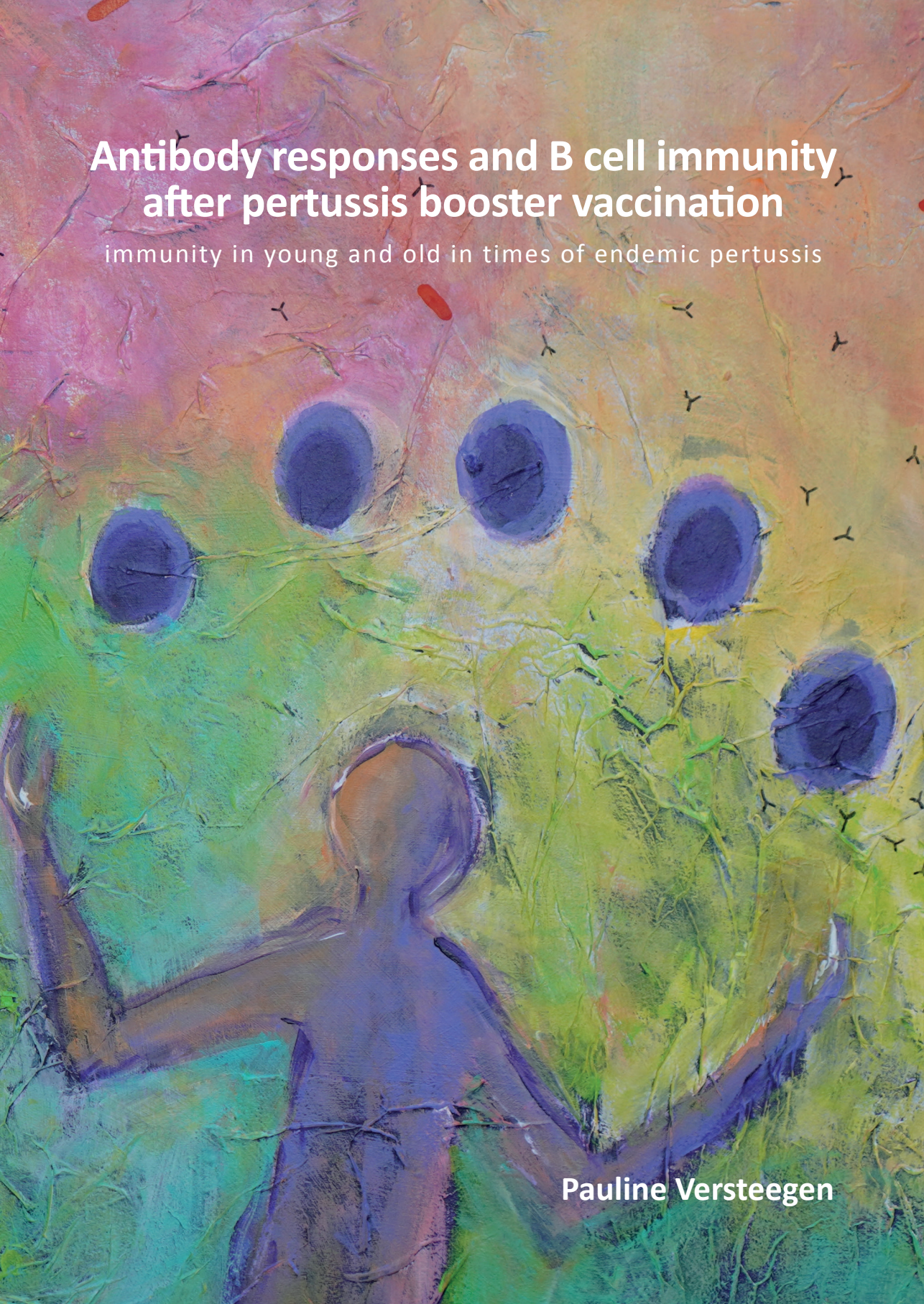
# Antibody responses and B cell immunity after pertussis booster vaccination

immunity in young and old in times of endemic pertussis



Pauline Versteegen

# Antibody responses and B cell immunity after pertussis booster vaccination

immunity in young and old in times of endemic pertussis

Pauline Versteegen

ISBN: 978-94-6506-024-8
DOI: https://doi.org/10.33540/2271
Cover design: Carla Frijnts
Lay-out: Ridderprint | www.ridderprint.nl
Print: Ridderprint | www.ridderprint.nl

**Antibody responses and B cell immunity after pertussis booster vaccination**
immunity in young and old in times of endemic pertussis

**Antistof respons en B-cel immuniteit na kinkhoest herhaal vaccinatie**
immuniteit in jong en oud ten tijde van endemische pertussis
(met een samenvatting in het Nederlands)

**Proefschrift**

ter verkrijging van de graad van doctor aan de
Universiteit Utrecht
op gezag van de
rector magnificus, prof. dr. H.R.B.M. Kummeling,
ingevolge het besluit van het college voor promoties
in het openbaar te verdedigen op

dinsdag 28 mei 2024 des middags te 2.15 uur

door

**Pauline Versteegen**

geboren op 27 december 1987
te Boxmeer

**Promotor:**
Prof. dr. E.A.M. Sanders

**Copromotoren:**
Dr. W.A.M. Berbers
Dr. A.M. Buisman

**Beoordelingscommissie:**
Prof. dr. L.J. Bont
Prof. dr. M.J.M. Bonten
Prof. dr. C.A.C.M. van Els
Prof. dr. M.I. de Jonge
Prof. dr. F. van Wijk (voorzitter)

## TABLE OF CONTENTS

# Chapter 1

# General introduction

## PERTUSSIS INFECTION AND DISEASE

Pertussis, or whooping cough, is a respiratory tract infection caused by the *Bordetella pertussis* bacterium [1]. After an incubation period of 7-10 days, it classically starts with a catarrhal stage characterised by coryza, low-grade fever and a mild, occasional cough [2]. This catarrhal stage is followed by the paroxysmal stage for 1-6 weeks characterised by a cough combined with either paroxysms of coughing and/or inspiratory whooping and/or post-tussive vomiting [2]. The final convalescent stage can take up to months and is characterised by gradual recovery and less persistent paroxysmal coughs [2]. Complications specifically described in infants are apnoea and refractory pulmonary hypertension, the latter might even lead to death [3]. In infants as well as children and adults complications like pneumonia, seizures, encephalopathy, anorexia, dehydration, epistaxis, hernias, otitis media and weight loss have been described [4]. In adolescents and adults syncope and rib fractures from severe coughing are reported complications as well[4]. Vaccinated individuals are more likely to have an atypical, mild or even asymptomatic course of infection without complications [5].

## BORDETELLA PERTUSSIS

*Bordetella pertussis* is a gram negative bacterium from the *Bordetella* genus [6]. The reservoir of this bacterium is the human. The bacterium *B. pertussis* has a reproduction number of 15-17 in a unvaccinated population ($R_0$) [7]. However in a vaccinated population ($R_t$) the reproduction number still is 5.5 [8]. Current acellular pertussis vaccines contain one or more antigens of *B. pertussis* that are somehow involved in the adhesion to airway epithelium like filamentous haemagglutinin (FHA), fimbriae types 2 and 3 (Fim), pertactin (Prn), and pertussis toxin (Ptx) [6]. Once the bacterium is attached to the airway epithelium, it can release its toxins of which Ptx is the most important one [6, 9]. Pertussis toxin (Ptx) is *B. pertussis* specific and is also the toxin most probably responsible for the specific symptoms of pertussis disease [10]. *B. pertussis* itself can effectively be eradicated using antibiotics, but it usually does not change the clinical course once Ptx is released [11].

## PERTUSSIS VACCINATION

In the 1950s a whole cell pertussis (wP) vaccine became available. This wP vaccine contained the whole bacterium and was quite effective in prevention of whooping cough [12]. However due to reactogenicity of the wP vaccine, many countries switched to acellular pertussis (aP) vaccines containing a subset of 1-5 purified *B. pertussis* antigens: pertussis toxin (Ptx), filamentous haemagglutinin (FHA), pertactin (Prn) and/or fimbriae types 2 and 3 (Fim) [13, 14]. Both wP and aP vaccines protect against clinical disease, however aP primed individuals were shown to be susceptible for infection sooner as compared to wP primed individuals [15]. From baboon studies, we know that wP vaccines as well as natural

infection protects better against colonisation and transmission compared with aP vaccines [16]. This study in baboons describes differentiation of CD4 T cells towards T helper (Th) 1 and Th17 memory in case of wP priming and priming by infection, while aP priming causes a shift towards Th 1 and Th2 memory. The immune response mismatch between infection and aP priming are likely to be at least partially responsible for the increased incidence. In order to improve protection against pertussis, alternative routes of administration are studied and new vaccines are under development like outer membrane vesicle vaccines and live attenuated vaccines [17].



