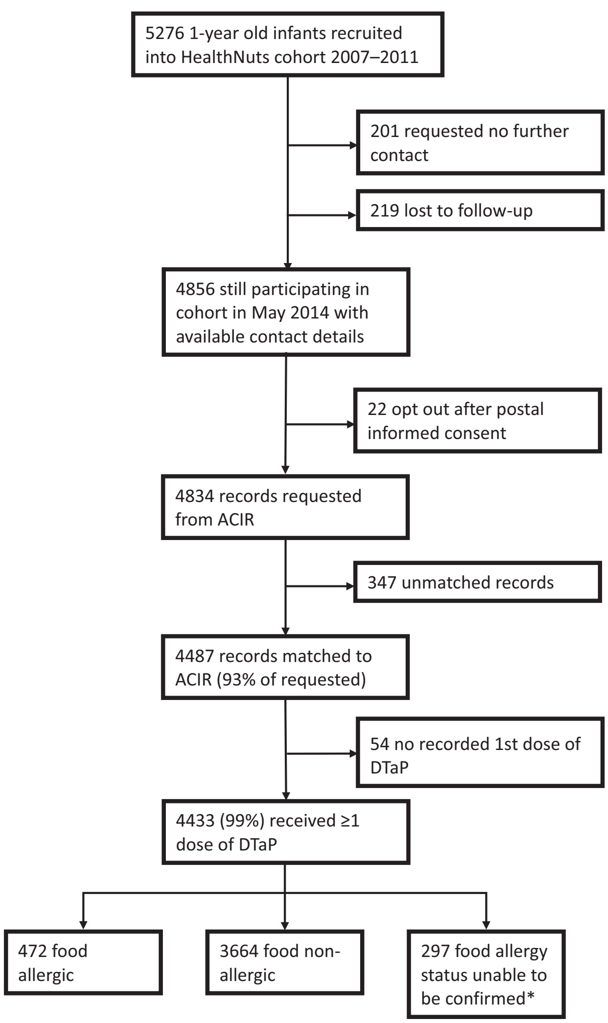

Figure 1 HealthNuts cohort and participant flow for the analysis of vaccination timing. ACIR, Australian Childhood Immunisation Register. *Unable to determine food allergy status due to failure to attend food challenge clinic (*n* = 230), inconclusive food challenge (*n* = 18), incomplete food challenge to all foods to which infant was sensitized (*n* = 22) or positive food challenge in the absence of atopic sensitization (*n* = 27).

Secondary outcomes

Eczema. Eczema was defined as established diagnosis by a doctor with the associated use of treatments (medications, topical steroids or moisturizers), or eczematous rash observed by a trained nurse at the time of recruitment. Children who were diagnosed with eczema before three months of age (prior to first scheduled vaccinations plus one-month window period) were excluded as eczema in these children was, by definition, unrelated to vaccination (*n* = 313, 21% of 1474 children who otherwise met criteria for eczema); thus, onset of eczema was between three and 12 months of age.

Eczema medication. Use of eczema medication was parent reported and included oral medication or topical steroids (but not moisturizers) to treat an itchy rash at any time in the first year of life. Similar to the eczema outcome, children diagnosed with eczema prior to three months of age were excluded from eczema medication analyses (*n* = 224, 19% of 1209 children who otherwise met criteria for the use of eczema medication).

Wheeze. 'Wheeze ever' reported by parents at one year of age.

Bronchiolitis admission. Hospital admissions for bronchiolitis at any time in the first year of life reported by parents at one year of age.

Statistical analyses

Demographic variables were compared between groups using chi-square or Kruskal–Wallis tests. The primary analyses were performed using logistic regression analysis to produce odds ratios (OR) for the association between delayed DTaP and allergic disease outcomes. Multivariate analyses were performed adjusting for prespecified potential confounders: sex (female/male), antibiotic use (categorized as yes/no due to excess missing data on number of antibiotic courses), daycare attendance (yes/no), number of siblings (0, 1–2, ≥3), birth country of the parents (both Australian, one or both parents born East Asia, either parent born elsewhere), presence of smokers at home (yes/no) and Socio-Economic Indexes for Areas (SEIFA; quintiles) (19). The SEIFA were used to estimate socioeconomic status on the basis of postcode (3, 19) due to incomplete parental income data. Other potential confounders (listed and categorized in Table 1) were not included in multivariate models because their inclusion did not alter any estimate by more than 10%. Analyses were stratified by sex as prespecified in the analysis plan, and a Wald test for homogeneity of effects was performed on all stratified analyses. A sensitivity analysis was performed designating children with indeterminate food allergy status as nonallergic unless they had a parent-reported history consistent with IgE-mediated food allergy at any age or a SPT wheal greater than the 95% positive predictive value for food allergy (≥4 mm to egg or ≥8 mm to peanut or sesame) (20), in which case they were designated as food allergic. A

