Review

Experience with monocomponent acellular pertussis combination vaccines for infants, children, adolescents and adults—A review of safety, immunogenicity, efficacy and effectiveness studies and 15 years of field experience☆

Birgit Thierry-Carstensen [a,*], Tine Dalby [a], Michael A. Stevner [a], John B. Robbins [b], Rachel Schneerson [b], Birger Trollfors [c]

[a] Statens Serum Institut, 5 Artillerivej, 2300 Copenhagen S., Denmark
[b] National Institute of Child Health & Human Development, National Institutes of Health, Bethesda, MD 20892, USA
[c] Department of Pediatric Medicine, Sahlgrenska University Hospital-East, 416 85 Gothenburg, Sweden

ARTICLE INFO

ABSTRACT

Combination vaccines containing a monocomponent acellular pertussis (aP) vaccine, manufactured at Statens Serum Institut (SSI), Denmark, have successfully controlled *Bordetella pertussis* infections in Denmark since 1997. The efficacy of this aP vaccine was 71% in a double-blind, randomised and controlled clinical trial. Its safety and immunogenicity have been demonstrated in infants, children, adolescents and adults. In approximately 500,000 children it was effective against pertussis requiring hospitalisation (VE: 93% after 3 doses) and against pertussis not requiring hospitalisation (VE: 78% after 3 doses). IgG antibodies against pertussis toxin (IgG anti-PT) response rates after booster vaccination of adults with tetanus, diphtheria and aP combination vaccine (TdaP) were considerably higher for this monocomponent aP vaccine containing 20 µg pertussis toxoid, inactivated by hydrogen peroxide (92.0%), than for two multi-component aP vaccines inactivated by formaldehyde and/or glutaraldehyde: 3-component aP with 8 µg pertussis toxoid (77.2%) and 5-component aP with 2.5 µg pertussis toxoid (47.1%), without compromising the safety profile. In Denmark where this monocomponent aP vaccine has been the only pertussis vaccine in use for 15 years, there has been no pertussis epidemic since 2002 (population incidence 36 per 100,000), in contrast to neighbouring countries, where epidemics have occurred. This monocomponent aP vaccine can be used in combination vaccines for primary and booster vaccination against pertussis in all age groups and is an important tool for successful pertussis control.

Contents

* Corresponding author. Tel.: +45 3268 3598; fax: +45 3268 3872.
  E-mail address: btc@ssi.dk (B. Thierry-Carstensen).

1. Introduction

This review summarises experience from clinical trials and post-marketing data of a monocomponent aP vaccine which contains pertussis toxoid in a less denatured form and at a higher dosage than other aP vaccines in use [1,2]. In spite of the introduction of universal infant vaccination [3], pertussis remains one of the leading causes of vaccine preventable deaths with an estimated 50 million cases and 300,000 deaths occurring worldwide annually. Over the past two decades, there has been a modest but steady increase in reports of pertussis incidence in Europe, the USA and other parts of the World [4,5]. This increase in incidence is mainly seen in adolescents and adults [6]. Several possible reasons for the apparent increase in incidence have been suggested [6]: low vaccine coverage in infants, insufficient administration of booster doses, increased awareness of pertussis, availability of new diagnostic tools, decreased chance of natural boosting in the vaccination era, reduced longevity or quality of the immune responses induced by the acellular pertussis (aP) vaccines currently in use, and lastly new circulating genotypes of *Bordetella pertussis* (*B. pertussis*) [5,7,8]. aP vaccines contain from one to five pertussis antigens. It is generally agreed that antibodies against pertussis toxin (anti-PT) play a major role in protection [9–11] and all aP vaccines contain pertussis toxoid. In addition to pertussis toxoid, multicomponent aP vaccines contain varying amounts of filamentous hemagglutinin (FHA), pertactin (PRN) and/or fimbriae (FIM) types 2 and 3. The roles of the additional antigens in protection against pertussis, and the possible advantages obtained are subject of debate [9–11]. Interestingly, the monocomponent aP vaccine, which is the subject of this review, has successfully controlled *B. pertussis* infections in Denmark, at least as effectively as in neighbouring countries where multicomponent aP vaccines are used, supporting that pertussis toxoid is both essential and sufficient for a pertussis vaccine [10,12].

2. Development history and aP vaccine particulars

The pertussis toxoid in the Danish aP vaccine is inactivated by hydrogen peroxide, whereas other aP vaccines are inactivated using formaldehyde and/or glutaraldehyde. It has been shown that chemical inactivation of pertussis toxin by hydrogen peroxide results in a lower degree of epitope impairment [1,2] and thus in a better immunogen. The development of the Danish vaccine, presently manufactured at Statens Serum Institut (SSI), Denmark, was initiated in the 1980s at the National Institute of Child Health and Human Development (NICHD), National Institute of Health (NIH), Bethesda, MD, USA [13]. It was found to be safe and immunogenic in initial dosage finding trials in adults [14], 18–24-months-old children [15], and in infants [1,16]. After these early trials, the efficacy was demonstrated in Gothenburg, Sweden, during 1991–94 [17],

Table 1 and Section 3. The selected dosage of 40 μg pertussis toxoid for the efficacy trial has subsequently remained unchanged in combination vaccine formulations for primary vaccination, whereas 20 μg has been established as sufficient for booster vaccination. All SSI combination vaccines are adsorbed to aluminium hydroxide and contain no preservative. During the last decade, the clinical development has continued with safety and immunogenicity trials in infants [18–20], children [21–23], adolescents [24] and adults [25,26] in various countries, Table 1 and Sections 4 and 5. The first regulatory approvals were obtained in 1996 in Denmark and Sweden, followed by other European countries in 1997 and the USA in 1998, and recently a booster formulation was approved for vaccination of children, adolescents and adults in 12 European countries. In Denmark combination vaccines containing monocomponent aP have been routinely used for primary vaccination of infants at 3, 5 and 12 months of age since 1997, and for booster vaccination of pre-school children since 2003. The results of two large post-marketing effectiveness studies [27,28] are presented in Table 1 and Section 6, and a description of the epidemiological situation of pertussis in Denmark is given in Section 7. In the following, DTaP refers to combination vaccines intended for primary vaccination with full doses of diphtheria (D), Tetanus (T) and pertussis (aP) antigens, with or without inactivated poliovirus vaccine (IPV) and *Haemophilus influenzae* type b conjugate vaccine (PRP-T). TdaP refers to combination vaccine formulations for booster vaccination where the diphtheria (d) and pertussis (aP) dosages are reduced, except in the dosage finding trials [22,23] where TdaP was investigated in dosages of 20 and 40 μg pertussis toxoid.

3. Vaccine efficacy of monocomponent aP

3.1. Double-blind, randomised, controlled trial, DTaP (SSI)

The efficacy of the monocomponent aP vaccine was demonstrated in a double-blind, randomised and placebo controlled trial with DTaP (SSI), in Gothenburg during 1991–94, when pertussis was endemic in Sweden [17], Study A, Table 1. After active and double-blind follow-up for pertussis for a median of 17.5 months, the efficacy was 71%, (95% CI: 63–78%) defining a pertussis case according to the WHO definition recommended at that time. In fully DTaP vaccinated subjects with or without pertussis in the subsequent follow-up period, but with no pertussis ± 2 months from the time of sampling, the one month post-3rd vaccination geometric mean concentration (GMC) of IgG anti-PT was 145 IU/mL, Study A. At the end of the double-blind follow-up period, trial subjects who were still at risk of pertussis (i.e. pertussis cases were considered no longer at 'risk' of pertussis from the first day of cough) were followed during an open extension follow-up period of 6 months where the vaccine efficacy was 77%, (95% CI: 65–85%) [29], Study A.

Table 1

Tabular overview of trials investigating the safety, efficacy, immunogenicity and postmarketing and effectiveness studies with monocomponent aP vaccine in infants, children, adolescents and adults. Including references, design, trial population, vaccination schedules, content of pertussis toxoid (PT, μg) in the vaccines; pertussis case definitions, number of included subjects (N), IgG anti-PT ELISA assay results or toxin neutralising anti-PT CHO cell assay results, IgG anti-PT GMCs, booster response, seroconversion and/or seropositivity rates, frequencies of injection site redness, swelling, pain and fever and other results or comments.