In the Netherlands, we switched from wP to aP vaccines in 2005 (**Table 1**). Currently, infants in the Netherlands receive their DTaP-IPV-Hib-HepB priming vaccinations at the age of 3 and 5 months, in case their mothers received a maternal dTap vaccination, and at the age of 2, 3, and 5 months if their mothers refrained from maternal vaccination or when born premature. All infants receive a DTaP-IPV-Hib-HepB booster at the age of 11 months and a dTap-IPV booster at the age of 4 years [18]. Recently the Health Council of the Netherlands advised to postpone booster vaccinations to respectively 1 year and 5-6 years of age. Across Europe, infants generally receive 2 or 3 infant priming vaccinations in the first 6 months of life, a first booster before the age of 2 years and a second booster between 4 and 8 year of age. After this second booster between 4-8 years, national immunisation programmes (NIP) differ in recommendations for additional booster vaccinations. While most NIPs offer a maternal dTap(-IPV) vaccination to all pregnant women, some countries also offer a dTap(-IPV) to adolescents and/or to the entire adult population every 10 years or even every 5 years for older adults [19]. In the Netherlands, in case of adults, the NIP only explicitly advises pregnant women to receive an adult dTap(-IPV) booster at around 22 weeks gestational age [18]. Healthcare workers who professionally have contact with infants under 6 months of age are also advised to get an dTap(-IPV) booster every 5 years, however this is not part of the NIP [20].

**Table 1:** Dutch pertussis vaccination schedule over time.

| Year | Change | Vaccination at age of | Vaccine type |
|---|---|---|---|
| 1954 | Pertussis vaccine became available | | wP |
| 1957 | Start of the NIP: wP 3+1 schedule | 3, 4, 5, and 11 months | wP |
| 1998 | Prepone of the NIP | 2. 3, 4, and 11 months | wP |
| 2001 | | 2, 3, 4, and 11 months | wP |
| | Addition of an aP containing pre-school booster | 4 years | aP |
| 2005 | Switch from wP to aP component in primary series | 2, 3, 4, and 11 months | aP |
| | | 4 years | aP |
| 2019 | Introduction of maternal dTap vaccination, combined with 2+1 primary series | 22 weeks gestational age | aP |
| | | 3, 5, and 11 months* | aP |
| | | 4 years | aP |

* Infants of not maternally vaccinated mothers receive an extra priming vaccination (3+1) at the age of 2 months.

## PERTUSSIS EPIDEMIOLOGY

After introduction of whole cell pertussis vaccination in the 1950s pertussis reported incidence and mortality dropped enormously [21]. However, since the 1990/early 2000s, resurgence of pertussis was observed in the Netherlands as well as globally. Increase in notifications might be caused by increased awareness, improved diagnostics, and improved registration, but also by evolution of the bacterium and increased transmission [22, 23]. Also the switch from wP to aP vaccines in infancy is likely to have contributed to the increased incidence [16, 24]. Priming in infancy using aP containing vaccines leads to slower clearance of *B. pertussis* compared to wP priming and also aP priming protects less against transmission compared to wP priming, which is likely caused by the difference in CD4 T cell response.

Within Europe huge notification rate differences have been observed between countries [25]. This is likely partly caused by their difference in pertussis vaccines and vaccination schedules, vaccination coverage, population density and contact patterns, but probably also differences in awareness, pertussis registration definition, registration tools, and diagnostic tools play a major role in this. Therefore notification rates are difficult to compare between countries. Seroprevalence studies are not influenced by factors like awareness and diagnostic tools but the comparability of these studies is still difficult since the participant selection methods are often different for these studies, no correlate of protection (CoP) is available, different serological cut-offs are used, and different assays are used [26-28]. However seroprevalence studies offer the opportunity to monitor infection prevalence over time. In the Netherlands we have seen an increase in the proportion of recently infected individuals in the Dutch population between 1995/1996 and 2006/2007 from 1 to 3,5% of the population over 9 years of age [29].

## HUMORAL IMMUNE RESPONSES

Generally, exposure to an antigen leads to activation and differentiation of B cells and subsequently immunoglobulins (Ig) are produced by plasma cells. Immunoglobulins, also known as antibodies, are the main effector molecule of the humoral immunity. Immunoglobulin M (IgM) is the first antibody isotype produced early during an immune response. After class switching IgG, IgA, and IgE are produced depending on the route of activation. When comparing total Ig in different age groups, an increase in Ig concentrations with age has been described [30]. This increase with age was most pronounced for IgA and less for IgG.

In case of intramuscular vaccination against pertussis, predominantly IgG will be produced, while respiratory infection with *B. pertussis* also induces mucosal and serological IgA production [31]. When pertussis specific antibodies post-vaccination are quantified, it

is common to measure antigen specific IgG in blood serum [32]. Pertussis specific IgG concentrations increase within a month upon vaccination. Rapidly waning antibody concentrations in serum are seen in the first year after booster vaccination in children and adults, but generally remain above pre-booster concentrations, similar to circulating memory B cells [33, 34]. Opposing differences in IgG concentrations against pertussis after aP versus wP primed individuals have been described. While aP primed children 4 years of age generally showed a stronger IgG response after an aP booster, children 9 years of age appeared to have a stronger responses when they had received wP vaccines in infancy [35]. Differences between aP and wP priming in infancy have been described for several functional serological assays as well. Bacterial adhesion inhibition in vitro seems to be positively affected by wP priming, while the neutralising capacity in vitro seems to be positively affected by aP priming [36, 37]. Not so much is known about the uphold of antibodies on the long-term. One study describes rapidly waning antibody concentrations in adults and adolescents while another study predicts a much slower antibody decline in adults [34, 38].



## B CELL RESPONSES

B cells derive from hematopoietic stem cells and mature in the bone marrow [39]. From there B cells migrate to secondary lymphoid organs where B cell activation begins by binding to an antigen and by T cell help [39]. Activated B cells proliferate and undergo somatic hypermutation. Some differentiate into long-lived plasma cells residing in the bone marrow to excrete large quantities of antibodies [40]. Others differentiate into antigen specific memory B cells circulating through the body. These memory B cells get reactivated upon encounter with a pathogen displaying the antigen the memory B cells recognises. Upon reactivation, memory B cells either directly differentiate into short-lived plasma cells to quickly produce lots of antibodies, or re-enter a germinal centre reaction, to become a renewed memory B cell or a long-lived plasma cell [40]. Dependent on the route of encounter, activated B cells can switch isotype by T cell help.

In case of intramuscular vaccination against pertussis, this will mainly be towards IgG producing B cells, while infection with *B. pertussis* will additionally cause isotype switching towards IgA producing B cells via the mucosal route [31]. As described for the pertussis specific antibody concentrations post-booster vaccination, the amount of antigen specific IgG memory B cells increases upon booster vaccination with a subsequent decrease in the first year post-booster though remaining above pre-booster frequencies in collected blood samples [33, 34]. Differences caused by priming vaccinations vary by age. Higher memory B cell frequencies were observed in aP primed children 4 years of age after an aP booster, while children 9 years of age appeared to have higher memory B cell frequencies when they had received wP vaccines in infancy. However, even in not recently vaccinated individuals,

pertussis specific memory B cells can be demonstrated pointing in the direction of long lasting immunity after priming vaccinations or pertussis infections [41].

## CORRELATES OF PROTECTION

A correlate of protection (CoP) for pertussis has not yet been identified. A household study in the 1990 suggested the CoP against disease to be IgG-Ptx ≥ 20 elisa units (EU) [42]. However, elisa units are not standardised and therefore the exact antibody quantity of this potential CoP is unclear. Furthermore, this household study pointing to a CoP of 20 EU/ml was performed in the era that wP vaccines were used and when the whooping cough epidemiology was completely different. Currently the proportion of aP primed individuals is increasing and whooping cough has a cyclic pattern, which might influence protection against infection and disease as well. Other studies searched for CoPs and described associations with antibody concentrations against Ptx, Prn, FHA, and/or Fim, but also cellular responses are described to be a candidate biomarker for protection [42-51]. However, to date, no golden standard for a pertussis CoP is generally accepted.

## AGEING AND VACCINES

Weaker immune responses after an encounter with a specific stimulus in older adults compared with other ages groups have been described [52]. This phenomenon is commonly known as immunosenescence and affects vaccine responses that are often reduced [53]. Aged memory B cells are less capable to differentiate into plasma cells post-challenge, causing antigen specific antibody production to decline with age [54, 55]. Not only quantitative antibody responses upon vaccination are often lower in older adults compared to younger adults, but also the functionality of these antibodies may be less [56, 57].

## SCOPE OF THIS THESIS

Despite high vaccination coverage, pertussis is still circulating, emphasising the need for better vaccines. To set out a desirable vaccine induced immune profile for future vaccines, it is important to know how the currently available aP vaccine influences both quantitative and functional antibody responses as well as cellular responses, and how those responses are affected by age, epidemiology and individual vaccination history.

### Research aim of this thesis

We started with exploring the current infection prevalence based on serology in the Netherlands followed by an evaluation of the immune response after an extra aP booster vaccination, making use of various age groups, vaccination backgrounds, and countries. This is relevant for future vaccination strategies.

The following five research questions were addressed:

- How did the infection prevalence in the Dutch population change over time? (Chapter 2)
- Do changes in infection prevalence have potential implications for the lifelong national immunisation programme (Chapter 2)?
- What is the value of different assays on B cell and humoral immunity in evaluating aP booster vaccination responses (Chapter 3-6)?
- Are aP booster vaccination responses and hybrid immunity influenced by age? (Chapter 3-7)
- Are aP booster vaccination responses influenced by type of priming vaccination? (Chapter 3-7)



## OUTLINE OF STUDIES

To address these research questions, we made use of multiple studies. The main study of this thesis is performed within the PERISCOPE project which is part of the Innovative Medicines Initiative 2 programme. The other studies that contribute to answer the research questions were performed at the Nation Institute for Public Health and the Environment (RIVM).

