Vaccine 32 (2014) 5931–5936

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Anti-pertussis antibody kinetics following DTaP-IPV booster vaccination in Norwegian children 7–8 years of age

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ARTICLE INFO

Article history:
Received 30 April 2014
Received in revised form 26 August 2014
Accepted 29 August 2014
Available online 13 September 2014

ABSTRACT

At the age of 7–8 years a booster of diphtheria, tetanus, acellular pertussis and polio vaccine is recommended for children in Norway. In this cross-sectional study we have analysed the antibody levels against pertussis vaccine antigens in sera from 498 children aged 6–12 years. The purposes of this study were to investigate the duration of the booster response against the pertussis vaccine antigens pertussis toxin (PT) and filamentous haemagglutinin (FHA); to determine the presence of high levels of pertussis antibodies in absence of recent vaccination; and to analyse how booster immunisation may interfere with the serological pertussis diagnostics. Prior to the booster the IgG antibody levels against PT revealed a geometric mean of 7.3 IU/ml. After the booster the geometric mean peak anti-PT IgG response reached to 45.6 IU/ml, followed by a steady decline in antibody levels over the next few years. The IgG anti-FHA levels followed the anti-PT IgG profiles. Three years after the booster the geometric mean IgG levels were only slightly above pre-booster levels. Prior to the booster 44% of the sera contained ≤5 IU/ml of anti-PT IgG compared to18% 3 years after and 30% 4 years after the booster. When recently vaccinated children were excluded, 6.2% of the children had anti-PT IgG levels above 50 IU/ml which may indicate pertussis infection within the last 2 years. This study indicates that the currently used acellular pertussis vaccines induce moderate immune responses to the pertussis antigens and that the antibodies wane within few years after the booster. This lack of sustained immune response may partly be responsible for the increased number of pertussis cases observed in this age group during the last years.

1. Introduction

Many countries experience increasing incidences of pertussis in spite of a high vaccine coverage [1]. The reasons for this increase are multifactorial as improved diagnostics, increased awareness, demographic changes, genetic adaptation of the causative bacteria *Bordetella pertussis* and vaccine failure, all may contribute [1,2]. The resurgence seems to coincide with the shift from the use of whole cell (wP) to acellular pertussis (aP) vaccines [3] although many clinical studies of aP and wP vaccines indicate that both types of vaccines induce comparable immunity [4,5]. However, studies comparing aP and wP vaccination that depend on immunogenicity data and non-inferiority criteria of antibody levels measured against the aP vaccine antigens rather than efficacy studies, must be interpreted with care as such studies may favour the aP vaccines. More recent studies suggest that the duration of protection following DTaP immunisation in the first year of life is lower than with DTwP [1,6–8].

Norway has been one of the countries with the highest number of reported pertussis cases in Europe, in spite of approximately 95% vaccination coverage. The incidence has been particularly high in the age groups 5–19 years. From 1998, a DTaP vaccine containing three-component pertussis antigens has been implemented in a three dose regimen at 3, 5 and 12 months in the first year of life instead of the DTwP vaccine. In 2006 a two-component pertussis DTaP booster to children at the age of 7–8 years was implemented in the Childhood Immunisation Program. This resulted in a drop in the incidence of pertussis particularly within the immunised group. However, previous studies indicate that the decay of antibodies against pertussis antigens both after primary and booster immunisation is rapid [9–12].

High anti-pertussis toxin (PT) IgG levels in the absence of recent vaccination may be used as a diagnostic test for recent or active pertussis [13]. The use of serology with detection of high levels of

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http://dx.doi.org/10.1016/j.vaccine.2014.08.069

anti-PT IgG may thus be a valuable tool for the diagnosis of pertussis even though polymerase chain reaction (PCR) now becomes more widespread in use and about 60% of recorded cases in Norway in 2012 were based on PCR. On the other hand, vaccination against pertussis in different age groups may complicate interpretation of serological diagnosis, particularly if the vaccine induced antibody levels are high. It is recommended not to use serology for diagnosis within the first 2 years after pertussis immunisation [14].