Study A: Efficacy trial – pivotal results, DTaP (SSI)

Reference, country & year	Design	Pertussis case definition	Population & schedule	Vaccines (PT, μg)	Trial subjects; N	Efficacy results Cases; n (%) Efficacy (95% CI)	1 mth post-vaccination GMC IgG anti-PT in DTaP × 3 serology subset		Other results/comments
							ELISA	CHO cell	
Trollfors et al. [17] Taranger et al. [29] Sweden 1991–94	- Double-blind, randomised, placebo-controlled - Double-blind follow-up (median 17.5 mth) from 1 mth after 3rd dose to 24 July 94 - Open extended follow-up from 25 July 94 to 31 Jan 95	WHO case definition: Trial subject: - Paroxysmal cough ≥21 days Laboratory confirmation: - Culture positive trial subject or family member - Statistically significant increase in serum anti-PT or anti-FHA in trial subject	Infants 3, 5, 12 mth	DTaP (40) DT DTaP (40) DT	Double-blind: 1724 1726 Open extended: 1530 1389	Double-blind: 72 cases (4.2%) 240 cases (13.9%) Efficacy: 71% (63–78) Open extended: 29 cases (1.9%) 110 cases (7.9%) Efficacy: 77% (65–85)	145 IU/mL (n = 566) - Subjects with or without pertussis in the subsequent follow-up period, but with no pertussis ±2 months from the time of sampling	240 (titre) (n = 488)	- In the DTaP × 3 serology subset the 1 mth post-vaccination anti-PT GMC was higher in subjects with no pertussis during follow-up (153 IU/mL) than in cases (96.9 IU/mL) (p = 0.001) - In cases, DTaP vaccinees had milder disease than DT vaccinees

Study B: Efficacy trial, household exposure after 2 or 3 doses, DTaP (SSI)

Reference, country & year	Design	Pertussis case definition	Population & schedule	Vaccines (PT, μg)	Trial subjects Household exposed; N	Efficacy results Cases, secondary; n (%) Efficacy (95% CI)	Other results/comments
Trollfors et al. [30] Sweden 1991–94	- Double-blind, randomised, placebo-controlled - Double-blind follow-up from day of 2nd dose to 24 July 94	Trial subject (household exposed): - WHO case definition - Onset of pertussis 6-60 d after onset of primary case Household contact (primary case): - Pertussis category 1, 2 or 3 as defined in [30]	- Infants 3, 5, 12 mth - Trial subjects household exposed after 2 or 3 doses	DTaP (40) DT DTaP (40) DT	After 2 doses: 32 35 After 3 doses: 99 79	After 2 doses: 4 cases (13%) 13 cases (37%) Efficacy: 66% (15–90) After 3 doses: 20 cases (20%) 64 cases (81%) Efficacy 75% (64–84)	DTaP provides protection against pertussis both after household and community exposure

Study C: Efficacy trial, correlation of post-vaccination anti-PT level and protection, DTaP (SSI)

Reference, country & year	Design	Pertussis case definition	Population & schedule	Vaccines (PT, μg)	1 mth post-vaccination GMC IgG anti-PT by ELISA in DTaP × 3 serology subset	Other results/comments
Taranger et al. [32] Sweden 1991–95	- Double-blind, randomised, placebo-controlled - Double-blind and open follow-up (21–33 mth) from 1 mth after 3rd dose to 31 March 95	Trial subject (not household exposed): - Severe pertussis: Paroxysmal cough ≥21 d - Mild pertussis: Paroxysmal cough <21 d Trial subject (household exposed): - Onset of severe or mild pertussis 6–60 d after onset of primary case Laboratory confirmation severe & mild pertussis: - Culture positive trial subject or family member - Statistically significant increase in serum anti-PT or anti-FHA in trial subject	- Infants 3, 5, 12 mth - DTaP × 3 serology subset - Not household exposed or household exposed	DTaP (40)	Not household exposed (N = 687): A: Severe pertussis (n = 85), 102 IU/mL B: Mild pertussis (n = 37), 115 IU/mL C: No pertussis (n = 566), 150 IU/mL A vs C (p < 0.0001) Household exposed (N = 126): A: Severe pertussis (n = 27), 79 IU/mL B: Mild pertussis (n = 24), 143 IU/mL C: No pertussis (n = 75), 212 IU/mL A vs C (p < 0.0001)	- Highly statistically significant correlation between post-vaccination anti-PT GMC and protection against pertussis during follow-up - 1 mth post-vaccination GMC of IgG anti-PT may be used as correlate of protection

ARTICLE IN PRESS

Table 1 (Continued)

Study D: Safety & immunogenicity trial in infants, primary vaccination 3, 5, 12 mths, DTaP-IPV (SSI)

Reference, country & year	Design	Population & schedule	Vaccines (PT, μg)	Trial subjects, N	Post-vaccination GMC IgG anti-PT by ELISA		Post-vaccination anti-PT seropositives (≥4 IU/mL)		Safety results: % subj with redness/swelling/fever 48 h after 3rd ds; DTaP-IPV vs wP	Other results/ comments
					After 1 mth	24 mth of age	After 1 mth	24 mth of age		
Gyhrs et al. [18] Denmark 1993–96	Open, randomised, controlled	- Infants 3, 5, 12 mth - Infants 5 wk, 9 wk 10 mth	DTaP-IPV (40)	186	267 IU/mL (n = 117)	34.5 IU/mL (n = 75)	100% (n = 117)	97.3% (n = 75)	Redness ≥2 cm: 53.6% (n = 168) Swelling ≥2 cm: 29.8% (n = 168) Fever ≥38.0 °C: 18.5% (n = 168)	DTaP-IPV was concluded to be safe and immunogenic in infants
			wP	84	11.1 IU/mL (n = 26)	6.8 IU/mL (n = 18)	73.1% (n = 26)	55.6% (n = 18)	Redness ≥2 cm: 59.0% (n = 61) Swelling ≥2 cm: 44.3% (n = 61) Fever ≥38.0 °C: 42.6% (n = 61)	

Study E: Safety & immunogenicity trial in infants, primary vaccination 3, 5, 12 mths, DTaP-IPV (SSI)

Reference, country & year	Design	Population & schedule	Vaccines (PT, μg)	Trial, subjects, N	1 mth post-vaccination GMC IgG anti-PT by ELISA		Post-vaccination anti-PT seropositives (≥10 IU/mL)		Safety results: % subj with redness/swelling/fever 7 d after 3rd ds/PRP-T vs +PRP-T	Other results/ comments
					After 2nd ds	After 3rd ds	After 2nd ds	After 3rd ds		
Knutsson et al. [19] Sweden 1997–99	Open, randomised, controlled	Infants 3, 5, 12 mth	DTaP-IPV (40)/PRP-T	204	127 IU/mL (n = 185)	261 IU/mL (n = 192)	100% (n = 185)	100% (n = 192)	Redness ≥2 cm: 13.3% (n = 203) Swelling ≥2 cm: 16.3% (n = 203) Fever ≥ 38.0 °C: 24.0% (n = 203)	Mixing caused moderate impairment of PRP-T response, not clinically significant, in infants
			DTaP-IPV (40) (+PRP-T)	203	121 IU/mL (n = 188)	236 IU/mL (n = 185)	100% (n = 188)	100% (n = 185)	Redness ≥2 cm: 24.9% (n = 197) Swelling ≥2 cm: 24.9% (n = 197) Fever ≥38.0 °C: 17.0% (n = 197)	

Table 1 (*Continued*)

Study F: Safety & immunogenicity trial in infants, primary vaccination 2, 3½, 5 and 16 mths, DTaP-IPV (SSI)

Reference, country & year	Design	Population & schedule	Vaccines (PT, μg)	Trial subjects, N	1 mth post-vaccination GMT anti-PT by CHO cell assay		Post-vaccination anti-PT seropositives (titre ≥4)		Safety results: % subj with redness/swelling/fever 72 h after any ds; Vero vs Mkc	Other results/ comments
					After 3rd ds	After 4th ds	After 3rd ds	After 4th ds		
Pietrzyk et al. [20] Poland 2003–05	Double-blind, randomised, controlled, non-inferiority trial	Infants 2, 3½, 5, 16 mth	DTaP-IPV (40) Vero	410	42.1 (titre) ($n=402$)	205 (titre) ($n=389$)	100% ($n=402$)	100% ($n=389$)	Redness ≥2 cm: 16.5% ($n=400$) Swelling ≥2 cm: 12.3% ($n=400$) Fever ≥38.0 °C: 31.0% ($n=410$)	The safety and immunogenicity of DTaP-IPV$_{Vero}$ was demonstrated to be non-inferior to DTaP-IPV$_{Mkc}$ in infants
			DTaP-IPV (40) Mkc	407	41.6 (titre) ($n=404$)	187 (titre) ($n=389$)	100% ($n=404$)	100% ($n=389$)	Redness ≥2 cm: 12.9% ($n=402$) Swelling ≥2 cm: 10.4% ($n=402$) Fever ≥38.0 °C: 35.1% ($n=407$)	

Study G: Safety & immunogenicity trial in children, booster vaccination 4–6-years, DTaP (Certiva)

Reference, country & year	Design	Population & schedule	Vaccines (PT, μg)	Trial subjects, N	Pre & post-vaccination GMC IgG anti-PT by ELISA		Anti-PT booster response (≥×4 rise & post ≥4 IU/mL): Anti-PT seroconversion (≥×4 rise):	Safety results: % subj with redness/swelling 7 d after booster ds	Other results/ comments
					Pre	Post			
Blatter et al. [21] USA 1995–96 Unpublished	Open, non-controlled	- Children 4–6 yr, booster vaccination- Previously wP at 2, 4, 6 & wP or aP at 12-24 mth	DTaP (40) - Certiva	222	3.7 IU/mL ($n=211$)	97 IU/mL ($n=211$)	Booster response: 93% ($n=211$) Seroconversion: 95% ($n=211$)	Redness ≥6 cm: 1.0% ($n=222$) Swelling ≥6 cm: 1.4% ($n=222$)	Booster vaccination with DTaP was safe and immunogenic in children

Table 1 (*Continued*)

Study H: Safety & immunogenicity trial in children, booster vaccination 6-years, TdaP (20 & 40), TdaP-IPV (20 & 40) (SSI)