### National immunisation programme evaluation study at the RIVM

To understand the magnitude of the problem of pertussis circulation the PIENTER (Peiling Immunisatie Effect Nederland Ter Evaluatie van het Rijksvaccinatieprogramma) study was used. This is a population-based cross-sectional serosurvey of a representative sample of Dutch residents performed by the RIVM every decade. Samples of the PIENTER 3 study were collected in 2016 and 2017, samples of the PIENTER 1 and 2 studies were collected in respectively 1995/1996 and 2006/2007. The samples were tested on the presence of Ptx-IgG antibody concentrations of which high levels are indicative for recent pertussis infection (**Chapter 2**).

### Innovative Medicines Initiative 2 Programme: PERISCOPE project

The aim of the PERISCOPE (PERtussIS COrrelates of Protection Europe) consortium is to identify a correlate of protection against pertussis infection, colonisation and disease and to create a platform where new pertussis vaccines can be evaluated against the two currently available vaccine types: aP and wP vaccines. Within the PERISCOPE consortium several vaccination studies are executed, as well as pre-clinical models and a human challenge model where volunteers get exposed to *B. pertussis*.

The main study of this thesis is performed within this PERISCOPE project and is called the BERT (Booster against pERTussis ) study. The BERT study is a longitudinal interventional study executed in the Netherlands, Finland and the United Kingdom. School-aged children, adolescents, young adults, and older adults were vaccinated with a three pertussis components containing vaccine and were followed-up for one year. We studied the quantitative **(Chapter 3)** and qualitative **(Chapter 4)** antibody responses as well as the qualitative (**Chapter 5**) and quantitative (**Chapter 6**) B cell responses extensively. Other partners within the consortium used the same samples to study mucosal immunity, T cell responses or myeloid cells subtypes activation.

**Pertussis vaccination studies at the RIVM**

For effectiveness of a pertussis booster vaccination on the long-term within different age groups, we followed-up three longitudinal intervention studies. The first study was the KIM (Kinkhoest IMmunisatie) study, where a cohort of aP primed children received an additional dTap booster at the age of 9 years. The second study concerned the Booster study and comprises a cohort of wP primed children who received an additional dTap booster at the age of 9 years. The third study was the VIKING (Volwassenen Immuniseren tegen KInkhoest, Nederland Gezond) study and comprises a cohort of whole cell primed adults who received an additional dTap booster between 25 and 29 years of age. All participants of the three studies were followed-up at approximately 6 years post additional booster. In these follow-up samples we measured pertussis antibody concentrations and the results were used to create a prediction model for pertussis antibody decay and the proportion of participants assumed to be protected (**Chapter 7**).

This thesis concludes with a general discussion summarising and evaluating the main findings of the clinical studies.

## REFERENCES


1. WHO. Health topics: Pertussis. [cited 2024 10 January]; Available from: https://www.who.int/health-topics/pertussis#tab=tab_1.
2. CDC. Pertussis (Whooping Cough) - Clinical Features. 2019 25-10-2019 25-07-2022]; Available from: https://www.cdc.gov/pertussis/clinical/features.html.
3. Cherry, J.D., Pertussis in Young Infants Throughout the World. Clin Infect Dis, 2016. 63(suppl 4): p. S119-S122. doi: 10.1093/cid/ciw550.
4. CDC. Pertussis (Whooping Cough) Clinical Complications. 2019 25-10-2019 25-07-2022]; Available from: https://www.cdc.gov/pertussis/clinical/complications.html.
5. Craig, R., et al., Asymptomatic Infection and Transmission of Pertussis in Households: A Systematic Review. Clin Infect Dis, 2020. 70(1): p. 152-161.
6. Finger, H. and C.H.W. von Koenig, Bordetella, in Medical Microbiology, S. Baron, Editor. 1996, University of Texas Medical Branch at Galveston Copyright © 1996, The University of Texas Medical Branch at Galveston.: Galveston (TX).
7. ECDC. Expert consultation on pertussis. 2014 12-05-2014 25-07-2022]; Available from: https://www.ecdc.europa.eu/en/publications-data/expert-consultation-pertussis.
8. Kretzschmar, M., P.F. Teunis, and R.G. Pebody, Incidence and reproduction numbers of pertussis: estimates from serological and social contact data in five European countries. PLoS Med, 2010. 7(6): p. e1000291.
9. Carbonetti, N.H., Contribution of pertussis toxin to the pathogenesis of pertussis disease. Pathog Dis, 2015. 73(8): p. ftv073.
10. Scanlon, K., C. Skerry, and N. Carbonetti, Association of Pertussis Toxin with Severe Pertussis Disease. Toxins (Basel), 2019. 11(7).
11. Altunaiji, S.M., et al., Antibiotics for whooping cough (pertussis). Cochrane Database of Systematic Reviews, 2007(3).
12. Standfast, A.F., Pertussis, typhoid-paratyphoid and cholera vaccines. Br Med Bull, 1969. 25(2): p. 189-94.
13. Lambert, L.C., Pertussis vaccine trials in the 1990s. J Infect Dis, 2014. 209 Suppl 1(Suppl 1): p. S4-9.
14. David, S., P.E. Vermeer-de Bondt, and N.A.T. van der Maas, Reactogenicity of infant whole cell pertussis combination vaccine compared with acellular pertussis vaccines with or without simultaneous pneumococcal vaccine in the Netherlands. Vaccine, 2008. 26(46): p. 5883-5887, DOI: 10.1016/j.vaccine.2008.07.105.
15. Sheridan, S.L., et al., Unexpectedly limited durability of immunity following acellular pertussis vaccination in preadolescents in a North American outbreak. Clin Infect Dis, 2012. 55(10): p. 1434-1435; author reply 1435-6. doi: 10.1093/cid/cis672.
16. Warfel, J.M., L.I. Zimmerman, and T.J. Merkel, Acellular pertussis vaccines protect against disease but fail to prevent infection and transmission in a nonhuman primate model. Proc Natl Acad Sci U S A, 2014. 111(2): p. 787-792, DOI: 10.1073/pnas.1314688110.
17. Locht, C., The Path to New Pediatric Vaccines against Pertussis. Vaccines (Basel), 2021. 9(3).
18. National Institute for Public Health and the Environment (RIVM), RVP-richtlijn Uitvoering Rijksvaccinatieprogramma. 2022: Bilthoven.

**19.** ECDC. Pertussis: Recommended vaccinations. 2020 [cited 2020 16 September]; Available from: https://vaccine-schedule.ecdc.europa.eu/Scheduler/ByDisease?SelectedDiseaseId=3&SelectedCountryIdByDisease=-1.

**20.** National Coordination Centre for Communicable Disease Control (LCI). Kinkhoestvaccinatie volwassenen - Factsheet. Available from: https://lci.rivm.nl/richtlijnen/kinkhoestvaccinatie-volwassenen.

**21.** WHO. 02-08-2022]; Available from: https://www.who.int/teams/health-product-policy-and-standards/standards-and-specifications/vaccine-standardization/pertussis.

**22.** Nieves, D.J. and U. Heininger, Bordetella pertussis. Microbiol Spectr, 2016. 4(3): p. EI10-0008-2015. doi: 10.1128/microbiolspec.EI10-0008-2015.

**23.** Cherry, J.D., Pertussis: challenges today and for the future. PLoS Pathog, 2013. 9(7): p. e1003418. doi: 10.1371/journal.ppat.1003418.

**24.** Sheridan, S.L., et al., Waning vaccine immunity in teenagers primed with whole cell and acellular pertussis vaccine: recent epidemiology. Expert Rev Vaccines, 2014. 13(9): p. 1081-1106. doi: 10.1586/14760584.2014.944167.

**25.** ECDC. Disease data from ECDC Surveillance Atlas for pertussis. 2019 [cited 2019 20 September]; Available from: http://ecdc.europa.eu/en/pertussis/surveillance-and-disease-data/atlas].

**26.** Berbers, G., et al., Circulation of pertussis and poor protection against diphtheria among middle-aged adults in 18 European countries. Nat Commun, 2021. 12(1): p. 2871.

**27.** Wehlin, L., et al., Pertussis seroprevalence among adults of reproductive age (20-39 years) in fourteen European countries. Apmis, 2021. 129(9): p. 556-565. doi: 10.1111/apm.13165.

**28.** Conde-Glez, C., et al., Seroprevalence of Bordetella pertussis in the Mexican population: a cross-sectional study. Epidemiol Infect, 2014. 142(4): p. 706-13.

**29.** de Greeff, S.C., et al., Seroprevalence of pertussis in The Netherlands: evidence for increased circulation of Bordetella pertussis. PLoS One, 2010. 5(12): p. e14183, DOI: 10.1371/journal.pone.0014183.

**30.** Blanco, E., et al., Age-associated distribution of normal B-cell and plasma cell subsets in peripheral blood. J Allergy Clin Immunol, 2018. 141(6): p. 2208-2219.e16.

**31.** Hendrikx, L.H., et al., Serum IgA responses against pertussis proteins in infected and Dutch wP or aP vaccinated children: an additional role in pertussis diagnostics. PLoS One, 2011. 6(11): p. e27681.