Table 1 Comparison of demography between children with on-time and delayed 1st dose of DTaP

		n	First-dose DTaP		
		4433	< 1 month late (*n* = 4324)	≥1 month late (*n* = 109)	*P*
Sex	Female	4433	2155/4324 (50%)	45/109 (41%)	0.08
SEIFA	median (10–90th centile)	4428	1057 (987–1108)	1057 (987–1108)	0.79
Ever had antibiotics		4289	2136/4183 (51%)	51/106 (48%)	0.55
Attends child care		4338	1231/4231 (29%)	18/107 (17%)	0.006
Siblings	None	4386	2150/4278 (50%)	20/108 (19%)	
	1–2	4386	1418/4278 (33%)	46/108 (43%)	
	3 or more	4386	710/4278 (17%)	42/108 (39%)	<0.001
Parent country of birth	Any parent East Asia	4308	469/4201 (11%)	17/107 (16%)	
	Both parents Australia	4308	2557/4201 (61%)	67/107 (63%)	
	Any parent other country	4308	1175/4201 (28%)	23/107 (21%)	0.16
Smoker at the home		4403	905/4296 (21%)	38/107 (36%)	<0.001
Maternal smoking during pregnancy		4311	183/4207 (4%)	8/104 (8%)	0.10
Atopic family history		4433	2942/4324 (68%)	77/109 (70%)	0.57
Birthweight (grams) *n* = 4349	Median (10–90th centile)	4349	3420 (2730–4060)	3432 (2855–4150)	0.39
Premature (<37 weeks of gestation)		4163	244/4066 (6%)	6/97 (6%)	0.94
Born in winter months		4433	1107/4324 (26%)	27/109 (25%)	0.84
Breastfed >6 months of age		4229	2436/4129 (59%)	55/100 (55%)	0.42
Ever had artificial formula		4114	3197/4011 (80%)	73/103 (71%)	0.03
Early introduction of egg into diet	≤6 months of age	4248	1007/4142 (24%)	39/106 (37%)	0.003
Cat at the house	No	4427	3585/4318 (83%)	88/109 (81%)	
	Outside	4427	134//4318 (3%)	4/109 (4%)	
	Inside	4427	599/4318 (14%)	17/109 (16%)	0.82
Dog at the house	No	4430	2945/4321 (68%)	84/109 (77%)	
	Outside	4430	459/4321 (11%)	5/109 (5%)	
	Inside	4430	917/4321 (21%)	20/109 (18%)	0.07
Date of recruitment (dd/mm/yy)	Median (10–90th centile)	4433	07/12/09 (07/10/08–24/03/11)	23/09/09 (21/10/08–24/03/11)	0.15
Age at recruitment (days)	Median (10–90th centile)	4433	378 (365–411)	391 (352–448)	<0.001
Age of 1st dose of DTaP (days)	Median (10–90th centile)	4433	63 (56–73)	103 (91–216)	<0.001

DTaP, diphtheria–tetanus–acellular pertussis vaccination; SEIFA, Socio-Economic Indexes for Areas (19), included in multivariate models as quintiles. Atopic family history defined as any first-degree relative eczema, asthma, hay fever. Data are *n*/*N* (%) unless otherwise specified. *n* indicates the number of participants with complete data.

sensitivity analysis for eczema was performed by including all children regardless of age of eczema diagnosis. To investigate for reverse causation between eczema and delayed DTaP, a Cox regression model was constructed including time at risk from birth until the age of DTaP vaccination, and including time as exposed from the age of doctor-diagnosed eczema; the proportional hazards assumption was not violated ($P = 0.30$, Schoenfeld residuals).