Reference, country & year	Design	Population & schedule	Vaccines (PT, μg)	Trial subjects, N	Pre & post-vaccination GMC IgG anti-PT by ELISA		Anti-PT seropositive (post ≥4 IU/mL): Anti-PT seroconversion (≥×4 rise):	Safety results: % subj with pain/redness/ swelling 14 d after booster ds	Other results/ comments
					Pre	Post			
Taranger et al. [22] Sweden 1997–99 Unpublished	Open, randomised, controlled, non-inferiority trial	- Children 5–6 yr, booster vaccination - Previously monocomponent aP at 3, 5, 12 mth	TdaP (20)	162	6.8 IU/mL (n = 161)	223 IU/mL (n = 152)	Seropositive: 99.3% (n = 152) Seroconversion: 97.4% (n = 152)	Pain (any): 55.6% (n = 162) Redness ≥6 cm: 20.4% (n = 162) Swelling ≥6 cm: 9.9% (n = 162)	- Anti-PT booster response of TdaP (20) non-inferior to TdaP (40) in children - Lower frequency injection site redness/swelling for TdaP (20) than TdaP (40) - Anti-PT GMC ratio of 32.8 from pre- to post-vaccination for TdaP (20)
			TdaP (40)	168	10.8 IU/mL (n = 168)	287 IU/mL (n = 166)	Seropositive: 100% (n = 166) Seroconversion: 98.2% (n = 166)	Pain (any): 63.1% (n = 168) Redness ≥6 cm: 30.4% (n = 168) Swelling ≥6 cm: 19.6% (n = 168)	
			TdaP-IPV (20)	148	10.7 IU/mL (n = 147)	222 IU/mL (n = 143)	Seropositive: 100% (n = 143) Seroconversion: 97.9% (n = 143)	Pain (any): 48.7% (n = 148) Redness ≥6 cm: 23.7% (n = 148) Swelling ≥6 cm: 10.8% (n = 148)	
			TdaP-IPV (40)	142	11.6 IU/mL (n = 142)	243 IU/mL (n = 141)	Seropositive: 100% (n = 141) Seroconversion: 95.7% (n = 141)	Pain (any): 64.8% (n = 142) Redness ≥6 cm: 27.5% (n = 142) Swelling ≥6 cm: 14.1% (n = 142)	

Study I: Immunogenicity trial in children, booster vaccination 10-years, TdaP (20 & 40) (SSI)

Reference & country	Design	Population & schedule	Vaccines (PT, μg)	Trial subjects, N	Pre & post vaccination median IgG anti-PT by ELISA Subject stratification by:		Other results/comments
					Pre < LOQ	Pre ≥ LOQ	
Trollfors et al. [23] Sweden	Open, randomised, controlled	- Children 10 yr, booster vaccination - Previously wP, aP or pertussis	TdaP (20)	166	Pre: <1 IU/mL (n = 30) Post: 16.5 IU/mL (n = 30)	Pre: 18 IU/mL (n = 136) Post: >400 IU/mL (n = 136)	- 10-yr olds with non-detectable antibodies before the booster vaccination had lower post-vaccination levels - The addition of aP to the Td vaccine did not affect anti-tetanus and anti-diphtheria antibody responses - An age of 10 yr for the first booster dose after primary DTaP vaccination may be to high
			TdaP (40)	170	Pre: <1 IU/mL (n = 21) Post: 36 IU/mL (n = 21)	Pre: 16 IU/mL (n = 149) Post: >400 IU/mL (n = 149)	
			Td	166	Pre: <1 IU/mL (n = 20) Post: <1 IU/mL (n = 20)	Pre: 16.5 IU/mL (n = 146) Post: 16 IU/mL (n = 146)	

Table 1 (*Continued*)

Study J: Safety & immunogenicity trial in adolescents, booster vaccination 15–16-years, TdaP (20) (SSI)

Reference, country & year	Design	Population & schedule	Vaccines (PT, μg)	Trial subjects, N	Pre & post-vaccination GMC IgG anti-PT by ELISA		Anti-PT booster response (≥×2 rise & post ≥4 IU/mL):	Safety results: % subj with pain/redness/swelling 14 d after booster ds	Other results/ comments
					Pre	Post			
Netterlid et al. [24] Sweden 2009 In publication	Open, randomised, controlled	- Adolescents 14–15 yr, booster vaccination - Previously 5-comp. aP at 3, 5, 12 mth & 5-comp. aP at 5½ yr	1-comp. TdaP (20)	115	2.9 IU/mL (n = 113)	74.2 IU/mL (n = 113)	Booster response: 95.6% (n = 113)	Pain (severe): 0.9% (n = 113) Extensive redness/swelling: 2.7% (n = 113)	Both TdaP vaccines were safe and immunogenic given as a 5th dose to adolescents
			5-comp. TdaP	115	In publication	In publication	Booster response: 87.7% (n = 113)	Pain (severe): 7.1% (n = 113) Extensive redness/swelling: 2.7% (n = 113)	

Study K: Safety & immunogenicity trial in adults, booster vaccination, TdaP (20) (SSI)

Reference, country & year	Design	Population & schedule	Vaccines (PT, μg)	Trial subjects, N	Pre & Post-vaccination GMC IgG anti-PT by ELISA		Anti-PT booster response (pre <20 IU/mL: ≥×4 rise; pre ≥20 IU/mL: ×2 rise): Anti-PT seropositive (post ≥5 IU/mL):	Safety results: % subj with pain/redness/swelling 28 d after booster ds	Other results/comments
					Pre	Post			
Thierry-Carstensen et al. [25] Denmark 2010	Double-blind, randomised, controlled, non-inferiority trial	- Adults, booster vaccination - Previously wP according to Danish schedule	TdaP (20)	401	6.9 IU/mL (n = 401)	122 IU/mL (n = 401)	TdaP (20): Booster response: 92.0% (n = 401)	Pain (severe): 0.7% (n = 401) Redness ≥5 cm: 0.7% (n = 401) Swelling ≥5 cm: 1.5% (n = 401)	- T and d immunogenicity of TdaP non-inferior to Td in adults - No differences in safety profile of TdaP (20) and Td
			Td	401	7.0 IU/mL (n = 399)	7.3 IU/mL (n = 399)	Seropositive: 98.3% (n = 401) GMC (rise): 17.7 (n = 401)	Pain (severe): 2.2% (n = 401) Redness ≥5 cm: 0.7% (n = 401) Swelling ≥5 cm: 1.5% (n = 401)	

Study L: Effectiveness and herd immunity – a massvaccination study, DTaP & aP (SSI)

Reference, country & year	Design	Pertussis case definition	Population & schedule	Vaccines (PT, μg)	Effectiveness results/herd immunity	Other results/comments
Taranger et al. [28] Trollfors et al. [43] Sweden 1995–99	- Open, prospective study with historic control - Gothenburg population (778,597) with pertussis, no vaccination 1975–95	The effect of vaccination on the incidence of pertussis evaluated by numbers of positive cultures and numbers of hospitalisations due to pertussis	Infants: 3, 5, 12 mth Target population: Children (≥1yr; up to 10 yr): 3 doses with 2 & 6 mth interval - Exclusion of subjects with a history of pertussis	DTaP/aP (40) - DTaP replacement by aP according to number of previous DT doses - 167,810 aP (40) doses administered to 61,219 subjects in target population	In target population: - Mean number of culture positives declined from 1214 per yr (1993–95) to 64 (1997–Jun 99 (p < 0.0001)) - Hospitalisations due to pertussis in same periods declined from 62 per yr to 6 (p < 0.0001) In all age groups of population: - Significant decreases in pertussis incidences in unvaccinated infants (<6 mth) and in adults (≥15 yr.) i.e. herd immunity	- In DTaP/aP × 3 serology subset post-vaccination GMC IgG anti-PT by ELISA was 198 IU/mL (N = 481) in infants - Mass vaccination decreased spread of *B. pertussis* in the population

Table 1 (Continued)

Study M: Effectiveness in Danish vaccination programme – a cohort study, DTaP-IPV (SSI)

Reference, country & year	Design	Pertussis case definition	Population & schedule	Vaccines (PT, µg)	Vaccine effectiveness (VE) results: Non-hospitalised pertussis		Hospitalised pertussis		Other results/comments
					Cases, n	VE, %	Cases, n	VE, %	
Hviid et al. [27] Denmark 1997–2001	Cohort study, linking vaccination history and cases of pertussis from a notification report system and a national hospital register	Vaccination effectiveness (VE = 1 − RR) against non-hospitalised pertussis and hospitalised pertussis according to number of received aP doses	- Infants 3, 5, 12 mth - Cohort born from 1 Jan 1994 to 31 Dec 2001	DTaP-IPV (40) Unvaccinated 1 dose 2 doses 3 doses	n = 85 n = 80 n = 95 n = 43	- 35% 59% 78%	n = 348 n = 110 n = 38 n = 5	- 37% 72% 93%	- For the VE analysis a total of 314 cases of non-hospitalised and 517 cases of hospitalised pertussis were identified during 529,861 person years

3.2. Household contact and transmission studies, DTaP (SSI)

The efficacy of DTaP under household exposure was investigated in 245 infants in the efficacy trial [30], Study B, Table 1. Included were infants who had secondary pertussis starting 6–60 days after the onset of a primary case of pertussis in their household. The vaccine efficacy was 66% in infants exposed to pertussis from the day of the 2nd vaccination and 75% in infants exposed from the day of the 3rd vaccination. Moreover, in a transmission study of parents and younger siblings of the efficacy trial participants, it was demonstrated that DTaP reduced the spread of pertussis to close contacts [31], a prerequisite for herd immunity.

3.3. Correlation between IgG anti-PT levels and protection against pertussis, DTaP (SSI)

In an extension study to the efficacy trial the correlation between the one month post-vaccination GMC of IgG anti-PT and protection against pertussis was investigated in fully DTaP vaccinated infants from whom a serum sample was available [32], Study C, Table 1. The follow-up period included double-blind and open follow-up for 21 to 33 months. A statistically significant correlation between the one month post-vaccination GMC of IgG anti-PT and subsequent protection against severe pertussis (paroxysmal cough $\geq$21 days) was demonstrated both in non-household exposed ($p < 0.0001$) and in household exposed ($p < 0.0001$) infants, Study C.