**32.** WHO TEAM Health Product Policy and Standards & Norms and Standards for Biological Products, Recommendations to assure the quality, safety and efficacy of acellular pertussis vaccines. 2014. p. 74.

**33.** Hendrikx, L.H., et al., Enhanced memory B-cell immune responses after a second acellular pertussis booster vaccination in children 9 years of age. Vaccine, 2011. 30(1): p. 51-8.

**34.** van der Lee, S., et al., Robust Humoral and Cellular Immune Responses to Pertussis in Adults After a First Acellular Booster Vaccination. Front Immunol, 2018. 9: p. 681.

**35.** van der Lee, S., et al., Whole-Cell or Acellular Pertussis Primary Immunizations in Infancy Determines Adolescent Cellular Immune Profiles. Front Immunol, 2018. 9: p. 51. doi: 10.3389/fimmu.2018.00051.

**36.** Zanaboni, E., et al., A novel high-throughput assay to quantify the vaccine-induced inhibition of Bordetella pertussis adhesion to airway epithelia. BMC Microbiol, 2016. 16: p. 215.

**37.** Giuliano, M., et al., Antibody responses and persistence in the two years after immunization with two acellular vaccines and one whole-cell vaccine against pertussis. J Pediatr, 1998. 132(6): p. 983-8.

**38.** Pool, V., et al., Humoral immunity 10years after booster immunization with an adolescent and adult formulation combined tetanus, diphtheria, and 5-component acellular pertussis vaccine in the USA. Vaccine, 2018. 36(17): p. 2282-2287. doi: 10.1016/j.vaccine.2018.03.029.

**39.** Parham, P., The Immune System. third edition ed. 1950, New York: Garland Science.

**40.** Palm, A.E. and C. Henry, Remembrance of Things Past: Long-Term B Cell Memory After Infection and Vaccination. Front Immunol, 2019. 10: p. 1787.

**41.** Buisman, A.M., et al., Long-term presence of memory B-cells specific for different vaccine components. Vaccine, 2009. 28(1): p. 179-186. doi: 10.1016/j.vaccine.2009.09.102.

**42.** Long, S.S., C.J. Welkon, and J.L. Clark, Widespread silent transmission of pertussis in families: antibody correlates of infection and symptomatology. J Infect Dis, 1990. 161(3): p. 480-6.

**43.** Cherry, J.D., et al., A search for serologic correlates of immunity to Bordetella pertussis cough illnesses. Vaccine, 1998. 16(20): p. 1901-6.

**44.** He, Q., et al., Protective role of immunoglobulin G antibodies to filamentous hemagglutinin and pertactin of Bordetella pertussis in Bordetella parapertussis infection. Eur J Clin Microbiol Infect Dis, 1996. 15(10): p. 793-798. doi: 10.1007/bf01701521.

**45.** Hewlett, E.L. and S.A. Halperin, Serological correlates of immunity to Bordetella pertussis, in Vaccine. 1998: Netherlands. p. 1899-900.

**46.** Marcellini, V., et al., Protection against Pertussis in Humans Correlates to Elevated Serum Antibodies and Memory B Cells. Front Immunol, 2017. 8: p. 1158.

**47.** Storsaeter, J., et al., Low levels of antipertussis antibodies plus lack of history of pertussis correlate with susceptibility after household exposure to Bordetella pertussis. Vaccine, 2003. 21(25-26): p. 3542-9.

**48.** Storsaeter, J., et al., Levels of anti-pertussis antibodies related to protection after household exposure to Bordetella pertussis. Vaccine, 1998. 16(20): p. 1907-16.

**49.** Taranger, J., et al., Correlation between pertussis toxin IgG antibodies in postvaccination sera and subsequent protection against pertussis. J Infect Dis, 2000. 181(3): p. 1010-3.

**50.** Tran Minh, N.N., et al., Cell-mediated immune responses to antigens of Bordetella pertussis and protection against pertussis in school children. Pediatr Infect Dis J, 1999. 18(4): p. 366-70.

**51.** Hellwig, S.M., et al., Crucial role of antibodies to pertactin in Bordetella pertussis immunity. J Infect Dis, 2003. 188(5): p. 738-742. doi: 10.1086/377283.

**52.** Gustafson, C.E., et al., Influence of immune aging on vaccine responses. J Allergy Clin Immunol, 2020. 145(5): p. 1309-1321. doi: 10.1016/j.jaci.2020.03.017.

**53.** Crooke, S.N., et al., Immunosenescence and human vaccine immune responses. Immun Ageing, 2019. 16: p. 25. doi: 10.1186/s12979-019-0164-9.

**54.** Pritz, T., et al., Plasma cell numbers decrease in bone marrow of old patients. Eur J Immunol, 2015. 45(3): p. 738-46.

**55.** Howard, W.A., K.L. Gibson, and D.K. Dunn-Walters, Antibody quality in old age. Rejuvenation Res, 2006. 9(1): p. 117-25.

**56.** Goodwin, K., C. Viboud, and L. Simonsen, Antibody response to influenza vaccination in the elderly: a quantitative review. Vaccine, 2006. 24(8): p. 1159-69.

**57.** Schenkein, J.G., S. Park, and M.H. Nahm, Pneumococcal vaccination in older adults induces antibodies with low opsonic capacity and reduced antibody potency. Vaccine, 2008. 26(43): p. 5521-6.

# Chapter 2

# More than 10 years after introduction of an acellular pertussis vaccine in infancy: a cross-sectional serosurvey of pertussis in the Netherlands

P. Versteegen, G. A. M. Berbers, G. Smits, E. A. M. Sanders, F. R. M. van der Klis, H. E. de Melker, and N. A. T. van der Maas.

The Lancet Regional Health – Europe 10 (Sept 05 2021): 100196.

## ABSTRACT

**Background:** Pertussis is a respiratory disease and still endemic despite high vaccination coverage. In the Dutch national immunisation programme (NIP) whole cell pertussis (wP) priming vaccines for infants were replaced by acellular pertussis (aP) priming vaccines in 2005. Serosurveillance gives the opportunity to objectively monitor effects of changes in the NIP on infection prevalence and vaccine response in the population over time.

**Methods:** For this population-based cross-sectional serosurvey a representative sample of Dutch residents (0-89 years) was drawn in 2016/2017. Primary outcome was the percentage of participants with pertussis toxin specific antibody concentrations ≥ 100 IU/ml as an indicator of recent infection, and to identify groups possibly more vulnerable to pertussis infection. Percentages were compared with previous results from 2006/2007.

**Findings:** In total 7621 persons were included in the analysis. An increase in recent infections from 3.5% to 5.9% was found in the population from 7 years and older (n=6013) in 2016/2017 compared with 2006/2007. Most noteworthy increase was seen in 12-18-year-olds who were wP primed and aP boosted.

**Interpretation:** Infection prevalence is still increasing in the Netherlands inducing a risk of pertussis disease in vulnerable (age) groups. Delaying the preschool booster might prolong the period of protection during primary school and thereby possibly protect younger siblings. Extra boosters might be considered for risk populations like older adults and people with (pulmonary) co-morbidities, since they have higher chances of complications and hospitalisation.


**Funding:** The Dutch Ministry of Health, Welfare, and Sport.

An unedited Dutch translation of the abstract is available in Supplementary text 1: Nederlandse samenvatting.

## RESEARCH IN CONTEXT

**Evidence before this study:** Pertussis is a severe respiratory disease. Despite available vaccines and high vaccination coverage, pertussis is still endemic. Two previous serosurveys approximately 10 and 20 years ago described an increase in pertussis infection prevalence from 1% to 3.4% in the Dutch population of 9 years and older. In the meantime, several changes have taken place in the Dutch national immunisation programme, with the most important ones being the switch from whole cell infant priming vaccinations to acellular infant priming vaccinations in 2005 and the addition of an acellular pertussis preschool booster in 2001. We searched PubMed for articles from 2005 up to March 2020 with no language restrictions, using the terms: (sero surveillance OR serosurvey OR seroprevalence OR seroepidemiology) AND pertussis AND population AND cross-sectional. This search revealed several studies from all over the world. Most studies described sero-positivity and some additionally described percentage of recent infection. Peak incidence was mostly in children and adolescents, dependent on age of last vaccination in combination with the epidemic pattern. One study in New South Wales reported results from three subsequent surveys representing different timings of the epidemic cycle: during an epidemic (1997/1998), post-epidemic (2002), and inter-epidemic (2007). Inter-epidemic the proportion of recent infections in the study population was lowest. Material of the study was dependent on residuals of specimens submitted for diagnostic testing. Our study also compares consecutive surveys, all executed in a similar manner with the advantage of a representative sample of the Dutch population and the availability of extensive questionnaire data, e.g. on demographics and vaccination status. The first survey (1995/1996) started before the first outbreak and continued during the first part of the very first outbreak since the introduction of pertussis vaccines in the national immunisation programme. The second survey (2006/2007) started two years after an epidemic and an increase in incidence took place during study inclusion. The current survey (2016-2017) started two years after the last epidemic we have had in the Netherlands.

**Added value of this study:** The here described population-based cross-sectional serosurvey showed an overall increase in pertussis infection prevalence from 3.5% to 5.9% in the Dutch population from 7 years and older during a decade. Whole cell primed adolescents of 12-18 years not only had the highest percentage of recent infections, but also showed the greatest increase. Acellular primed 7-11-years-olds and whole cell primed 50-64-year-olds showed significant increases as well.