Children with missing data on potential confounders were excluded from the multivariate regression analyses. *P* values less than 0.05 were considered statistically significant. The population sample size of 5000 was derived for recruitment into the original cohort (3). Given that DTaP data were available for 4433 children and 109 (2.5%) had a one-month delay in DTaP, the study ultimately had a power of 0.80 to detect a 73% reduction in food allergy associated with delayed vaccination in the unadjusted analysis. Statistical analyses were conducted in Stata version 11 (College Station, TX, USA).

Ethics and consent

The protocol for the original HealthNuts study was approved by the Human Research Ethics Committees at the Office for Children, Government of Victoria, the Department of Human Services, Government of Victoria, and at the Royal Children's Hospital, Melbourne. Approval for linkage with immunization data was granted by the Human Research Ethics Committee at the Royal Children's Hospital, Melbourne. Written information was provided to parents of participants with the option to opt out. The methodology, outcomes and analysis plan for this observational study were registered prior to data linkage with ANZCTR (trial ID ACTRN12614001193662).

Results

Background and demography

Of the 5276 children included in the original cohort, vaccination data were sought for 4834 (92%) with current contact details, and complete data on vaccinations were available for 4487 (85%) children (Fig. 1). There were differences between those with and without vaccination data available, including increased prevalence of food allergy and eczema amongst those with available vaccination data (Table S1).

Vaccination exposure and predictors of delayed vaccination

Overall, 4433 of 4487 (99%) children were recorded as having received a first dose of DTaP. Of these children, all received at least two doses and 4402 (99.3%) received all three doses. The first dose of DTaP was co-administered with IPV in 4415 children (99.6%), with hepatitis B vaccine in 4400 (99.3%), with Hib in 4416 (99.6%) (usually as part of *Infanrix Hexa*®), with PCV in 4396 (99.2%) and with rotavirus vaccine in 4173 (94.1%).

One hundred and nine children (2.5%) received the first dose of DTaP one month late (delayed DTaP). Median age of DTaP was 63 days in the on-time group and 103 days in the delayed DTaP group (Table 1). Vaccine timeliness improved somewhat over the period of the study; 70 (2.9%) of 2378 children born before 1/1/2009 had delayed DTaP, whereas 39 (1.9%) of 2055 children born after 1/1/2009 had delayed DTaP. Factors associated with delayed DTaP were older age at recruitment, lack of attendance at child care, having siblings, smokers at the home and never having had artificial formula (Table 1). Of these factors, only having siblings was also associated with food allergy (an inverse association, $P = 0.04$ for 1–2 siblings and $P = 0.001$ for ≥3 siblings) (2) and only smokers at the home was associated with eczema (an inverse association, $P = 0.02$).

Primary outcomes

There was no significant association between delayed DTaP and food allergy (adjusted odds ratio (aOR): 0.77; 95% CI: 0.36–1.62, $P = 0.49$, Table 2). There was no overall association between delayed DTaP and atopic sensitization (Table 2). In the preplanned sex-stratified analyses, females tended to have less atopic sensitization if they had delayed DTaP (aOR: 0.25; 0.06–1.04, $P = 0.06$), and this association tended to be different from the association in males (P for interaction = 0.09; Table 2). In a sensitivity analysis assuming food allergy status on the basis of skin prick wheal size amongst those with indeterminate food allergy status, the association between delayed DTaP and food allergy was similar (aOR: 0.87; 0.45–1.68, $P = 0.69$).

Secondary outcomes

Children with delayed DTaP had reduced odds of eczema compared to those vaccinated on time (aOR: 0.57; 0.34–0.97, $P = 0.04$, Table 3), with a similar magnitude of association in boys and girls (Table S2). Similarly, there was an association

Table 2 Association between timing of 1st dose of DTaP, food allergy and atopic sensitization

First-dose DTaP	Food allergy	OR (95% CI)	*P*	*n*	aOR (95% CI)	*P*
All children						
<1 month late	463/4041 (11%)					
≥1 month late	9/103 (9%)	0.74 (0.37–1.48)	0.39	3846	0.77 (0.36–1.62)	0.49
Females						
<1 month late	206/1998 (10%)					
≥1 month late	1/41 (2%)	0.22 (0.03–1.59)	0.14		0.23 (0.03–1.73)	0.15
Males						
<1 month late	256/2027 (13%)					
≥1 month late	8/62 (13%)	1.02 (0.48–2.18)	0.95		1.15 (0.50–2.63)	0.74
P for same effect in males and females			0.15			0.15