We have performed a cross-sectional study to measure the antibody immune response against pertussis in 498 children aged 6–12 years who were scheduled to receive a DTaP booster vaccine at the age of 7–8 years. The aim of the current work was to study the duration of the booster response against the different pertussis vaccine antigens; to determine the proportion of high antibody levels in absence of recent vaccination thus reflecting recent infection; and to analyse how booster immunisation may interfere with the serological diagnosis.

2. Materials and Methods

2.1. Serum sample collection

Serum samples from 503 children submitted to the laboratory at the Department of clinical biochemistry for analysis at Akershus University Hospital from December 2009 to January 2011 were collected. They were leftover volumes after clinical biochemistry analysis and were randomly picked out during the 14 months period. The children were born between 1998 and 2003 and were scheduled to have a DTaP-polio booster vaccination at the age of 7–8 years. Approximately half of the samples (46%) were from general practitioners (GPs), the rest were from in-patients. One third of the samples from the GPs lacked any information regarding diagnosis and medical records were not available. Medical records were checked for all in-patients, leading to the exclusion of five patients suffering from diagnoses likely to cause immunodeficiency (acute lymphatic leukaemia, lymphoma, former spleen extirpation). The two dominating indications for sampling were allergy investigation and acute infection, followed by unspecified stomach pain, neurological/psychiatric disease and endocrine disorders. A total of 498 children were thus included. Date of blood sampling and date of birth and personal identification number for each person were recorded, and linked to the Norwegian Immunisation Registry (SYSVAK) to obtain the vaccine history and to calculate the number of days between last pertussis booster and blood sampling. The study was approved by the Norwegian Regional Committee for Medical Research Ethics.

2.2. Vaccination

The childhood pertussis vaccination program in Norway consists of three doses of DTaP-polio at 3, 5 and 12 months of age, containing the pertussis antigens pertussis toxoid, filamentous haemagglutinin (FHA) and pertactin (Prn) (Infanrix-polio, GSK). At the age of 7–8 years the children are offered a booster dose consisting of pertussis toxoid and FHA (Tetravac, Sanofi Pasteur MSD).

2.3. Serological analysis

Anti-PT IgG antibodies were analysed using a validated *in-house* enzyme-linked immunosorbent assay (ELISA) slightly modified from previous publications [15,16]. Briefly, PT (List Biological labs, CA, USA) was coated to 96 wells micro-titer plates at 1 μg/ml in 0.05 M bicarbonate buffer pH 9.6 for 48 h at 4 °C. Blocking was performed with 250 μL 1% powdered skimmed milk (Oxoid, UK) in PBS for 30 min at room temperature. Two-fold serial dilutions of patients sera were analysed, and bound antibody was detected with an anti-human IgG (gamma chain-specific) alkaline phosphatase conjugate (Sigma, USA). The WHO International Standard Pertussis Antiserum (NIBSC 06/140) was used to generate the standard curve. Interpolation of unknown sera was done by four-parameter curve analysis (Softmax Ver. 2.35, Molecular Devices Corp., UK). Values from at least two dilutions showing parallelism to the standard curve were used to calculate the IgG level, expressed as IU/ml. The lower limit of detection was 1 IU/ml, and sera with values below this were assigned a value of 1 IU/ml.

IgG antibodies against pertactin (Prn) (RIVM, the Netherlands) were measured with a similar method as for the anti-PT IgG, with a Prn coat at 1 μg/ml [17]. The sera were diluted in four two-fold dilutions and the results were calculated against the WHO reference serum 06/140, containing 65 IU/ml anti-Prn IgG by the use of four-parameter curve analysis.