**Implications of all the available evidence:** This study emphasises that priming with either a whole cell or an acellular vaccine is not the only factor involved in the vulnerability for pertussis infection but also in the degree of circulation. Since pertussis infections in vaccinated individuals manifest itself usually mild or subclinical, extra booster vaccinations for school aged children, adolescents, or adults do not seem beneficial to add to the

NIP. However, it might be considered to delay the 4 year olds booster with two years to extend the period of vaccine-induced protection and thereby possibly protect younger unprotected siblings. Extra booster doses should be considered only for risk populations like older adults and people with (pulmonary) comorbidities like is common in the Netherlands for the flu vaccine.

## INTRODUCTION

Pertussis is a severe respiratory disease caused by *Bordetella pertussis* and is transmitted between humans by coughing and sneezing [1]. Pertussis presents typically with paroxysmal coughing, inspiratory whooping, and posttussive vomiting and can affect individuals of all ages, although infants are at greatest risk of serious complications [2]. Older adults and people with (pulmonary) comorbidities are also at risk of complications and hospitalisation [3]. Since the start of the national immunisation programme (NIP) in 1957 in the Netherlands with a whole cell pertussis (wP) vaccine, disease incidence and mortality dropped enormously, but from 1996 onwards pertussis epidemics have been observed regularly (**Figure 1**). Since then, several changes have been implemented in the NIP.



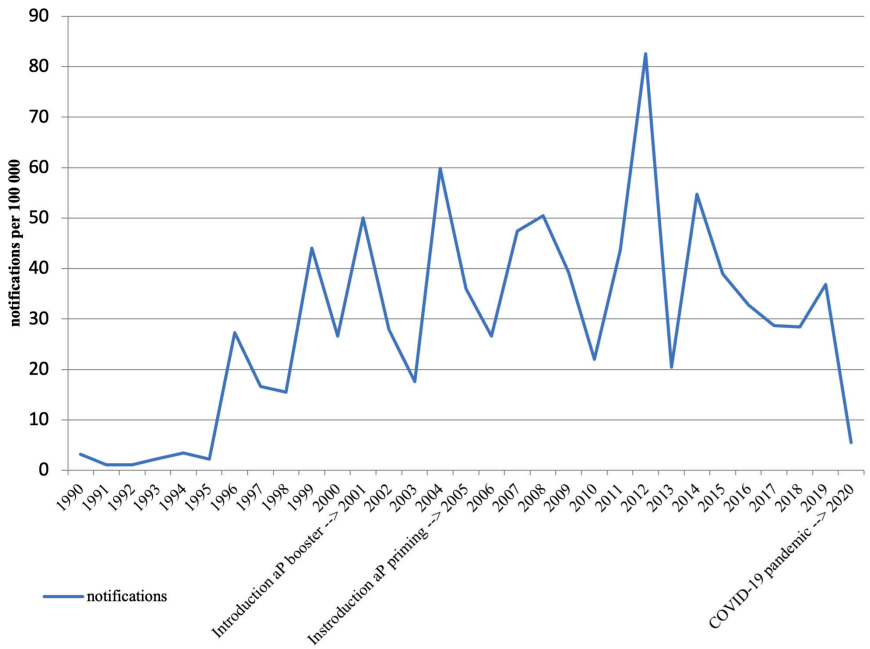



**Figure 1:** Pertussis notifications.

The most important changes in the NIP with a possible impact on this serosurveillance study were the switch from wP priming in infancy to priming with acellular pertussis (aP) vaccines in 2005 and the addition of an aP booster at 4 years of age in 2001 [4]. The switch from wP to aP priming has been made because of reactogenicity of the Dutch wP vaccine [5]. Moreover, the Dutch wP vaccine had a low estimated vaccine effectiveness since the early 1990s [6]. The aP vaccines in the Dutch NIP contained at least pertussis toxin (Ptx),

filamentous haemagglutinin (FHA), and pertactin (Prn) (aP3), and sometimes additionally fimbriae types 2 and 3 (Fim2/3) (aP5). Over the years, different aP3/5 vaccines have been used with various amounts of antigens. Vaccination with aP vaccines provides higher antibody concentrations to the vaccine antigens, but appears to be less protective on the long term [7]. Other adjustments in the NIP were acceleration of the priming schedule from 3, 4, 5 and 11 months of age to 2, 3, 4, and 11 months in 1999 and the implementation of the maternal pertussis vaccination late 2019. An overview of all changes in the Dutch NIP concerning pertussis vaccines is illustrated in **Supplementary Table 1**.

Serosurveillance gives the opportunity to monitor infection prevalence and vaccine response in a population over time, while notification rates are dependent on factors like awareness of disease and the tendency of the public to visit a doctor. In New South Wales three subsequent serosurveys showed a decrease in recent infections over time, representing consecutively a survey during an epidemic, a survey post-epidemic, and a survey inter-epidemic [8]. To monitor the impact of the NIP in the Netherlands at antibody level, several serosurveillance studies were performed over time as well [9, 10]. Previously, an increase in infection prevalence -as indicated by a Ptx IgG level 100 IU/ml- from 1.0% to 3.4% in the population over 9 years of age was observed between 1995-1996 (1996 first epidemic) and 2006-2007 (last epidemic 2004, next epidemic 2008) [11].

In the Netherlands a relatively high vaccination coverage was continually achieved (92-96%) despite the existence of low vaccination coverage (LVC) areas [12]. The country average vaccination coverage for pertussis at one year of age was 95% and 94% during study inclusion in 2016 and 2017, respectively. The LVC areas have a relatively high percentage of vaccination-refusers based on religious grounds [12].

In the current cross-sectional serosurveillance study performed in 2016-2017, we investigated the change in seroepidemiology ten years after the previous serosurvey and whether changes were possibly related to the switch from wP to aP priming in 2005 or to the in 2001 implemented aP booster vaccination at 4 years of age. Other possible influencing factors, like religion, were also analysed.

## METHODS

### Study design and participants

From February 2016 through October 2017 a national serumbank for cross-sectional population-based serosurveillance studies was established, as previously described [13]. In short, for the national sample (NS) an age-stratified two-stage cluster sample was drawn from the population register in forty municipalities and an additional sample in nine LVC municipalities, resulting in a NS of 5745 Dutch residents (0-89 years of age) and 1354 persons living in LVC areas. Participants were asked to donate blood, fill in a questionnaire

and bring their vaccination certificate. The study was approved by the Medical Ethics Committee Noord-Holland (METC number: M015-022) and designed and conducted in accordance with the guidelines of the Declaration of Helsinki (1996). Written informed consent was obtained from all adult participants and from parents or legal guardians of minors. A summary of the vaccination background of the different age groups can be viewed in **Table 1**.

**Table 1:** Vaccination background.

| Age category | Serosurvey 2006/2007 | Serosurvey 2016/2017 |
|---|---|---|
| 0 y | aP priming<br>- | aP priming<br>- |
| 1 y | aP priming<br>- | aP priming<br>- |
| 2 y | wP priming<br>- | aP priming<br>- |
| 3 y | wP priming<br>- | aP priming<br>- |
| 4-6 y | wP priming<br>aP booster | aP priming<br>aP booster |
| 7-11 y | wP/aP primed<br>- | aP priming<br>aP booster |
| 12-18 y | wP primed<br>- | wP priming<br>aP booster |
| 19-34 y | wP primed<br>- | wP priming<br>- |
| 35-49 y | wP primed<br>- | wP priming<br>- |
| 50-64 y | wP primed/unvaccinated<br>- | wP priming/unvaccinated<br>- |
| 65-79 y | wP primed/unvaccinated<br>- | wP priming/unvaccinated<br>- |
| 80 + y | N/A | wP priming/unvaccinated<br>- |

## Serological analysis

Serum IgG concentrations against Ptx (NVI), FHA (Kaketsuken), and Prn [14] were quantified using the fluorescent-bead-based multiplex immunoassay (MIA) as previously described [15, 16]. The measurement was performed using a BioPlex 200 combined with BioPlex Manager 6.1 (Bio-Rad Laboratories). To express antibody concentrations in IU/ml, an in-house standard, calibrated on the Pertussis Antiserum (human) 1st WHO International Standard was used. The lower limits of quantification (LLOQs) were 1.0 IU/ml for all antigens. Values below LLOQs were analysed as 1/2 LLOQ.

### Serosurveillance study 2006-2007

Results from the current serosurveillance study were compared with those from the 2006/2007 study, which was conducted in a similar way [10, 11]. Briefly, 5,740 participants were included in the NS and 1,518 in the LVC group. IgG-Ptx levels were measured using the same MIA, but were expressed in EU/ml [15]. EU/ml was transformed to IU/ml as earlier described [16]. A summary of the vaccination background of the different age groups can be viewed in **Table 1**.

### Statistical analysis

Every participant was assigned a sampling weight incorporating the probability of selection and adjustment for age, sex, urbanisation degree, and ethnicity [13]. Participants were divided in age categories, mainly based on differences in vaccination history of the participants of the current study (**Table 1**). Participants 0-11 years of age received a similar pertussis vaccination schedule but were divided in small age groups to show the presence or absence of vaccination effects. Participants 12-18 years of age received a similar schedule and were therefore taken together. Participants 19 years and older all received the same vaccination schedule and were divided in age groups spanning 15 years.