First-dose DTaP	Atopic sensitization	OR (95% CI)	*P*	*n*	aOR (95% CI)	*P*
All children						
<1 month late	734/4175 (18%)					
≥1 month late	13/107 (12%)	0.65 (0.36–1.16)	0.15	3971	0.66 (0.35–1.24)	0.19
Females						
<1 month late	348/2070 (17%)					
≥1 month late	2/44 (5%)	**0.24 (0.06–0.98)**	**0.05**		0.25 (0.06–1.04)	0.06
Males						
<1 month late	385/2087 (18%)					
≥1 month late	11/63 (17%)	0.94 (0.48–1.81)	0.84		1.00 (0.49–2.05)	0.99
P for same effect in males and females			0.09			0.09

DTaP, diphtheria–tetanus–acellular pertussis vaccination; OR, univariate odds ratio; aOR, odds ratio adjusted for sex, SEIFA quintile, siblings, antibiotic use, child-care attendance, smokers at the home and parent country of birth. *n* indicates the number of participants included in multivariate analyses. Results in boldface indicate $P < 0.05$.

Table 3 Association between timing of 1st dose of DTaP and secondary outcomes

	First-dose DTaP						
	< 1 month late	≥1 month late	OR (95% CI)	*P*	*n*	aOR (95% CI)	*P*
Eczema	1127/3735 (30%)	19/99 (19%)	**0.55 (0.33–0.91)**	**0.02**	3550	**0.57 (0.34–0.97)**	**0.04**
Eczema medication	961/3838 (25%)	12/99 (12%)	**0.41 (0.22–0.76)**	**0.004**	3725	**0.45 (0.24–0.83)**	**0.01**
Bronchiolitis admission	112/4160 (3%)	4/105 (4%)	1.43 (0.52–3.96)	0.49	4038	1.17 (0.41–3.33)	0.77
Wheeze ever	677/3786 (18%)	20/100 (20%)	1.15 (0.70–1.89)	0.59	3691	1.16 (0.69–1.95)	0.57

DTaP, diphtheria–tetanus–acellular pertussis vaccination; OR, univariate odds ratio; aOR, odds ratio adjusted for sex, SEIFA quintile, siblings, antibiotic use, child-care attendance, smokers at the home and parent country of birth. *n* indicates the number of participants included in multivariate analyses. Results in boldface indicate $P < 0.05$.

between delayed DTaP and reduced use of eczema medication (aOR: 0.45; 0.24–0.83, $P = 0.01$, Table 3). In a sensitivity analysis, including all children regardless of the age of eczema diagnosis made a minimal difference to these estimates (eczema: aOR 0.60; 0.37–0.96, $P = 0.03$; eczema medication: aOR 0.47; 0.27–0.82, $P = 0.008$). In *post hoc* analyses, there were no large differences in the association between delayed DTaP and eczema amongst subgroups (Table S3). There was no association between doctor-diagnosed eczema and subsequent delayed DTaP (hazard ratio: 0.98; 0.86–1.11, $P = 0.70$), giving no suggestion of reverse causation. There were no significant associations between delayed DTaP and bronchiolitis admissions or wheeze (Table 3).

Discussion

Overall, we found no significant associations between delayed DTaP and our primary outcomes of food allergy or atopic sensitization at one year of age. However, we found that children with their first dose of DTaP-containing vaccine delayed by one month had significantly reduced eczema and reduced use of eczema medication, even after accounting for a variety of potential confounding factors. Additionally, there was some evidence of a differential association by sex, where girls with delayed DTaP tended to have reduced atopic sensitization, whereas boys did not. Other vaccinations were invariably co-administered, including diphtheria, tetanus, IPV, hepatitis B, Hib, PCV, rotavirus; thus, if the observed associations are causal, it is unclear whether DTaP, another vaccine or a combination of vaccines, is responsible.

This study is the most comprehensive investigation into vaccinations and food allergy to date, and the lack of overall association between DTaP timing and food allergy is reassuring. However, this study was limited by statistical power as only 2.5% of children had delayed vaccination, considerably fewer than 4.9% found in a 2001 Australian cohort (21). This improvement in timeliness may be specific to the HealthNuts population or may represent a trend over time, especially given that the recent introduction of rotavirus vaccine has apparently increased timeliness of other co-administered vaccines (22).