IgG antibodies against FHA were analysed using Pertusscan 2 + 2 (Euro-Diagnostica AB, Malmö, Sweden), and the results were reported as a percentage of the negative cut-off (i.e., an optical density of 0.3 equals 100%). This is the preferred kit to measure anti-FHA IgG by the Norwegian diagnostic laboratories. The performance was according to manufacturer's instruction and one dilution (1:500) of test sera was used in the analysis.

In-house positive control serua were included in all ELISA plates and demonstrated good reproducibility of the assays, with a coefficient of variation of <10% for the anti-PT IgG, 16% for the anti-FHA-IgG, and 17% for the anti-PRN-IgG.

2.4. Statistical analysis

The sera were grouped into three subsets: sera from subjects who had received the booster dose at scheduled time (booster group), sera from subjects who had not received the booster (pre-booster group), and sera from subjects who had no recorded pertussis vaccine history. Linear regression analysis was used to assess the relationship between antibody levels and time since booster dose. The sera in the booster group were congregated into groups of 100 days after booster vaccination. Geometric mean (GM) levels and 95% confident intervals (CI) of GM were determined for IgG antibodies against the pertussis antigens PT, FHA and Prn for all groups. Anti-PT IgG ≤5 IU/ml was used as a measure of low specific antibody level.

3. Results

3.1. Immunisation history

The vaccination history of the 498 children is summarised in Table 1. According to the immunisation register 485 individuals (97%) had received three doses in the primary immunisation series during their first year of life. Of the patients born in the years from 1998 to 2002, 89% had received the fourth booster dose according to schedule at the age of 6–8 years. The patients born in 2003 had not yet been offered the booster dose. Thirteen children had no recorded vaccine history.

3.2. Antibody decay after booster

Fig. 1 shows the individual serum IgG levels against PT, FHA and Prn plotted against time since the booster dose (red circles) or since the primary immunisation series (blue triangles). Previous to the booster, the GM anti-PT IgG level was 7.3 IU/ml (95% CI: 6.0, 9.0 IU/ml) of the 104 participants who had only received the primary immunisations. As expected, the PT and FHA levels increased following the booster dose and were highest in the sera from those most recently immunised, with a peak anti-PT IgG GM level of 45.6 IU/ml (95% CI: 24.8, 83.9 IU/ml) and a peak anti-FHA IgG GM

Table 1
Pertussis immunisation history of the 498 participants included (number of children in each birth year).

Vaccine history	1998	1999	2000	2001	2002	2003
Primary vaccination	94	92	92	74	67	66
+Booster at age 7–8 years	90	89	85	66	51	0
No vaccine recorded	4	1	2	2	1	3
Total	98	93	94	76	68	69

level of 336.6 AU/ml (95% CI: 284.3, 398.6 AU/ml) within the first 100 days after the booster (Fig. 2A and B). After the peak response, there was a steady decline in anti-PT and anti-FHA IgG levels. But even in the samples collected 1001–1745 days after the 4th booster, the anti-PT- and anti-FHA IgG levels were still significantly higher ($P < 0.05$) than in sera collected before the booster (Fig. 2A and B). The anti-PT IgG GM levels from samples collected within the first year post booster was 32.3 IU/ml (95% CI: 25.6, 40.8 IU/ml), and 33% of these sera had an anti-PT IgG level ≤20 IU/ml. The number of sera with anti-PT IgG levels ≤5 IU/ml increased with time since the booster. The first 300 days after the booster, none of the sera contained an anti-PT IgG level ≤5 IU/ml (Fig. 3), whereas from 300 to 1000 days after the booster 14–16% of the samples displayed levels ≤5 IU/ml and from 1000 to 1745 days even 18–30%. Of the 104 subjects who had not received the booster dose, 43% had an anti-PT IgG level ≤5 IU/ml (6.4 geometric mean years since previous (primary) pertussis vaccination of the whole group).