3.4. Interpretation of results of efficacy trials with aP vaccines

The efficacy trials with the aP vaccines, monocomponent and multicomponent, conducted in the 1990s have left important questions unresolved. Most importantly, none of the three double-blind, randomised and controlled efficacy trials [17,33,34] were designed to answer the question of how many components an aP vaccine should contain. Moreover the fact that all multicomponent aP vaccines contain FHA introduced a bias, as paired FHA serology was used for laboratory confirmation of pertussis cases in the trials. The bias was caused by the presence of vaccine induced anti-FHA in acute phase sera from multicomponent aP recipients, reducing the likelihood of statistically significant increases in anti-FHA between acute and convalescent phase sera, resulting in missed pertussis cases and artificially high efficacy estimates for the multicomponent aP vaccines. Correction of this bias can be done by excluding pertussis cases confirmed solely by paired FHA serology from the efficacy calculations. Calculated this way the efficacy of monocomponent aP is 78% [17,35].

In a review by Martha Granström [36] the corrected efficacies for the mono-, three-and five-component aP vaccines investigated in the three double-blind, randomised and controlled efficacy trials [17,33,34] were all estimated to be approximately 80%.

4. Safety in infants, children, adolescents and adults

Adverse reactions to vaccination were compared among DTaP vaccines. The multicentre acellular pertussis trial (MAPT) performed in the 1990s investigated the safety of 13 DTaP vaccines in infants. Monocomponent DTaP (SSI) was not included in the original MAPT, but was subsequently tested according to the same protocol [37]. Although there were differences among the DTaP vaccines, none was consistently the most or least reactogenic; however, all were associated with substantially fewer and less severe adverse reactions than the DTwP vaccines [37]. The reactogenicity of DTaP combination vaccines has been shown to increase with the number of received doses, mainly attributed to the D and aP components [38]. To decrease reactogenicity, the amounts of diphtheria

and pertussis antigens in TdaP and TdaP-IPV formulations intended for booster vaccination have been reduced. A broad range of clinical trials have documented the safety of these booster formulations in populations of children, adolescents and adults [39–41]. In the safety and immunogenicity trials [18–20,22,24,25] with combination vaccines containing monocomponent aP reviewed below, no vaccine related serious adverse events were reported.

4.1. Safety of DTaP-IPV and DTaP-IPV/PRP-T (SSI) in infants

The safety profiles of the DTaP-IPV (SSI) and DTaP-IPV/PRP-T (SSI) primary combination formulations in Danish, Swedish and Polish infants [18–20] are shown in Table 1, Studies D, E and F. The frequencies of injection site redness and swelling and of fever were lower in DTaP-IPV recipients than in wP recipients [18], STUDY D. Approximately 15% of the infants who completed the primary vaccination schedules with DTaP-IPV or DTaP-IPV/PRP-T experienced injection site redness and/or swelling ≥ 2 cm and 20–30% experienced fever $\geq 38.0\,^{\circ}$C [18–20], Studies D, E and F. In Study F it was demonstrated that the frequencies of redness and swelling increased with the number of DTaP-IPV dose [20]. In Study D there was one case of persistent crying ≥ 3 h, whereas in Study F three such cases were reported [18,20].

4.2. Safety of TdaP and TdaP-IPV (SSI) in children

Swedish pre-school children who had previously received 3 doses of DTaP (SSI) were booster vaccinated with either TdaP (20 μg), TdaP (40 μg), TdaP-IPV (20 μg) or TdaP-IPV (40 μg) [22], Study H, Table 1. Injection site pain (of any intensity) was experienced by 55.6% whereas injection site redness (≥ 6 cm) occurred in 20.4% and swelling (≥ 6 cm) in 9.9% of the TdaP (20 μg) recipients, Study H. No extensive swelling (≥ 10 cm) reactions were reported in any group [22]. Injection site redness and swelling frequencies were lower in TdaP (20 μg) than in TdaP (40 μg) recipients, Study H. The most commonly reported systemic symptoms were somnolence, malaise, irritability and rhinitis, with no obvious patterns or trends [22].

4.3. Safety of TdaP (SSI) in adolescents

TdaP (SSI) and 5-component TdaP were investigated in two parallel groups in 14–15-year-old Swedish adolescents who were vaccinated with 3 doses 5-component DTaP and 1 dose 5-component TdaP in previous clinical trials [24], Study J, Table 1. After the TdaP (SSI) booster vaccination, the frequency of severe injection site pain was 0.9% and of extensive redness and swelling (of more than half the circumference of the upper arm) 2.7%, Study J. The most frequently reported systemic symptoms were moderate to severe fatigue (15.2%) and moderate to severe headache (17.0%) [24].

4.4. Safety of TdaP (SSI) in adults

In a trial in Danish adults the investigated TdaP (SSI) and Td (SSI) vaccines contained identical tetanus and diphtheria toxoid batches, which allowed for an unbiased double-blind evaluation of the contribution of the aP component to the safety profile of TdaP [25], STUDY K, Table 1. In TdaP recipients, the frequencies of severe injection site pain, redness ≥ 5 cm and swelling ≥ 5 cm were 0.7%, 0.7% and 1.5%, respectively, with comparable frequencies in Td recipients, Study K. No TdaP recipients experienced injection site swelling or redness ≥ 10 cm whereas two Td recipients (0.5%) did. The three most frequent systemic symptoms in TdaP recipients, whether related or not to the vaccination and including all intensities were: headache (20.4%), fatigue (17.0%) and myalgia (10.0%),

with similar frequencies in the Td group. There was thus no indication of the aP component contributing to higher rates of local or systemic adverse events in this clinical setting [25]. The safety profile was similar to that reported for 3- and 5-component TdaP in American adults [25,42].

5. Immunogenicity in infants, children, adolescents and adults

The immunogenicity of the combination vaccines containing monocomponent aP was evaluated measuring IgG anti-PT levels by ELISA coated with native PT in most trials whereas the toxin neutralising anti-PT Chinese hamster ovary (CHO) cell assay was used in a few [17,20]. Across trial comparisons of post-vaccination IgG anti-PT concentrations are not always possible. In the early Swedish trials A, B, C, E, H and I, the IgG anti-PT determinations were performed by the same laboratory using the FDA reference pertussis antiserum lot 3 as standard and are comparable. In other trials the assays were performed by different laboratories either using the FDA or NIBSC (06/140) international standards. IgG anti-PT levels are presented here as reported.

5.1. Immunogenicity of DTaP-IPV and DTaP-IPV/PRP-T (SSI) in infants

Three trials investigated the immunogenicity of DTaP-IPV (SSI), administered according to primary infant vaccination schedules [18–20], Studies D, E and F, Table 1. Before introduction of DTaP-IPV into the Danish programme in 1997, the vaccine was investigated in the suggested new 'Nordic schedule' of 3, 5 and 12 months, in comparison to the previous Danish wP vaccination schedule of wP at 5, 9 weeks and 10 months, STUDY D. Unintentionally, there are unequal numbers in the two trial groups in this trial. At 24 months of age, approximately one year after the 3rd aP/wP vaccination, 97.3% of aP and 55.6% of wP vaccinated infants were seropositive (IgG anti-PT concentration ≥ 4 IU/mL), Study D. In a subsequent trial, PRP-T (Act-HIB) was reconstituted with DTaP-IPV (SSI) and administered as one injection, DTaP-IPV/PRP-T, and compared to DTaP-IPV + PRP-T, injected separately, in Swedish infants during 1997–99 [19], Study E. The one month post 3rd vaccination GMCs of IgG anti-PT were 261 IU/mL (DTaP-IPV/PRP-T) and 236 IU/mL (DTaP-IPV + PRP-T) in the two groups. Finally, a trial was conducted in Polish infants during 2003–05 [20], Study F. Herein, the non-inferiority of DTaP-IPV$_{Vero}$ (SSI) to DTaP-IPV$_{Mkc}$ (SSI) was demonstrated for all vaccine antigens [20]. The toxin neutralising anti-PT geometric mean titre (GMT) was 205 in DTaP-IPV$_{Vero}$ vaccinated infants one month after completion of the Polish 2, 3½, 5 and 16 months schedule, Study F. In comparison, it was 240 in fully DTaP vaccinated infants one month after completion of vaccination in the efficacy trial, Study A.

5.2. Immunogenicity of DTaP (Certiva), TdaP and TdaP-IPV (SSI) in children

During 1995–96, Certiva, an American DTaP (40 μg) combination vaccine (North American Vaccine Inc. Beltsville, MD, now acquired by Baxter), containing the same monocomponent aP as the SSI vaccines, was investigated in 4–6-year old American children, previously vaccinated according to the US programme [21], Study G, Table 1. A booster response (4-fold increase in IgG anti-PT & post-vaccination level ≥ 4 IU/mL) was obtained by 93% after the DTaP vaccination, Study G. In Sweden, children 5–6 years of age, previously vaccinated with DTaP (SSI) at 3, 5 and 12 months in the efficacy trial [17], were enrolled in a dosage finding trial during 1997–99 [22], Study H, Table 1, and booster vaccinated with either TdaP (20 μg), TdaP (40 μg), TdaP-IPV (20 μg) or TdaP-IPV (40 μg)

(SSI). The trial demonstrated that the immunogenicity of aP (20 μg) was non-inferior to aP (40 μg), Study H. In a subsequent trial, TdaP (20 μg), TdaP (40 μg) and Td (SSI) were investigated in 10-year-old Swedish children with previous histories of wP or aP vaccination or pertussis [23], Study I, Table 1. Herein, TdaP (20 μg), TdaP (40 μg) and Td recipients were analysed in subgroups with or without detectable IgG anti-PT before the vaccination. One month post vaccination, median IgG anti-PT concentrations were, as expected, higher in the subgroups of children with detectable IgG anti-PT before the booster vaccination, Study I.