Other important limitations exist. There was a bias towards participation amongst those with food allergy and eczema, and thus, population at risk of allergic diseases were slightly overestimated (1). However, the overall participation rate was high, and there is no reason to suggest that this participation bias would have affected our analysis of vaccination timing. Over 20% of eczema cases were excluded due to having a diagnosis of eczema prior to the age of scheduled vaccination, and thus, the eczema results presented here pertain only to eczema between three and 12 months of age. Importantly, our primary objective was to study timing of the acellular pertussis vaccine, but the almost invariable co-administration of other vaccinations means that we are unable to determine which component may be responsible for modifying risk of eczema. Furthermore, our findings are unable to attribute any risk of allergic disease to vaccination *per se* as all included children were vaccinated.

By comparing early *vs* late vaccination, we have eliminated confounding associated with reasons for receipt or refusal of vaccination. However, our results have potential to be confounded by reasons for delayed vaccination. Febrile episodes are associated with vaccination delay, but fever only after six months of age is linked to protection against allergic disease (23, 24), and it is unlikely that vaccination would be delayed by one month due to fever in many children. Rotavirus vaccine is often withheld when vaccination is delayed because of strict upper age limits for its use (22), but there was no evidence that differential vaccination with rotavirus vaccine affected the results. We investigated many other factors associated with vaccination delay (25) and found no evidence that confounding was responsible for the findings; however, we are unable to exclude residual confounding due to the observational design of the study, and therefore, causation cannot be ascribed. We have not identified any sources of bias that could explain these findings, and there was no association between early doctor-diagnosed eczema and a subsequent vaccination delay, indicating that reverse causation is unlikely.

The rationale behind this investigation was evidence that delayed administration of diphtheria–tetanus–whole-cell pertussis (DTwP) vaccination in infancy might reduce the subsequent risk of asthma (15), hay fever (13) and possibly atopic sensitization (14). Additionally, delayed DTwP vaccination provided a survival advantage for girls in a high mortality setting (26), while DTaP-IPV-Hib vaccination was a risk factor for infectious disease hospital admissions in a low mortality setting (27). The World Health Organization has recently acknowledged the importance of the nontargeted (also called nonspecific) effects of vaccinations and has called for further research into the epidemiological and underlying immunological mechanisms of such effects (28).

Various observational studies of pertussis vaccination and atopic disease have found protection, no association or increased risk associated with receipt of vaccination (6–9, 29). One international study reported reduction in eczema severity associated with pertussis vaccinations, with an apparent dose response to the total number of vaccinations received (30). Many of these studies did not report age of vaccination, and thus, heterogeneity in vaccination timing may have led to conflicting results in studies of pertussis vaccination *vs* no vaccination. No previous studies have examined the timing of pertussis vaccination and food allergy, and only one study examined eczema (16). In a large UK cohort, no association between DTwP-IPV timing and eczema was found (16); however, vaccination timing was divided into quartiles and age of vaccination was not reported; thus, it is unclear whether there was any practical difference in vaccination timing between groups. The association between delayed DTaP and eczema in the present study resembles a previous observation where delayed DTwP was associated with reduced risk of childhood asthma (15).

These findings need to be considered in the context of pertussis disease. In the USA, pertussis has a case fatality of 0.6% in two-month-old infants (31), and a single dose of acellular pertussis vaccine is highly protective against pertussis disease and pertussis mortality (31, 32). There is a movement to advance the age of the first pertussis vaccine to reduce pertussis morbidity and mortality in infants (33), and the primary immunization series is now encouraged from six weeks of age in Australia. If delayed vaccination is proven beneficial for allergic disease, such benefits would need to be carefully measured against the specific advantage of early vaccination in the relevant population (31, 33) while considering impact on other vaccines in the schedule (22). It should also be noted that Victoria has recently introduced a maternal pertussis immunization programme recommended in the third trimester of pregnancy (34), which may have implications on the immune response to the infant dose of pertussis vaccine and any future evaluations on vaccination timing and atopic disease.

While trained innate immunity and T-cell cross-reactivity may explain some nontargeted effects of vaccination in relation to susceptibility to infection (35, 36), mechanisms to explain altered risk of allergic disease are unknown. However, T helper (T_H) 2 stimulation by vaccinations could theoretically offer an explanation, as acellular pertussis vaccines are strong T_H2 stimulants (32, 37, 38) and T_H2 polarization is associated with the development of food allergy and atopic eczema. Components of the *Bordetella pertussis* cell wall may have an inhibitory role on the development of IgE in relation to other vaccine components (39), suggesting that DTaP, which lacks the cellular components, has greater potential to be allergenic than DTwP. Also promoting a T_H2 response to nonvaccine antigens are pneumococcal vaccines (40) and aluminium adjuvant (41), used in both DTaP and pneumococcal vaccines. Therefore, multiple components of the primary immunization series are theoretically capable of initiating a generalized T_H2 bias.