According to the records from SYSVAK, 13 subjects had not received any pertussis vaccine ever. The GM anti-PT IgG level for this group was 11.8 IU/ml (95% CI: 6.0, 23.2), and 31% had an anti-PT IgG level ≤5 IU/ml (Fig. 3).

The vaccine used for booster at 7–8 years contains only the pertussis antigens PT and FHA; consequently there was no increase in the anti-Prn IgG level after the booster (Figs. 1C and 2C). Although there seemed to be an increase in anti-Prn IgG levels in the years following the booster (Fig. 1C red circles), no significant difference could be observed between the sera collected within the first 365 days and the sera collected 1101 to 1745 days after the booster. The anti-Prn IgG GM level of the whole booster group was 25.1 IU/ml (CI: 22.5, 28.1 IU/ml) and for the pre-booster group 22.0 IU/ml (CI: 18.5, 26.3 IU/ml).

3.3. *High anti-PT IgG and anti-Prn IgG as markers for recent pertussis*

A high level of anti-PT IgG in absence of recent vaccination is used as indication of recent pertussis. For seroepidemiological studies an anti-PT IgG cut-off of 80 IU/ml may be used to identify pertussis infection within the last year, whereas a cut-off of 50 IU/ml may indicate infection within the last two years [18]. Analysis of sera from patients, who had not been vaccinated within the last 2 years, revealed that 6 of 369 sera (1.6%) had anti-PT IgG levels higher than the recommended Norwegian cut-off of 80 IU/ml, and 23 sera (6.2%) were above 50 IU/ml.

Since the vaccine used at this age does not contain Prn, high levels of anti-Prn IgG might indicate recent infection. Forty-nine of the 498 sera (10%) displayed an anti-Prn IgG level ≥100 IU/ml and 39 of these subjects had not been immunised within the last 2 years. Two sera with anti-Prn IgG levels >100 IU/ml also had anti-PT IgG higher than 80 IU/ml; one had been boosted 573 days ago and the other one with anti-PT IgG of 505 IU/ml and anti-Prn IgG of 175 IU/ml had been immunised only 18 days earlier.

In Norway a diagnostic cut-off of anti-PT IgG level at 80 IU/ml is recommended (established with the Virion\Serion Bordetella Pertussis Toxin IgG assay). Within the first 2 years after the booster only 9 of 130 subjects had anti-PT IgG values above this level; however, 4 of these also had an anti-Prn IgG level above 50 IU/ml possibly indicating recent infection with *B. pertussis*.

4. Discussion

Antibodies against pertussis vaccine antigens were measured in a cross-sectional study in sera from children aged 6–12 years. Most of the children received a DTaP booster vaccine at age 7–8 years. At 6.4 geometric mean years after primary vaccination, the pre-booster anti-PT IgG GM level was 7.3 IU/ml. In the first 100 days after the booster dose a rather moderate peak response was observed reaching up to an anti-PT IgG GM level of 45.6 IU/ml, which was followed by a subsequent decline the following years. Three years after the booster dose almost 20% of the sera contained an anti-PT IgG level less than 5 IU/ml.

These anti-PT IgG levels are lower than the corresponding levels reported in a Danish study where adults were given a booster vaccine with a single-component pertussis antigen (PT), in spite of the lower PT-antigen content in the Danish vaccine [10]. Also, in a Dutch study using an aP booster vaccine with a similar dose of PT and FHA [19], higher anti-PT IgG levels (187 EU/ml 28 days post booster) were found than we did in our study. The shorter interval between primary immunisation series and the booster dose in the Dutch study (4 years versus 6 years) and the shorter and exact blood sample timing after the booster (28 days versus 0–100 days (mean 59 days)) might possibly explain the more pronounced booster response. In line with our results they also noted a significant decline in the anti-PT IgG level 2 years after the booster.