5.3. Immunogenicity of TdaP (SSI) in adolescents

TdaP (SSI) and 5-component TdaP vaccine were investigated in two parallel groups in Swedish 14–15-year-olds enrolled in a randomised and controlled trial in 2009 [24], Study J, Table 1. The adolescents had a history of vaccination with 5-component DTaP at 3, 5 and 12 months and 5-component TdaP at 5½ years of age in previous trials. TdaP (SSI) resulted in a booster response (2-fold increase in IgG anti-PT & post-vaccination level ≥4 IU/mL) in 95.6%, with a GMC of IgG anti-PT of 74.2 IU/mL, Study J, which was approximately 4 times higher than for the 5-component TdaP vaccine [24].

5.4. Immunogenicity of aP and TdaP (SSI) in adults

A dosage finding trial investigating the safety and immunogenicity of five aP vaccines in American healthy adults was published in 1999 [26]. For the monocomponent aP vaccine dosages of 4, 12.5 and 40 μg pertussis toxoid were investigated. All investigated aP vaccines were shown to be safe and immunogenic and were considered possible candidates for future use in adults [26].

More recently, TdaP (SSI) was compared to Td (SSI) in 802 healthy Danish adults, previously immunised with wP [25] Study K, Table 1. The percentages of subjects with protective tetanus and diphtheria antibody levels after the TdaP vaccination (tetanus: 100%; diphtheria: 98.5%) were non-inferior to those after vaccination with Td (tetanus: 100%; diphtheria: 99.5%) [25]. In the TdaP recipients, an IgG anti-PT booster response (4-fold increase, if initially <20 IU/mL or 2-fold increase if initially ≥20 IU/mL) was elicited in 92.0% and the post-vaccination GMC was 122 IU/mL, Study K.

In a trial in American adults the IgG anti-PT booster response (≥5 IU/mL if initially <5 IU/mL, 4-fold increase if initially ≥5 and <20 IU/mL, or 2-fold increase if initially ≥20 IU/mL) rates and corresponding GMCs were 77.2% and 63.6 IU/mL for 3-component aP containing 8 μg pertussis toxoid and 47.1% and 32.2 IU/mL for 5-component aP containing 2.5 μg pertussis toxoid [42].

6. Postmarketing vaccine effectiveness studies and safety experience with monocomponent aP

6.1. Effectiveness and herd immunity, mass vaccination study, aP and DTaP (SSI)

A mass vaccination (effectiveness) study with monocomponent aP and DTaP was conducted in the Gothenburg area during 1995–99 [28,43], Study L, Table 1. Infants were vaccinated with DTaP (SSI) at 3, 5 and 12 months of age, whereas children aged ≥1 year were offered 3 doses of aP (SSI), as they had already completed the DT vaccination. 12 month old children (who had already received 2 doses of DT) were offered one dose of aP at 12 months followed by 2 doses of DTaP. From June 1995 through February 1999, 167,810 doses of monocomponent aP were administered to 61,219 children (56% received 3 doses). The number of

B. pertussis isolates from patients per year in the Gothenburg area declined from 1214 (January 1993–December 1995) to 64 (January 1997–June 1999), and hospitalisations due to pertussis declined from 62 to 5 in these two periods. Significant decreases in the number of isolates occurred in all age groups, including adults and non-vaccinated infants. Thus mass vaccination of the children with monocomponent aP decreased the spread of B. pertussis in the entire population, Study L, Table 1. In 2000 a combination vaccine with two-component aP was introduced for general vaccination of infants in the Gothenburg area. Pertussis returned in 2004 with high reported incidences in all age groups [43]. The reason for this outbreak is probably that no pre-school booster vaccination was part of the Swedish vaccination programme until 2007. From pertussis surveillance data covering incidences during 1997–2011, reported by The Swedish Institute for Infectious Disease Control (SMI) [44,45], it is seen that pertussis incidences in Gothenburg were significantly higher than in the rest of Sweden, especially during 2004. Contributing factors were the earlier use of PCR for diagnosis, a higher nasopharyngeal sampling rate and more reports of mild pertussis cases in Gothenburg compared to the rest of Sweden [45]. The possible contribution of the previous use of monocomponent aP in Gothenburg was analysed in two groups with different vaccination histories: monocomponent aP primed at 3, 5 and 12 months of age (during 1996–98) and two-component aP primed according to the same schedule (during 2000–11), and since 2007 including a pre-school aP booster. The risks of pertussis in these two Gothenburg groups relative to the risk in the rest of Sweden (RRs) were calculated [45]. In young partly vaccinated infants (e.g. 5–12 months old), the relative risk of pertussis in Gothenburg compared to the rest of Sweden was lower in the monocomponent aP group [RR = 3.2 (95% CI: 1.8–5.4)] than in the two-component aP group [RR = 9.2 (95% CI: 7.4–11.4)], whereas in children from 5 to 8 years of age the RRs were lower in the two-component aP group, which could be due to the effect of the pre-school aP booster vaccination [45]. During 2005–11 there were no significant differences between reported pertussis incidences in Gothenburg and the rest of Sweden [45].

6.2. Effectiveness against pertussis, cohort study, DTaP-IPV (SSI)

The effectiveness of monocomponent aP in the Danish vaccination programme, vaccine effectiveness (VE), was evaluated in a large cohort of 541,525 children [27], Study M, Table 1. DTaP-IPV (SSI) was effective against pertussis requiring hospitalisation (VE: 93% after 3 doses) and against pertussis not requiring hospitalisation (VE: 78% after 3 doses), Study M, Table 1. Infant vaccination coverage rates in Denmark are approximately 90%. The higher effectiveness against severe pertussis than against mild pertussis is in line with results from the efficacy trial where the efficacy against mild pertussis (any cough ≥7 days) was lower than that against WHO defined pertussis (paroxysmal cough ≥21 days), i.e. 54% vs 71% [17].

6.3. Itching injection site nodules in the Gothenburg region

In 2003 a high rate of itching injection site nodules and contact allergy to aluminium was reported from Gothenburg, where aluminium adsorbed DTaP and aP vaccine from SSI had been administered in clinical trials including the mass vaccination study in the 1990s [46,47]. It was suggested that these reactions could be related to the type of aluminium salt adsorbent. This was further investigated in a trial including 25,232 children 10 years of age who were followed for itching injection site nodules and contact allergy for 6 months after booster vaccination with Td (SSI) and a standard Swedish Td vaccine, adsorbed to aluminium hydroxide and aluminium phosphate, respectively. 3–6 children with itching
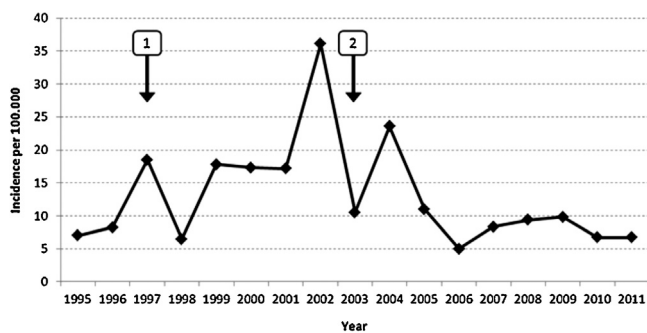
Fig. 1. Total incidence of laboratory confirmed pertussis per 100,000 (population) in Denmark through the years 1995–2011. The arrows mark changes to the vaccination programme. (1) Substitution of the wP vaccine with the monocomponent aP vaccine. (2) Introduction of the preschool monocomponent aP booster.

injection site nodules per 10,000 were identified in both groups with no differences between them and no detection of contact allergy to aluminium [48]. Itching injection site nodules after vaccination with similar aluminium adsorbed DTaP vaccines from other manufacturers have been reported both from Sweden [49,50] and from other countries [51,52]. In conclusion, itching injection site nodules occur infrequently following vaccination with aluminium adsorbed vaccines.

6.4. Safety experience from spontaneous reporting systems

Since the introduction of combination vaccine containing monocomponent aP in 1997 in the Danish vaccination programme, more than 2.5 million doses have been administered as primary vaccinations to infants, and since 2003 approximately 600,000 doses of aP have been administered as booster vaccinations to preschool children. The total number of adverse reaction reports related to the aP containing vaccines since 1997 is approximately 1100 related to primary vaccination, and 200 related to booster vaccination. The majority of these reports concern events such as transient injection site reactions and fever. In light of the experience from the Gothenburg region the post marketing surveillance of itching injection site nodules has been of particular interest. Approximately 100 reports concerning such cases have been received since 1997, supporting a very low frequency of this vaccine related adverse reaction. There were just below 60 reports of extensive swelling reactions related to the booster vaccinations.