Primary study outcome is pertussis infection prevalence in the national sample and in different age groups. Participants were divided in three categories based on the level of their Ptx IgG antibody concentration. Serological cut-offs to indicate pertussis infection are not unanimous since there is no known correlate of protection [17]. We used an IgG-Ptx level of 100 IU/ml to be indicative for recent infection in absence of a vaccination in the last few years, which is used as diagnostic cut-off in the Netherlands for pertussis infection using a single serum sample [18]. IgG-Ptx levels 50-100 IU/ml are also shown to enable comparison with studies using a different cut-off [16, 19]. Infection prevalence estimates with 95% confidence intervals (95% CI) were calculated in the participant group of 7 years and older based on previously published literature [20, 21]. In our study we noticed low proportions of IgG-Ptx 100 IU/ml in 7 and 8 year olds (**Supplementary Figure 1**) confirming this age limit. Sample size was calculated to estimate infection prevalence with a precision of 2.5% in the NS and 10-15% in age groups spanning at least 5 years [13].

Secondary study outcomes were: 1. A risk analysis for contracting pertussis 2. A comparison between infection prevalence rates and notification rates; 3. A comparison between the GMCs of the three pertussis vaccine antigens; and 4. A comparison with the serosurvey 10 years ago. For all participants of 7 years and older of the NS and the LVC areas together of the 2016/2017 survey (n=6013), we determined whether there were risk factors that were independently associated with an increased chance to contract pertussis, i.e. Ptx 100 IU/ml, using logistic regression analysis. All variables were first tested in a univariate model and variables with a p-value < 0.1 were included in the multivariable model. By stepwise

backward selection, variables independently associated with Ptx antibody concentrations 100 IU/ml were identified. Participants from LVC areas were divided into two groups based on religion, one group containing the orthodox-reformed individuals (ORI) who (partly) refuse vaccination and the second group containing the non-ORI. Infection prevalence estimates of both groups were compared with the NS. Calculated odds ratios (ORs) were presented with 95% CI. To gain insight in differences between serum infection prevalence and reported disease incidence in the national sample of 7 years and older and per age group in 2016/2017, weighted infection prevalence was compared with the disease incidence rates calculated from mandatory notifications. Comparisons are presented as rate ratios (RR) with 95% CI. GMCs for three pertussis vaccine antigens (Ptx, FHA, and Prn) with 95% CI were calculated to show vaccination effects and to explore trends between the different pertussis vaccine antibody levels within the national sample in 2016/2017. Additionally, a comparison between the 2006/2007 and the 2016/2017 survey was performed using only the results from participants 7-79 years of age, causing slight differences in sampling weight. Comparisons were made between the NS and per age group.



Analyses were performed using Microsoft Excel and SAS version 9.4.

## ROLE OF THE FUNDING SOURCE

The Dutch government as study sponsor had no role in study design, data collection, data analysis, data interpretation, nor in the writing of the report or in the decision to submit the paper for publication. All authors had full access to all the data in the study and accept responsibility to submit for publication.

## RESULTS

### Infection prevalence 2016/2017

Infection prevalence in the national sample 7 years of age (n=5745) [13] was 5.9% (95% CI 5.3-6.6). Highest proportions were found in the paediatric population divided in 7-11-year-olds (n=414) having an infection prevalence of 8.7% (95% CI 4.5-12.8) and 12-18-years-olds (n=565) 11.5% (95% CI 8.0-15.0). In the adult cohorts infection prevalence was 3.9% (95% CI 2.4-5.4) in 19-34-year-olds (n=1565), 4.7% (95% CI 3.5-5.9) in 35-49-years-olds (n=1252), 5.5% (95% CI 4.0-6.9) in 50-64-year-olds (n=1159), 6.3% (95% CI 4.3-8.4) in 65-79-year-olds (n=931), and 7.1% (95% CI 1.8-12.4) in the 80+ cohort (n=127).

**Table 2:** Potential risk factors for pertussis infection prevalence in the population ≥ 7 years of age.

| | n (%)<br>n = 6013 | % Recent pertussis infection<br>(95% CI) | Univariate Crude OR<br>(95% CI) | p-value | Multivariate Adjusted OR<br>(95% CI) | p-value |
|---|---|---|---|---|---|---|
| Sex | | | | 0.04 | | |
| Male | 2677 (44.5) | 6.7 (5.8-7.6) | Ref. | | | |
| Female | 3336 (55.5) | 5.4 (4.6-6.2) | 0.80 (0.64-0.99) | | | |
| Age group, years | | | | 0.001 | | 0.0004 |
| 7-11 | 414 (6.9) | 8.9 (6.5-12.0) | Ref. | | Ref. | |
| 12-18 | 565 (9.4) | 13.1 (10.5-16.1) | 1.54 (1.01-2.33) | | 1.55 (1.02-2.37) | |
| 19-34 | 1565 (26.0) | 4.3 (3.4-5.4) | 0.46 (0.30-0.69) | | 0.23 (0.06-0.85) | |
| 35-49 | 1252 (20.8) | 4.6 (3.5-5.8) | 0.49 (0.32-0.75) | | 0.23 (0.06-0.85) | |
| 50-64 | 1159 (19.3) | 4.8 (3.7-6.2) | 0.52 (0.34-0.80) | | 0.29 (0.08-1.06) | |
| 65-79 | 931 (15.5) | 6.0 (4.6-7.7) | 0.65 (0.42-1.01) | | 0.42 (0.11-1.59) | |
| 80+ | 127 (2.1) | 7.9 (4.1-13.6) | 0.87 (0.42-1.81) | | 0.54 (0.13-2.32) | |
| Region | | | | 0.57 | | |
| North | 973 (16.2) | 6.99 (5.51-8.72) | Ref. | | | |
| Midwest | 838 (13.9) | 5.37 (3.99-7.06) | 0.76 (0.51-1.11) | | | |
| Mideast | 921 (15.3) | 5.97 (4.57-7.65) | 0.84 (0.59-1.22) | | | |
| Southwest | 937 (15.6) | 5.12 (3.84-6.68) | 0.72 (0.49-1.05) | | | |
| Southeast | 1233 (20.5) | 5.76 (4.56-7.17) | 0.81 (0.58-1.15) | | | |
| LVC | 1111 (18.5) | 6.30 (4.98-7.85) | 0.90 (0.63-1.26) | | | |
| aP booster around 4y of age | | | | <0.0001 | | 0.001 |
| No, but eligible (7-18 year-olds) | 157 (2.6) | 19.1 (13.5-25.8) | Ref. | | Ref. | |
| Yes, and eligible (7-18 year-olds) | 820 (13.6) | 9.5 (7.6-11.7) | 0.45 (0.28-0.71) | | 0.44 (0.27-0.70) | |
| Not eligible (19 years and older) | 5013 (83.8) | 4.9 (4.4-5.6) | 0.22 (0.15-0.33) | | 1.19 (0.33-4.27) | |
| Coughing >2w | | | | 0.09 | | |
| Yes, 0-5m before sampling | 1192 (19.8) | 7.2 (5.8-8.8) | Ref. | | | |
| Yes, 6-11m before sampling | 266 (4.4) | 7.1 (4.5-10.7) | 0.99 (0.59-1.66) | | | |
| No | 4245 (70.6) | 5.4 (4.8-6.2) | 0.74 (0.57-0.96) | | | |
| Unknown | 310 (5.2) | 6.8 (4.4-10.0) | 0.94 (0.57-1.53) | | | |

| | n (%)<br>n = 6013 | % Recent pertussis infection<br>(95% CI) | Univariate Crude OR<br>(95% CI) | p-value | Multivariate Adjusted OR<br>(95% CI) | p-value |
|---|---|---|---|---|---|---|
| Number of contacts | | | | 0.8 | | |
| ≤median number by age group | 3113 (51.8) | 6.0 (5.2-6.8) | Ref. | | | |
| >median number by age group | 2900 (48.2) | 5.9 (5.1-6.8) | 0.97 (0.79-1.21) | | | |
| Number of household members | | | | 0.0002 | | 0.0099 |
| 1-2 | 2417 (40.2) | 4.4 (3.6-5.3) | Ref. | | Ref. | |
| 3-5 | 2137 (35.5) | 6.5 (5.5-7.6) | 1.52 (1.17-1.97) | | 1.59 (1.15-2.20) | |
| >5 | 1025 (17.1) | 7.6 (6.1-9.4) | 1.80 (1.33-2.43) | | 1.53 (1.04-2.25) | |
| unknown | 434 (7.2) | 7.8 (5.6-10.7) | 1.85 (1.24-2.77) | | 1.86 (1.22-2.82) | |
| Child <4y in the household | | | | 0.367 | | |
| No | 5438 (90.4) | 5.8 (5.2-6.5) | Ref. | | | |
| Yes | 575 (9.6) | 6.8 (4.9-9.1) | 1.17 (0.83-1.65) | | | |
| Underlying disease | | | | | | |
| No | 1666 (27.7) | 5.5 (4.4-6.6) | Ref. | | | |
| Yes | 4265 (70.9) | 6.0 (5.3-6.7) | 1.10 (0.86-1.40) | | | |
| Unknown | 82 (1.4) | 14.6 (8.2-23.6) | 2.97 (1.55-5.67) | | | |
| Priming by vaccination | | | | 0.0049 | | |
| Whole cell vaccination | 2071 (34.4) | 6.3 (5.3-7.4) | Ref. | | | |
| Acellular vaccination | 385 (6.4) | 9.4 (6.7-12.6) | 1.03 (0.75-1.42) | | | |
| Unvaccinated or unknown | 3557 (59.2) | 5.3 (4.6-6.1) | 1.06 (0.90-1.24) | | | |
| Ethnicity* | | | | 0.4749 | | |
| 1st and 2nd generation Western people, including Dutch origin | 5293 (88.0) | 5.9 (5.2-6.5) | Ref. | | | |
| 1st and 2nd generation non Western people | 720 (12.0) | 6.5 (4.9-8.5) | 1.12 (0.82-1.54) | | | |
| (Maternal) education level | | | | 0.2016 | | |
| High | 257 (17.2) | 3.11 (1.59-6.02) | Ref. | | | |
| Middle | 355 (23.7) | 3.38 (1.94-5.81) | 1.24 (0.94-1.63) | | | |
| Low | 756 (50.6) | 3.97 (2.79-5.61) | 1.29 (0.98-1.72) | | | |
| Unknown | 127 (8.5) | 3.15 (1.23-7.82) | 1.46 (0.93-2.31) | | | |
| Religion | | | | 0.3702 | | |
| Non-orthodox protestant religion or no religion | 5719 (95.1) | 5.9 (5.2-6.5) | Ref. | | | |
| Orthodox protestant religion | 294 (4.9) | 7.1 (4.6-10.5) | 1.23 (0.78-1.95) | | | |