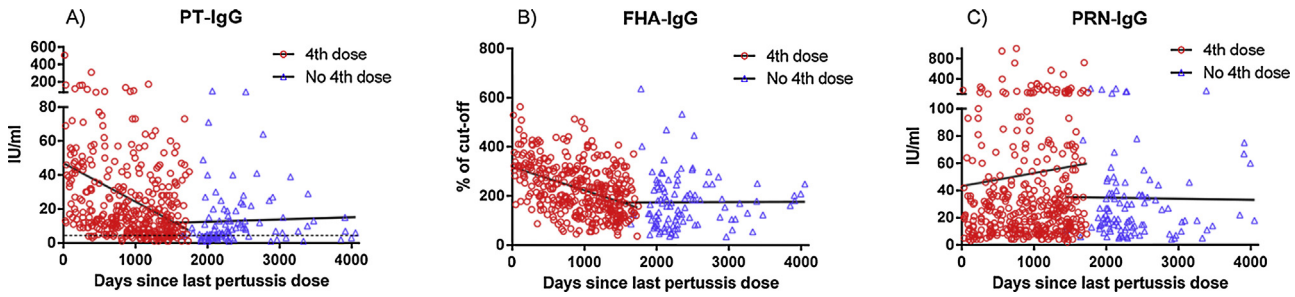

Fig. 1. Individual IgG levels against pertussis toxin (PT)(A), filamentous haemagglutinin (FHA)(B), and pertactin (Prn)(C) in sera from 485 subjects and days since booster dose as age 7–8 years (red circles), or since primary immunisation (no 4th dose) (blue triangles). The straight lines represent the linear regression lines of each data set. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