7. Description of the epidemiological situation of pertussis in Denmark

Since 1995 Denmark has experienced one epidemic of pertussis which occurred in 2002 where the reported incidence was 36 per 100,000 (population) [53]. An incidence of 24 per 100,000 was seen in 2004 [53], while the incidence ranged between 6 and 11 per 100,000 in the remaining years up to 2011 [54,55], Fig. 1. Infant deaths attributed to pertussis are rare in Denmark. The latest two deaths occurred in 2010 [55] and 2005 [53] in infants too young to have been eligible for vaccination. The highest reported incidence in a single age-group is among <1 year old infants of whom the majority were not or only partially vaccinated. The incidence of pertussis among <1 year old infants ranged between 84 and 202 per 100,000 during the years 2005–11. For children ≥1 year of age, the incidence of pertussis peaked in the 3–6-year-olds in the period when wP vaccination was still in use (1995–96). Since the introduction of combination vaccine containing monocomponent aP in 1997, the peak has gradually shifted towards the young teenagers 13–14 years old, Table 2 and Fig. 2. This moving peak correlates with the last age-cohort vaccinated with the wP vaccine.

Table 2
Incidence per 100,000 (population) of laboratory confirmed pertussis in Denmark by age group in the years 1995 and 1996 (before introduction of monocomponent aP in 1997) and in the years 2010 and 2011. The major age-group, besides the infants, has shifted from the 3–6-year-olds to the 13–14 year-olds.

	1995	1996	2010	2011
0 years	115	94	111	96
1 years	28	28	14	16
2 years	34	34	12	15
3 years	41	67	17	31
4 years	48	61	9	11
5 years	53	72	3	6
6 years	33	72	9	3
7 years	44	36	9	12
8 years	41	38	14	9
9 years	25	17	28	14
10 years	9	16	7	22
11 years	4	18	21	13
12 years	13	6	19	22
13 years	5	11	17	25
14 years	2	5	21	26
15 years	5	3	23	11
16 years	2	3	24	19
17 years	0	2	10	8
18 years	0	3	12	4
19 years	0	0	3	4
20 years	0	0	6	3
20+ years	1	1	3	3
Total	7	8	7	7

The incidence among young children has decreased markedly, from 43 and 44 per 100,000 among the 1–4-year and 5–9-year-olds, respectively, during 1995–96 to 15 and 11 for the same age-groups during 2010–11. The total population incidences in these two periods are comparable, Table 3. In 1998 PCR was introduced as a diagnostic method for pertussis in Denmark followed by serology in 2010. Both PCR and serology have been shown to be superior to culture [56]. PCR is in general more sensitive [57] and serology is in particular superior when it comes to diagnosis of adults [58,59]. In 2011, 78% of all laboratory-confirmed cases of pertussis in Denmark were identified by PCR, 17% by serology, 3% by culture and 1% by combinations of two methods. The use of serology was still at a low level in 2011, but as the use expands it is expected to have a similar effect on the number of confirmed cases of pertussis as has been seen in other countries [60]. Similar to other countries [4], Denmark has experienced an increase in incidence of confirmed pertussis among adults since the 1990s. Whether this increase is true or merely an effect of improved diagnostic methods and increased awareness is unknown. It is not possible to directly compare the above Danish incidence rates to those in neighbouring countries due to marked across country and region differences in surveillance systems, use of diagnostic methods and awareness of pertussis [4].

Table 3
Incidence per 100,000 (population) of laboratory confirmed pertussis in Denmark by age group in the years 1995 and 1996 (before introduction of monocomponent aP in 1997) and in the years 2010 and 2011. Relative changes between the two time periods are shown.

	1995 and 1996	2010 and 2011	Relative change
0 years	104.4	103.2	1.0
1–4 years	42.5	15.4	0.4
5–9 years	43.8	10.8	0.2
10–14 years	9.0	19.4	2.2
15–19 years	1.7	12.0	7.0
20+ years	1.3	2.9	2.2
Total	7.7	6.7	0.9
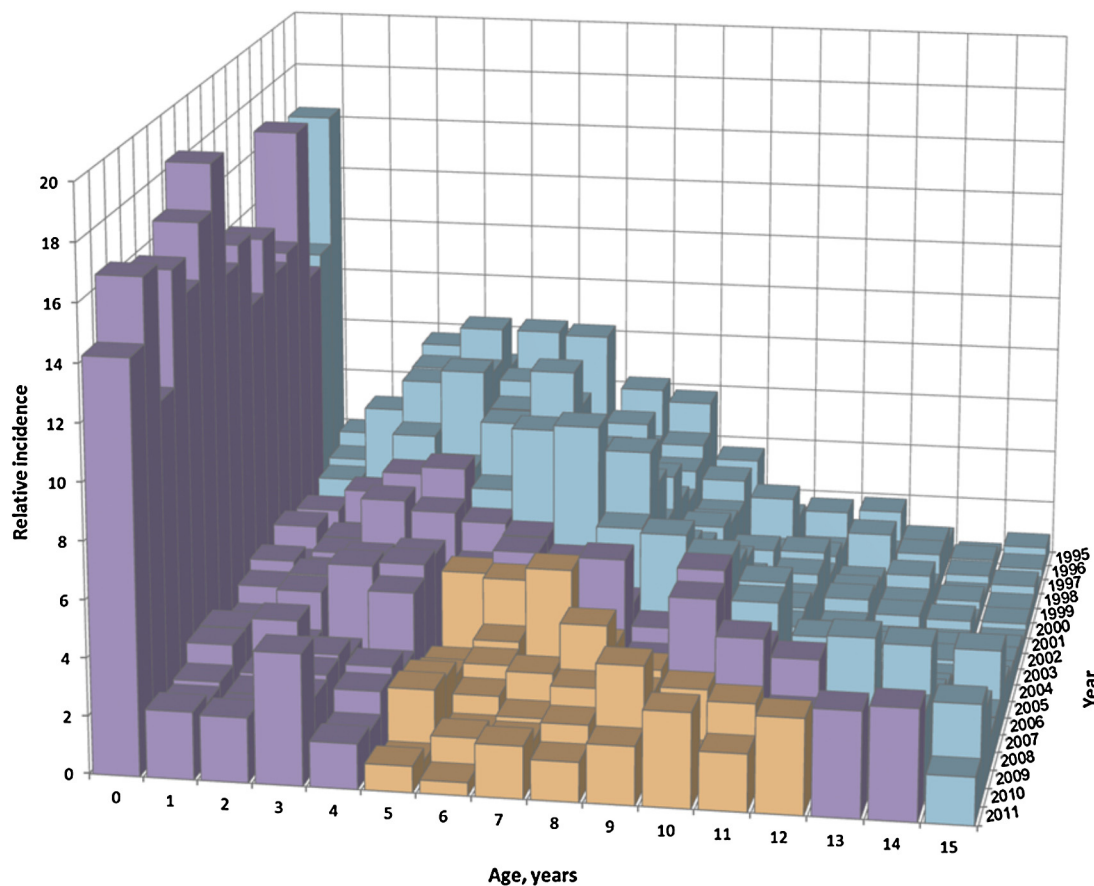
Fig. 2. Incidence by age group of laboratory confirmed pertussis in relation to total incidence that year. Columns indicate children who were vaccine-eligible in the three different periods with regards to vaccines: Turquoise: The whole-cell period (until 1996). Lilac: aP period without booster (1997–2003). Orange: aP period with preschool booster (introduced September 2003, colour markings from 2004). (For interpretation of the references to color in the text, the reader is referred to the web version of the article.)

8. Discussion

In Denmark, the introduction of wP in 1961 (Potency: ≥4 IU, opacity before inactivation ≤20 OU, manufactured by SSI) caused a rapid decline in notified pertussis cases in all age groups, however minor epidemics continued to occur every 3–4 years with wP coverage rates of around 80% [61]. Combination vaccines containing monocomponent aP have been used for primary vaccination of infants at 3, 5 and 12 months of age since 1997 and for booster vaccination of pre-school children at 5 years of age since 2003. The incidence of pertussis in Denmark has remained stable around 6–11 per 100,000 during 2005–11 [54,55]. The most recent epidemic occurred in 2002, the year before introducing the 5-years aP booster vaccination, with an incidence of 36 per 100,000 [53]. Thus, the 3–4 years outbreak intervals seen in the wP vaccination period seem to have changed. The highest incidence among children ≥1 year of age has shifted from the 3–6-year-olds in the wP vaccination period to the 13–14-year-olds. This change has occurred gradually and coincides with the introduction of aP vaccination. This could imply that the aP vaccine induces better immunity than the previously used wP vaccine in Denmark, which contradicts recent reports from Australia and the USA where it was suggested that wP primed individuals experience less pertussis than aP primed individuals [62,63]. The changing epidemiology of *B. pertussis* infection in Europe and in the USA, with increasing numbers of cases in older populations, stresses the importance of booster vaccination of children, adolescents and adults [4]. Infection-acquired immunity against pertussis has been reported to wane after 4 to 20 years and immunity following vaccination seems to wane after 4–12 years [64]. Several trials have documented that a booster response is induced by TdaP vaccines in adolescents and adults [65–71]. As decennial booster vaccination against tetanus and diphtheria during adolescence and adulthood is well established in many countries, the recent trials documenting the safety and immunogenicity of decennial vaccination with TdaP vaccines [66,71] are promising, as they support the feasibility of a shift from decennial Td to decennial TdaP booster vaccination. The clinical implications of the amount of and different manufacturing techniques of pertussis toxoid were investigated in the MAPT in the 1990s [72]. Herein, post-vaccination GMCs of IgG anti-PT could not be shown to correlate with the quantity of pertussis toxoid in the investigated aP combination vaccines. However, the immunogenicity of pertussis toxoid was highly dependent on antigen derivation and vaccine formulation including inactivation techniques. This was most clearly demonstrated for an aP combination vaccine containing genetically inactivated pertussis toxoid, which was the most immunogenic vaccine although it contained the lowest amount (5 μg) of pertussis toxoid [72]. Recent studies suggest that the impact of varying chemical inactivation on pertussis toxin epitopes need to be further investigated [73,74]. In two trials in adults, IgG anti-PT booster response rates and post-vaccination GMCs were higher for TdaP (SSI) containing 20 μg pertussis toxoid inactivated by hydrogen peroxide, than for 3- and 5-component aP vaccines containing 8 and 2.5 μg pertussis toxoid inactivated by formaldehyde and/or glutaraldehyde [25,42]. The higher amount of pertussis toxoid, the fact that it is less denatured in the TdaP (SSI) vaccine or both may play a role [1,2]. IgG anti-PT levels achieved after vaccination with TdaP predict antibody persistence [67], and it was recently

suggested that the increased pertussis toxin production of the new circulating P3 strains of *B. pertussis* would be less likely to cause pertussis in the presence of higher levels of circulating neutralising anti-PT in the populations [8]. Anti-PT levels have been shown to decline rapidly during the first years after TdaP booster vaccination of adults [65,67]. Interestingly, a recent small investigation in Danish adults indicated that anti-PT levels declined more rapidly in patients who had experienced pertussis than in healthy adults booster vaccinated with the Danish aP vaccine [75]. Whether the successful control of pertussis in Denmark can be attributed to the monocomponent aP vaccine, the only vaccine in use in Denmark during the last 15 years, should be followed closely, including the need of an additional adolescent aP booster vaccination, as well as the effectiveness of the monocomponent aP vaccine in the other European countries where it is presently being introduced.