Staphylococcus aureus colonization is implicated in the pathogenesis of infant eczema, as infants with eczema have excessive T_H2 cytokine responses to staphylococcal superantigens (42). Thus, it is possible that a T_H2 stimulant such as DTaP or another vaccine component given during a critical window in infancy could have a bystander effect whereby predisposed infants who are colonized with *S. aureus* become sensitized to staphylococcal superantigens and develop eczema, without having the stronger T_H2 bias required to cause other allergic diseases (42).

In conclusion, delayed DTaP vaccination was not associated with food allergy or atopic sensitization overall, but was associated with less eczema and less use of eczema medication. Additionally, there was a borderline association between delayed DTaP and reduced atopic sensitization amongst girls. This finding is consistent with the observation that the nontargeted effects of vaccines tend to be sex differential with females generally being more susceptible than males (43). These results warrant further investigation. Timing of routine immunizations in infancy may affect susceptibility to allergic disease.

Acknowledgments

The authors thank the parents and children participating in the HealthNuts study, the staff at Melbourne's local council immunization clinics, ALK-Abelló SA, Madrid, Spain, for supplying the skin prick testing reagents, Thao Nguyen (SAEFVIC, Murdoch Childrens Research Institute) for invaluable help with data acquisition, Dr Paul Geil (School of Physics, University of Melbourne) for expert assistance with data processing, Megan Mathers for generous administrative assistance and the HealthNuts safety committee: Associate Professor Noel Cranswick (Australian Paediatric Pharmacology Research Unit/Murdoch Childrens Research Institute), Dr Jo Smart (Department of Allergy and Immunology, Royal Children's Hospital, Melbourne, Australia) and Associate Professor Jo Douglass (Head of Allergy, Alfred Hospital, Melbourne, Australia). The complete HealthNuts study group are gratefully acknowledged: Deborah Anderson, Nadine Bertalli, Maia Brewerton, Sonia Chhabra, Hayley Crawford, Helen Czech, Thanh Dang, Terry Dwyer, Jana Eckert, Justine Ellis, Manuel Ferreira, Carley Garner, David Hill, Paul Licciardi, Pamela Martin, David Martino, Melanie Matheson, John Molloy, Nicholas Osborne, Rachel L Peters, Susan Prescott, Colin Robertson, Marnie Robinson, Richard Saffery, Jeeva Sanjeevan, Holly Shaw, Noor Suaini, Tina Tan, Dean Tey, Leone Thiele, Kate Tilbrook, Kaye Trembath, Peter Vuillermin, Giovanni Zurzolo.

Author contributions

All authors interpreted the results, critically reviewed and approved the final manuscript as submitted. NK conceptualized

and designed this data linkage study, coordinated collection of vaccination data, analysed the data and drafted the manuscript. JJK is a co-investigator for the original HealthNuts study, designed this data linkage study and assisted with statistical analyses. NWC coordinated vaccination data collection. SB assisted with data management and processing. LCG is a co-investigator for the original HealthNuts study and assisted with statistical analyses. AJL, MLKT, MW and A-LP are all co-investigators of the original HealthNuts study. SCD is a co-investigators of the original HealthNuts study and assisted with study design and statistical analyses. KJA is the principle investigator of the original HealthNuts study and conceptualized and designed this data linkage study.

Funding

The HealthNuts study was funded by the National Health and Medical Research Council of Australia, the Ilhan Food Allergy Foundation, AnaphylaxiStop, the Sylvia and Charles Viertel Charitable Foundation and the State Government of Victoria's Operational Infrastructure Support Program.

Conflict of interest

The authors declare that they have no conflicts of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1 Australian National Immunisation Program Schedule over the birth years of the HealthNuts cohort.

Table S1 Comparison of demography for children included and excluded from the current analysis of vaccination timing in the HealthNuts cohort

Table S2 Association between delay in 1st dose of DTaP and secondary outcomes in females and males

Table S3 Association between delayed 1st dose of DTaP and eczema amongst subgroups

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