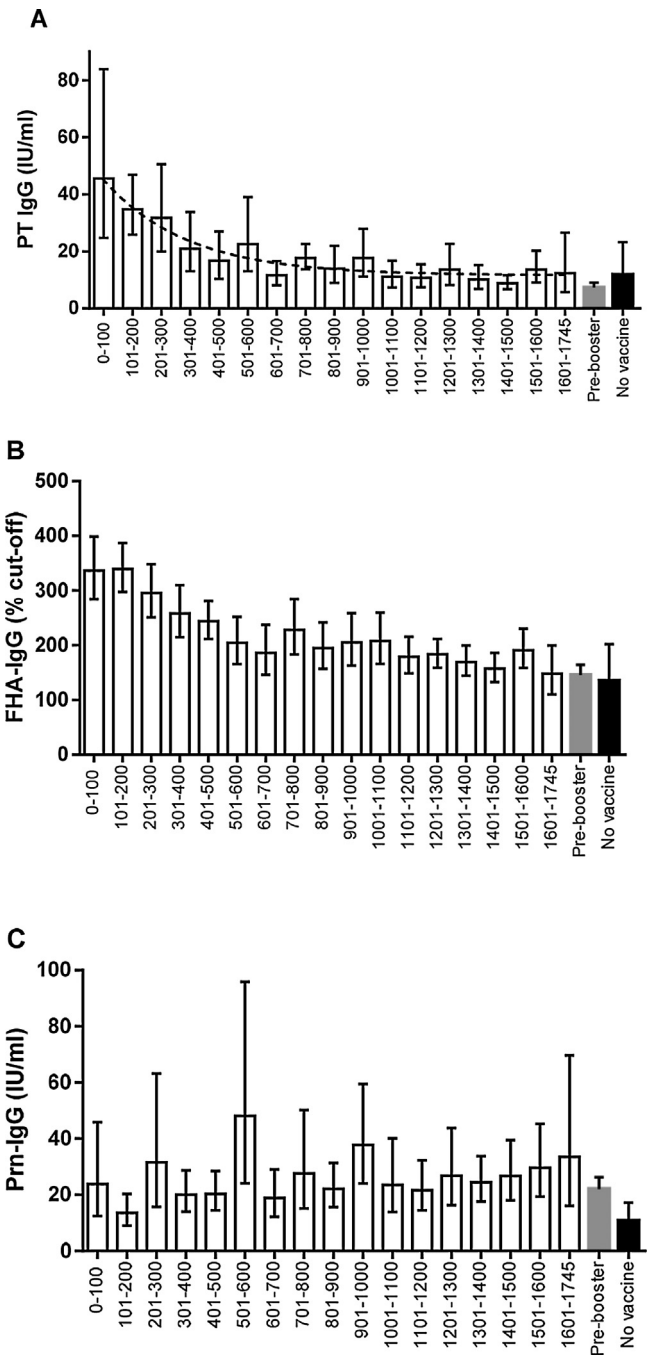

Fig. 2. Geometric mean and 95% CI of IgG antibody levels against PT (A), FHA (B), and Prn (C) and days since last DTaP-booster (4th dose). The stippled line in A indicates the non-linear fit against the geometric mean of the booster group. ("Pre-booster" means that no DTP vaccine has been recorded since the primary immunisation, and "No vaccine" means that no DTP vaccine is recorded ever.).

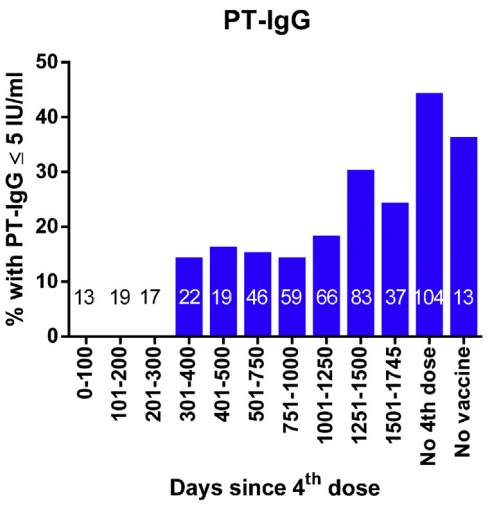

Fig. 3. Proportion of samples with anti-PT IgG ≤5 IU/ml and number of days since last DTP. The figures above the *X*-axis show the total number of sera in each aggregated group (total of 498 samples). (The first three groups contain 0% with anti-PT IgG ≤5 IU/ml.).

Caution should nevertheless be taken when results from different laboratories are compared; however the methods used are similar and have been compared through inter-laboratory evaluations. The differences observed are more likely explained by different vaccine history, different vaccines, different age groups, and possible interference from other vaccine antigens.

In line with the decrease of pertussis-specific antibodies, a higher number of sera with an anti-PT IgG level ≤5 IU/ml were found with increasing time since booster. Although there is no established serological correlate of protection against pertussis, it is likely that subjects with low vaccine-induced anti-PT IgG levels are less protected than subjects with higher levels [20,21]. This is also supported by data from the Norwegian Surveillance System for Communicable Diseases that show a peak in the incidence of pertussis at 6 year of age (prior to the booster), followed by a drop after the booster at age 7–8 years, and a subsequent large increase from the age of 11 to 15–16 years (Fig. 4) (Statistics from the Norwegian Surveillance System for Communicable Diseases, MSIS, Norwegian Institute of Public Health: http://www.msis.no/). It must be emphasised that the booster DTaP vaccine at age 7–8 years was implemented in 2006, and that the increase observed within the 11–15 years olds, most likely relates to individuals that were too old to have received this booster. However, the incidence figures from 2012 show an increased incidence starting already at the age of 10 years, i.e. in subjects who most likely have achieved the booster vaccine. These data thus indicate that the booster introduced in 2006 only protects for about 3–4 years. This is comparable to what have been observed in other countries recently [22,23].