References

[1] Heron I, Chen FM, Fusco J. DTaP vaccines from north american vaccine (NAVA): composition and critical parameters. Biologicals 1999;27(June (2)):91–6.

[2] Ibsen PH. The effect of formaldehyde, hydrogen peroxide and genetic detoxification of pertussis toxin on epitope recognition by murine monoclonal antibodies. Vaccine 1996;14(April (5)):359–68.

[3] Vaccine Assessment and Monitoring team of the Department of Vaccines and Biologicals. WHO-recommended standards for surveillance of selected vaccine-preventable diseases. World Health Organization; 2003. Report No.: WHO/V&B/03.01.

[4] Zepp F, Heininger U, Mertsola J, et al. Rationale for pertussis booster vaccination throughout life in Europe. Lancet Infect Dis 2011;11(July (7)):557–70.

[5] Octavia S, Sintchenko V, Gilbert GL, et al. Newly emerging clones of *Bordetella pertussis* carrying prn2 and ptxP3 alleles implicated in Australian pertussis epidemic in 2008–2010. J Infect Dis 2012;205(April (8)):1220–4.

[6] Heininger U, Cherry JD. Pertussis immunisation in adolescents and adults – *Bordetella pertussis* epidemiology should guide vaccination recommendations. Expert Opin Biol Ther 2006;6(July (7)):685–97.

[7] Mooi FR, van Loo IH, van GM, et al. *Bordetella pertussis* strains with increased toxin production associated with pertussis resurgence. Emerg Infect Dis 2009;15(August (8)):1206–13.

[8] Mooi FR, van der Maas NA, De Melker HE. Pertussis resurgence: waning immunity and pathogen adaptation – two sides of the same coin. Epidemiol Infect 2013;(February):1–10.

[9] Poolman JT, Hallander HO. Acellular pertussis vaccines and the role of pertactin and fimbriae. Expert Rev Vaccines 2007;6(February (1)):47–56.

[10] Robbins JB, Schneerson R, Keith JM, Miller MA, Kubler-Kielb J, Trollfors B. Pertussis vaccine: a critique. Pediatr Infect Dis J 2009;28(March (3)):237–41.

[11] Vidor E, Plotkin SA. Immunogenicity of a two-component (PT & FHA) acellular pertussis vaccine in various combinations. Hum Vaccin 2008;4(September (5)):328–40.

[12] Taranger J, Trollfors B, Bergfors E, et al. Immunologic and epidemiologic experience of vaccination with a monocomponent pertussis toxoid vaccine. Pediatrics 2001;108(December (6)):E115.

[13] Sekura RD, Fish F, Manclark CR, Meade B, Zhang YL. Pertussis toxin, affinity purification of a new ADP-ribosyltransferase. J Biol Chem 1983;258(December (23)):14647–51.

[14] Sekura RD, Zhang YL, Roberson R, et al. Clinical, metabolic, and antibody responses of adult volunteers to an investigational vaccine composed of pertussis toxin inactivated by hydrogen peroxide. J Pediatr 1988;113(November (5)):806–13.

[15] Krantz I, Sekura R, Trollfors B, et al. Immunogenicity and safety of a pertussis vaccine composed of pertussis toxin inactivated by hydrogen peroxide, in 18- to 23-month-old children. J Pediatr 1990;116(April (4)):539–43.

[16] Isacson J, Trollfors B, Taranger J, et al. Safety, immunogenicity and an open, retrospective study of efficacy of a monocomponent pertussis toxoid vaccine in infants. Pediatr Infect Dis J 1994;13(January (1)):22–7.

[17] Trollfors B, Taranger J, Lagergard T, et al. A placebo-controlled trial of a pertussis-toxoid vaccine. N Engl J Med 1995;333(October (16)):1045–50.

[18] Gyhrs A, Lyngholm E, Larsen SO, Aggerbeck H, Heron I. Immunogenicity and safety of a tetravalent diphtheria-tetanus-acellular pertussis-inactivated poliovirus vaccine. Scand J Infect Dis 1999;31(6):579–85.

[19] Knutsson N, Trollfors B, Taranger J, et al. Immunogenicity and reactogenicity of diphtheria, tetanus and pertussis toxoids combined with inactivated polio vaccine, when administered concomitantly with or as a diluent for a Hib conjugate vaccine. Vaccine 2001;19(August (31)):4396–403.

[20] Pietrzyk JJ, Wysocki J, Pejcz J, et al. Safety and immunogenicity of a DTaP-IPV(Vero) (serum-free) combination vaccine in comparison to DTaP-IPV(Mkc) when administered simultaneously with *Haemophilus influenzae* type B conjugate vaccine (PRP-T) in children at 2, 3.5, 5 and 16 months of age. Vaccine 2008;26(September (41)):5296–303.

[21] Blatter M. Open trial to evaluate the safety and immunogenicity of a fifth (booster) dose of diphtheria, tetanus, acellullar pertussis vaccine in healthy children; 1996. Unpublished; Jun 26. Report No.: Internal clinical trial report (014).

[22] Taranger J, Trollfors B. Safety and Immunogenicity of combined vaccines against diphtheria, tetanus, pertussis and polio at six years of age; 2001. Unpublished; Jan 12. Report No.: Internal clinical trial report (007B).

[23] Trollfors B, Knutsson N, Taranger J, et al. Diphtheria, tetanus and pertussis antibodies in 10-year-old children before and after a booster dose of three toxoids: implications for the timing of a booster dose. Eur J Pediatr 2006;165(January (1)):14–8.

[24] Netterlid E, Hallander HO, Nilsson L, Ljungman M, Gothefors L, Gustafsson L. An immunogeneicity and safety study of combined adsorbed tetanus, low dose diphtheria and acellular partussis vaccine given as a school leaving booster to 14–15 year olds primed with a five component acellular pertussis vaccine at 3, 5 and 12 months of age and a booster dose at 5½ years of age. In: Poster session at Nordic Vaccine meeting 5–7 September 2012. 2012.

[25] Thierry-Carstensen B, Jordan K, Uhlving HH, et al. A randomised, double-blind, non-inferiority clinical trial on the safety and immunogenicity of a tetanus, diphtheria and monocomponent acellular pertussis (TdaP) vaccine in comparison to a tetanus and diphtheria (Td) vaccine when given as booster vaccinations to healthy adults. Vaccine 2012;30(August (37)):5464–71.

[26] Keitel WA, Muenz LR, Decker MD, et al. A randomized clinical trial of acellular pertussis vaccines in healthy adults: dose-response comparisons of 5 vaccines and implications for booster immunization. J Infect Dis 1999;180(August (2)):397–403.

[27] Hviid A, Stellfeld M, Andersen PH, Wohlfahrt J, Melbye M. Impact of routine vaccination with a pertussis toxoid vaccine in Denmark. Vaccine 2004;22(September (27/28)):3530–4.

[28] Taranger J, Trollfors B, Bergfors E, et al. Mass vaccination of children with pertussis toxoid – decreased incidence in both vaccinated and nonvaccinated persons. Clin Infect Dis 2001;33(October (7)):1004–10.

[29] Taranger J, Trollfors B, Lagergard T, et al. Unchanged efficacy of a pertussis toxoid vaccine throughout the two years after the third vaccination of infants. Pediatr Infect Dis J 1997;16(February (2)):180–4.

[30] Trollfors B, Taranger J, Lagergard T, et al. Efficacy of a monocomponent pertussis toxoid vaccine after household exposure to pertussis. J Pediatr 1997;130(April (4)):532–6.

[31] Trollfors B, Taranger J, Lagergard T, et al. Immunization of children with pertussis toxoid decreases spread of pertussis within the family. Pediatr Infect Dis J 1998;17(March (3)):196–9.

[32] Taranger J, Trollfors B, Lagergard T, et al. Correlation between pertussis toxin IgG antibodies in postvaccination sera and subsequent protection against pertussis. J Infect Dis 2000;181(March (3)):1010–3.

[33] Gustafsson L, Hallander HO, Olin P, Reizenstein E, Storsaeter J. A controlled trial of a two-component acellular, a five-component acellular, and a whole-cell pertussis vaccine. N Engl J Med 1996;334(February (6)):349–55.