### Risk factors for contracting pertussis

In **Table 2** all tested risk factors for contracting pertussis, like sex, age, and religion, are listed. Using the multivariable model, it turned out that in 2016/2017 19-49-year-olds had 0.23 times lower odds to contract pertussis compared with 7-11-year-olds, but 12-18-yearolds had 1.55 times higher odds compared with 7-11-year-olds. The model also showed that 7-18-year-olds who have had the 4-year-olds booster, had 0.44 times lower odds to have Ptx antibody concentrations 100 IU/ml compared with 7-18-year-olds who did not receive the preschool booster. Finally, when a household consisted of more than two members, the likelihood of having Ptx antibody concentrations 100 IU/ml increased. Although religion was not a significant risk factor, an additional analysis was performed between the NS, ORI, and non-ORI since we have a large cluster of LVC areas, also known as the Bible Belt, covering a substantial part of the Netherlands. This analysis did not reveal any unexpected outcomes (**Supplementary Figure 2**).

### Reported incidence versus infection prevalence

Our study indicates an infection prevalence of 5.9% in the study population from 7 years and older in 2016/2017. Reported incidence percentages of pertussis notifications in the corresponding years showed that 0.029% in the Dutch population from 7 years and older was diagnosed with pertussis in 2016/2017 [4]. Therefore, infection prevalence is approximately factor 200 higher compared with reported incidence (**Table 3**). Reported incidence and infection prevalence was highest in 7-18-year-olds, discrepancy between infection prevalence and reported incidence was greatest in older adults.

**Table 3:** Reported incidence of pertussis notifications versus serum infection prevalence.

| Age category | Incidence of pertussis notifications in the Dutch population 2016/2017 (%) | Infection prevalence in the study population (%) | Rate ratio (95% CI) |
|---|---|---|---|
| 7-11 y | 0.07 | 8.6 | 119.9 (82.9-173.5) |
| 12-18 y | 0.07 | 11.5 | 166.9 (128.3-217.2) |
| 19-34 y | 0.02 | 3.9 | 200.7 (148.1-272.0) |
| 35-49 y | 0.03 | 4.7 | 141.6 (107.8-185.9) |
| 50-64 y | 0.02 | 5.5 | 313.8 (244.9-401.9) |
| 65-79 y | 0.02 | 6.3 | 290.8 (220.1-384.2) |
| 80+ | 0.01 | 7.1 | 651.8 (377.3-1126) |
| Total | 0.03 | 5.9 | 200.3 (178.6-224.7) |

### Comparing different pertussis antigens

Reflecting the pertussis immunisation schedule (2, 3, 4, and 11 months, and 4 years of age), geometric mean concentrations (GMCs) of IgG-Ptx in infants showed high levels from 3 months onwards, with a peak at 5 months followed by a steady decrease up to 10 months, and again an increase at 11 months (**Table 4**). The high IgG-Ptx GMC of the 4-6-year-olds reflect the acellular booster at 4 years of age. The IgG-Ptx GMCs of the 7-11 (GMC 14 IU/ml; 95% CI 12-17 IU/ml) and 12-18-year-olds (GMC 15 IU/ml; 95% CI 12-18 IU/ml) were higher compared with those of the 19-34 (GMC 8 IU/ml; 95% CI 7-9 IU/ml) and 35-49-year-olds (GMC 9 IU/ml; 95% CI 9-10 IU/ml). The pattern observed for Ptx was comparable with the patterns for FHA and Prn with the exception that FHA showed higher GMCs in the population of 50 years and older and Prn was already decreased in the 12-18 year olds.



**Table 4:** Pertussis antigens in the different age groups.

| Age category | N= 5727 (%) | Ptx GMC (95% CI) in IU/ml | FHA GMC (95% CI) in IU/ml | Prn GMC (95% CI) in IU/ml |
|---|---|---|---|---|
| 0 y | 397 (6.9) | 37 (30-44) | 33 (28-39) | 42 (35-53) |
| 1 m | 11 (0.2) | 2.1 (1.0-4.2) | 3.3 (1.2-9.3) | 4.1 (2.8-6.0) |
| 2 m | 32 (0.6) | 5.1 (3.0-8.8) | 7.5 (5.0-11) | 14 (7.3-25) |
| 3 m | 42 (0.7) | 60 (36-99) | 34 (18-64) | 76 (40-145) |
| 4 m | 36 (0.6) | 58 (40-83) | 50 (38-65) | 71 (47-107) |
| 5 m | 46 (0.8) | 88 (67-116) | 75 (53-106) | 125 (101-154) |
| 6 m | 35 (0.6) | 56 (44-72) | 51 (41-62) | 59 (39-91) |
| 7 m | 39 (0.7) | 45 (32-65) | 49 (37-66) | 52 (39-68) |
| 8 m | 41 (0.7) | 34 (23-51) | 34 (27-42) | 39 (28-54) |
| 9 m | 44 (0.8) | 39 (27-56) | 24 (18-28) | 23 (11-50) |
| 10 m | 32 (0.6) | 26 (18-38) | 22 (18-28) | 20 (9.0-43) |
| 11 m | 39 (0.7) | 62 (35-110) | 62 (34-111) | 72 (33-155) |
| 1 y | 105 (1.8) | 56 (45-70) | 66 (54-82) | 91 (67-123) |
| 2 y | 69 (1.2) | 9.0 (6.5-13) | 19 (14-26) | 22 (18-30) |
| 3 y | 70 (1.2) | 9.0 (6.5-13) | 25 (16-39) | 20 (13-29) |
| 4-6 y | 201 (3.5) | 28 (24-33) | 61 (52-72) | 77 (60-100) |
| 7-11 y | 324 (5.7) | 14 (12-17) | 35 (30-41) | 29 (25-35) |
| 12-18 y | 443 (7.7) | 15 (12-18) | 33 (29-38) | 15 (13-17) |
| 19-34 y | 1243 (21.7) | 7.9 (7.2-8.6) | 20 (19-22) | 14 (12-15) |
| 35-49 y | 1009 (17.6) | 9.4 (8.6-10) | 19 (18-21) | 10 (8.8-11) |
| 50-64 y | 976 (17.0) | 12 (11-13) | 27 (25-29) | 11 (10-13) |
| 65-79 y | 784 (13.7) | 12 (11-13) | 40 (36-44) | 10 (9.1-11) |
| 80+ y | 106 (1.9) | 12 (8.7-16) | 30 (24-37) | 5.5 (4.1-7.7) |

### Differences between 2006/2007 and 2016/2017

A comparison of the proportions of the three IgG-Ptx categories for different age groups between the two studies revealed an overall significant increase of the infection prevalence in the population of 7 years and older (**Figure 2**). Previously in 2006/2007 (n=5740) [10] 3.5% showed IgG-Ptx concentrations indicating a recent infection, in the current study of 2016/2017 (n=5745) [13] this percentage increased to 5.9% ($p < 0.001$). For the different age categories, a significant increase in infection prevalence, varying between 1.6 and 3.3 fold change, was observed in 7-11 years olds ($p = 0.012$), 12-18 years olds ($p < 0.001$), and 50-64-year-olds ($p = 0.040$).