About 10% of the sera revealed anti-Prn IgG levels >100 IU/ml. Such high anti-Prn IgG levels may be a result of the primary immunisations 6–11 years earlier, but this seems unlikely considering

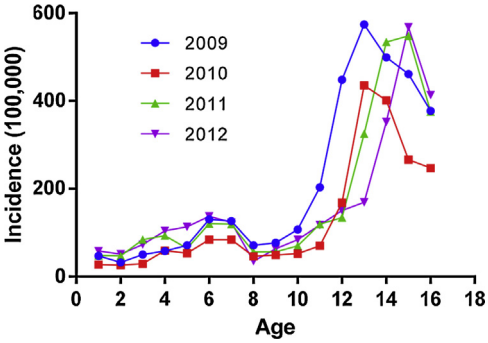

Fig. 4. The incidence of pertussis in the years 2009 to 2012 as notified to the Norwegian Surveillance System for Communicable Diseases (MSIS). A booster DTaP started in 2006 to 7–8 year old children, hence, the increased incidence seen between 11 and 15 years of age is of children that for the most were too old to have got the booster at age 7–8 years. The curves of the year 2011 and 2012 indicate that the booster only protects for about 3–4 years.

the rapid waning of pertussis specific antibody levels after vaccination [19]. This proportion of high Prn antibody levels can better be explained by infection with circulating Prn-expressing strains like *B. pertussis* or *Bordetella parapertussis* [24]. However, there was no significant correlation between the level of IgG against Prn and PT in these sera with high anti-Prn IgG. Prn is a very immunogenic antigen that readily gives rise to high antibody levels which may last for a long time [25,26]. Also, PRN antibodies might be induced earlier in infection and prevent disease, while PT antibodies are later induced in infection and after early signs of disease. Consequently, antibodies against Prn cannot be used to diagnose active pertussis, at least not from a single serum sample. Of importance in this regard is also the high frequency of circulating Prn-negative *B. pertussis* strains that have been observed in many countries recently [27,28]. In Norway around 20% of the analysed isolates from the last 5 years were found to be Prn-negative (unpublished observations).

For serological diagnostics, we have recommended a cut-off at 80 IU/ml in absence of recent vaccination. Only 9 of 130 sera (7%) had anti-PT IgG above this level within the two first years after the booster, and 6 of these samples were collected within the first year after the booster and thus most likely vaccine induced. This indicates that booster immunisation with aP vaccine interferes marginally with serological diagnostic, as previously described by others [12,14].

A limitation of this study might be that the sera were randomly picked from leftovers volumes of samples for clinical chemistry analysis. They were thus not from healthy children but rather from children under evaluation for different diseases/illnesses. It may thus be argued that such left-over sera may not be representative for the general population regarding the immune response against pertussis following infection or vaccination. However, the peak response observed in this study is very similar to what we observed in a previous vaccine study [17] Such left-over samples are often used for these kinds of studies, as in a recent seroprevalence study of pertussis involving sixteen European countries (Organization of a sereoprevalence study of pertussis in the member states and EEA countries (WP6); Specific Contract ECDC/2011/013). Sera from children where the medical record indicated possible immunodeficiency were excluded.

Another limitation may be associated to the reported pertussis incidence peak in 2009 compared to the next years. This may have caused an increased transmission of pertussis during the first months of collection. However, when the average anti-PT IgG levels were compared among sera collected at the start of the project with sera collected at the end of the project no differences were seen (data not shown).

In conclusion our data indicate that the immunity against pertussis is low 5 years after primary vaccination and that the DTaP-booster administered at age 7–8 years gives a moderate anti-pertussis immune response that wanes to near pre-booster level in a few years. This sero-epidemiological study contributes to the conclusion that some, if not all, of the aP vaccines are inadequate to reduce the burden of pertussis. Although serious disease in the smallest, most vulnerable, not completely vaccinated children still is rare due to mass vaccinations with aP, improved pertussis vaccines are needed. Improved vaccines should leave a longer-lasting immune response and should also harbour additional antigens that minimise the problems with vaccine escape mutant *B. pertussis* strains.

Acknowledgements

We gratefully acknowledge Samuel Merino at the Norwegian Institute of Public Health, for doing the anti-FHA IgG analysis.

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