[34] Greco D, Salmaso S, Mastrantonio P, et al. A controlled trial of two acellular vaccines and one whole-cell vaccine against pertussis. Progetto Pertosse Working Group. N Engl J Med 1996;334(February (6)):341–8.

[35] Taranger J, Trollfors B, Lagergard T, Robbins JB. Protection against pertussis with a monocomponent pertussis toxoid vaccine. Int J Infect Dis 1997;1: 148–51.

[36] Granstrom M. The history of pertussis vaccination: from whole cell to subunit vaccines. Vaccinia, vaccination and vaccinology: Jenner, Pasteur and their successors. Paris: Elsevier; 1996. p. 104–14.

[37] Decker MD, Edwards KM, Steinhoff MC, et al. Comparison of 13 acellular pertussis vaccines: adverse reactions. Pediatrics 1995;96(September (3 Pt 2)):557–66.

[38] Rennels MB, Deloria MA, Pichichero ME, et al. Extensive swelling after booster doses of acellular pertussis-tetanus-diphtheria vaccines. Pediatrics 2000;105(January (1)):e12.

[39] Knuf M, Habermehl P, Faber J, et al. Assessment of nine candidate DTP-vaccines with reduced amount of antigen and/or without adjuvant as a fourth (booster-) dose in the second year of life. Vaccine 2006;24(July (27/28)):5627–36.

[40] Jacquet JM, Begue P, Grimprel E, et al. Safety and immunogenicity of a combined DTPa-IPV vaccine administered as a booster from 4 years of age: a review. Vaccine 2006;24(March (13)):2440–8.

[41] Zepp F, Habermehl P, Knuf M, Mannhardt-Laakman W, Howe B, Friedland LR. Immunogenicity of reduced antigen content tetanus-diphtheria-acellular pertussis vaccine in adolescents as a sixth consecutive dose of acellular pertussis-containing vaccine. Vaccine 2007;25(July (29)):5248–52.

[42] Blatter M, Friedland LR, Weston WM, Li P, Howe B. Immunogenicity and safety of a tetanus toxoid, reduced diphtheria toxoid and three-component acellular pertussis vaccine in adults 19–64 years of age. Vaccine 2009;27(January (5)):765–72.

[43] Trollfors B, Dotevall L, Sundh V, Welinder-Olsson C. Pertussis after end of a mass vaccination project-End of the "vaccination honey-moon". Vaccine 2011;29(February):2444–50.

[44] Carlsson RM, Trollfors B. Control of pertussis – lessons learnt from a 10-year surveillance programme in Sweden. Vaccine 2009;27(September (42)):5709–18.

[45] Nilsson L, von Segebaden K, Kling A-M, Grünewald M. Fourteen year report – pertussis in Gothenburg study area. Sweden: Smittskyddsinstitutet; 2012 July. Report No.: 2012-18-2.

[46] Bergfors E, Trollfors B, Inerot A. Unexpectedly high incidence of persistent itching nodules and delayed hypersensitivity to aluminium in children after the use of adsorbed vaccines from a single manufacturer. Vaccine 2003;22(December (1)):64–9.

[47] Thierry-Carstensen B, Stellfeld M. Itching nodules and hypersensitivity to aluminium after the use of adsorbed vaccines from SSI. Vaccine 2004;22(May (15/16)):1845.
[48] Netterlid E, Bruze M, Hindsen M, Isaksson M, Olin P. Persistent itching nodules after the fourth dose of diphtheria-tetanus toxoid vaccines without evidence of delayed hypersensitivity to aluminium. Vaccine 2004;22(September (27/28)):3698–706.
[49] Trollfors B, Bergfors E, Inerot A. Vaccine related itching nodules and hypersensitivity to aluminium. Vaccine 2005;23(January (8)):975–6.
[50] Bergfors E, Trollfors B. Sixty-four children with persistent itching nodules and contact allergy to aluminium after vaccination with aluminium-adsorbed vaccines-prognosis and outcome after booster vaccination. Eur J Pediatr 2013;172(February (2)):171–7.
[51] Frederiksen MS, Tofte H. Immunisation with aluminium-containing vaccine of a child with itching nodule following previous vaccination. Vaccine 2004;23(November (1)):1–2.
[52] Croce S, Lhermitte B, Tomasetto C, Guillard O, Bellocq JP, Chenard MP. [Late-onset vaccination-induced subcutaneous pseudolymphoma]. Ann Pathol 2008;28(April (2)):146–9.
[53] Andersen PH. Pertussis 2002–2004. EPI-NEWS 2005 Nov 16;Week 46.
[54] Andersen PH. Pertussis 2008. EPI-NEWS 2009 Oct 28;Week 44.
[55] Andersen PH. Pertussis 2010. EPI-NEWS 2011 Oct 26;Week 42–43.
[56] Andre P, Caro V, Njamkepo E, Wendelboe AM, Van RA, Guiso N. Comparison of serological and real-time PCR assays to diagnose Bordetella pertussis infection in 2007. J Clin Microbiol 2008;46(May (5)):1672–7.
[57] Dragsted DM, Dohn B, Madsen J, Jensen JS. Comparison of culture and PCR for detection of Bordetella pertussis and Bordetella parapertussis under routine laboratory conditions. J Med Microbiol 2004;53(August (Pt 8)):749–54.
[58] van der Zee A, Agterberg C, Peeters M, Mooi F, Schellekens J. A clinical validation of Bordetella pertussis and Bordetella parapertussis polymerase chain reaction: comparison with culture and serology using samples from patients with suspected whooping cough from a highly immunized population. J Infect Dis 1996;174(July (1)):89–96.
[59] Yih WK, Lett SM, des Vignes FN, Garrison KM, Sipe PL, Marchant CD. The increasing incidence of pertussis in Massachusetts adolescents and adults, 1989–1998. J Infect Dis 2000;182(November (5)):1409–16.
[60] Quinn HE, McIntyre PB. Pertussis epidemiology in Australia over the decade 1995–2005 – trends by region and age group. Commun Dis Intell 2007;31(June (2)):205–15.
[61] Nielsen A, Larsen SO. Epidemiology of pertussis in Denmark: the impact of herd immunity. Int J Epidemiol 1994;23(December (6)):1300–8.
[62] Sheridan SL, Ware RS, Grimwood K, Lambert SB. Number and order of whole cell pertussis vaccines in infancy and disease protection. JAMA 2012;308(August (5)):454–6.
[63] Klein NP, Bartlett J, Fireman B, Rowhani-Rahbar A, Baxter R. Comparative effectiveness of acellular versus whole-cell pertussis vaccines in teenagers. Pediatrics 2013;131(June (6)):e1716–22.
[64] Wendelboe AM, Van RA, Salmaso S, Englund JA. Duration of immunity against pertussis after natural infection or vaccination. Pediatr Infect Dis J 2005;24(May (5 Suppl.)):S58–61.
[65] McIntyre PB, Burgess MA, Egan A, Schuerman L, Hoet B. Booster vaccination of adults with reduced-antigen-content diphtheria, Tetanus and pertussis vaccine: immunogenicity 5 years post-vaccination. Vaccine 2009;27(February (7)):1062–6.
[66] Booy R, Van Der Meeren O, Ng SP, Celzo F, Ramakrishnan G, Jacquet JM. A decennial booster dose of reduced antigen content diphtheria, tetanus, acellular pertussis vaccine (Boostrix) is immunogenic and well tolerated in adults. Vaccine 2010;29(December (1)):45–50.
[67] Edelman K, He Q, Makinen J, et al. Immunity to pertussis 5 years after booster immunization during adolescence. Clin Infect Dis 2007;44(May (10)):1271–7.
[68] Barreto L, Guasparini R, Meekison W, Noya F, Young L, Mills E. Humoral immunity 5 years after booster immunization with an adolescent and adult formulation combined tetanus, diphtheria, and 5-component acellular pertussis vaccine. Vaccine 2007;25(November (48)):8172–9.
[69] Tran Minh NN, He Q, Edelman K, et al. Immune responses to pertussis antigens eight years after booster immunization with acellular vaccines in adults. Vaccine 2000;18(April (19)):1971–4.
[70] Edelman KJ, He Q, Makinen JP, et al. Pertussis-specific cell-mediated and humoral immunity in adolescents 3 years after booster immunization with acellular pertussis vaccine. Clin Infect Dis 2004;39(July (2)): 179–85.
[71] Mertsola J, Van Der Meeren O, He Q, et al. Decennial administration of a reduced antigen content diphtheria and tetanus toxoids and acellular pertussis vaccine in young adults. Clin Infect Dis 2010;51(September (6)):656–62.
[72] Edwards KM, Meade BD, Decker MD, et al. Comparison of 13 acellular pertussis vaccines: overview and serologic response. Pediatrics 1995;96(September (3 Pt 2)):548–57.
[73] Sutherland JN, Chang C, Yoder SM, Rock MT, Maynard JA. Antibodies recognizing protective pertussis toxin epitopes are preferentially elicited by natural infection versus acellular immunization. Clin Vaccine Immunol 2011;18(June (6)):954–62.
[74] Sutherland JN, Maynard JA. Characterization of a key neutralizing epitope on pertussis toxin recognized by monoclonal antibody 1B7. Biochemistry 2009;48(December (50)):11982–93.
[75] Dalby T, Petersen JW, Harboe ZB, Krogfelt KA. Antibody responses to pertussis toxin display different kinetics after clinical Bordetella pertussis infection than after vaccination with an acellular pertussis vaccine. J Med Microbiol 2010;59(September (Pt 9)):1029–36.