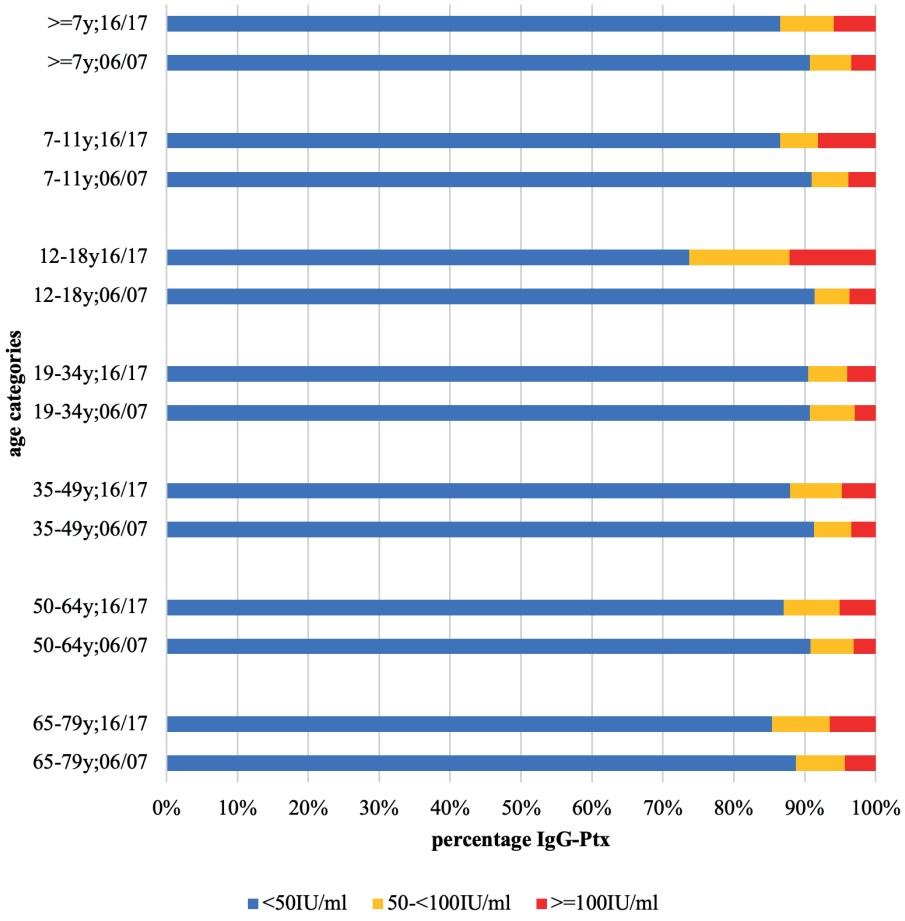



**Figure 2.** Pertussis infection prevalence in 2016/2017 compared to 2006/2007.

## DISCUSSION

In this 2016/2017 serosurveillance study we observed a still increasing proportion of participants with an IgG-Ptx concentration 100 IU/ml indicative of recent pertussis infections in the Dutch population of 7 years and older compared with two similar studies one and two decades ago. Most outstanding finding was the increase in IgG-Ptx seroprevalence suggestive for recent infection in the 12-18-year-olds who had received an extra 4-years-olds aP booster compared with their peers in the study ten years earlier.

As previously mentioned, the 12-18-year-olds showed the most striking increase in proportion of recently infected individuals. This seems remarkable since in 2006/2007 this age group was just wP primed and in the current study (2016/2017) they additionally received a preschool aP booster. Some adolescents received one or more aP priming vaccines, but since this was only a small proportion (6.5%) it is not likely to influence the results significantly. Since the introduction of the preschool booster, children become vulnerable for pertussis infection from the age of 7-9 years [20]. The 12-18-yearolds in this 2016-2017 study were 7-14 years of age during the 2012 and/or 2014 epidemics and therefore were vulnerable for pertussis infection at that time. Antibody concentrations induced by infection can reach high levels and are described to decrease approximately with 50% every 7 months, indicating that antibody concentrations can remain high for quite some years [22]. The relative lower GMC for Prn might be explained by the increasing proportion of Prn negative strains in the circulation [23]. At this age, the source of infection is mostly from schoolmates. Considering that all schoolmates are vulnerable around the same age, *B. pertussis* can be easily transmitted between peers during an epidemic.

The increase in the 7-11-year-olds during the decade between the two studies probably reflects a real increase in recently infected individuals [20]. During the 2nd serosurvey, this group was wP primed and a proportion received an aP booster at 4-years of age. In the current study, this group was completely aP primed and aP boosted. Infant priming with an aP vaccine is associated with a higher risk of pertussis later in childhood [7]. As a result, the 7-11 year old children in the current study might be more vulnerable than those from the previous study resulting in a higher infection prevalence. To prolong the duration of vaccine induced protection and thereby possibly also protect unprotected younger siblings in the household, it can be considered to delay the 4-year-olds booster. Serologic and cellular data indicate that the preschool booster can probably be safely delayed with two years [21, 24]. In Europe, the timing of the booster varies between 4 and 8 years [25] and infant notification rates do not seem to correspond to the timing of this booster [25, 26]. Additionally, aP vaccines seem to protect less against transmission of *B. pertussis* than wP vaccines do [27]. If the increase in infection prevalence is related to the increase in aP primed individuals, we might expect a further increase in the next decade. School aged children tend to have mostly assortative physical contacts of long

duration which makes them vulnerable for close-contact infections and therefore they might have a large contribution to *B. pertussis* transmission.

In the adult population, only an increase of recent infections is observed in the 50-64-year-olds. Next to assortative contacts, this age group mixes more with other age groups than younger or older individuals [28]. In this age group, the source of pertussis infection is most often relatives and workplace [29]. The increase might be due to potential contact of middle-aged adults with an increasing proportion of aP primed relatives. From the age of 50 onwards, an increase in FHA GMCs was observed. Since we do not see this increase for other pertussis antigens, this is likely due to infection with other microbes that contain FHA or FHA-like proteins [30].

Next to certain ages and the absence of a preschool aP-booster, a larger household also increased the risk to contract pertussis. Living in a larger household is associated with higher number of contacts and additionally children and adolescents are more likely to live in larger households [28]. Therefore, this risk factor interacts with age and therefore also with vaccination background. LVC areas caused by religious groups that refuse vaccination, did not influence the risk of pertussis as it did for measles and poliomyelitis [31, 32]. This might be caused by the effectiveness of the vaccines used. Nor pertussis vaccines nor pertussis infection cause lifelong protection, where measles vaccines tend to protect lifelong. Besides the risk factors discussed in this article, molecular changes on the pathogen level to escape the vaccine are known to have increased the circulation of *B. pertussis* as well [33].

Every surveillance method has its shortcomings, but combining reported incidence with infection prevalence, we can monitor both disease and infection pressure. The latter is important to estimate to risk for vulnerable (age) groups. Discrepancy between reported pertussis incidence in the Dutch population and infection prevalence in this study population was possibly caused by asymptomatic pertussis infection, or atypical presentation of pertussis disease in immunised individuals [34], and by limited awareness of pertussis in the population over 7 years of age. Costs might play a role as well, since in the Netherlands general practitioner visits are covered by insurance, but additional lab diagnostics usually involves costs for the patient. Therefore, general practitioners do not always confirm diagnosis by lab diagnostics and consequently do not report.

Since vaccinated individuals usually only experience mild or no symptoms when they get infected [35], there is only limited benefit of booster vaccinations for the individual. Ptx antibodies obtained by aP vaccination decay in less than 5 years [36]. Vaccinating everyone every 5 years is not cost effective and will probably result in a low vaccination coverage amongst adults, as shown in Austria and France [37]. Therefore, in order to increase herd immunity there is a need for new vaccines which induce long term protection as well as

protection against transmission, without increasing reactogenicity. Meanwhile, the focus of the NIP is protecting populations that are at risk of complications due to *B. pertussis* infection, e.g. the very young unvaccinated infants by vaccinating expectant mothers. Maternal vaccination has been implemented in many countries already including the Netherlands late 2019 and has proven to be very effective to protect the very young infants [38]. Next to protecting infants it can be considered to protect other risk groups by offering extra booster vaccines to older adults and people with (pulmonary) co-morbidities like is common in the Netherlands for the flu vaccine.

One of the strengths of this study is the large randomly chosen study population recruited in a relatively small timeframe, which makes it possible to reliably extrapolate data to the general Dutch population. Also, the availability of serum in combination with extensive questionnaires is unique in such a large cohort. A limitation of the study is the cross-sectional design, fluctuations in *B. pertussis* infection pressure over the years, will cause cohort effects influencing the differences between age groups now and a decade ago. Furthermore, we cannot prove causality between the increased infection related prevalence and the switch from wP to aP vaccination and the introduction of the aP preschool booster, because other factors also changed over time. Other limitations are the stepwise model selection which might give an over-optimistic impression [39], recent infection is arbitrarily defined, and different diagnostic cut-offs are used in different countries. High Ptx antibody concentrations at young age are probably due to vaccination, but infection cannot be ruled out.

In conclusion, despite the switch to aP vaccines and the addition of a preschool aP booster more than 10 years ago, seroprevalence of Ptx antibody levels indicating to recent pertussis infection is still increasing especially in school aged children and adolescents. Since pertussis infection often presents itself mild or even subclinical in vaccinated individuals, extra booster vaccinations for school aged children, adolescents, or adults do not seem beneficial to add to the NIP. However, it might be considered to delay the 4-year-olds booster with two years to extend the period of vaccine-induced protection. Extra booster doses might be considered for risk populations like older adults and people with (pulmonary) co-morbidities, since they have higher chance of complications and hospitalisation.

## CONTRIBUTORS

Conceptualisation by PV, NATvdM, GAMB, HEdM, FRMvdK, and EAMS. Luminex data generated by GS. Underlying data verified by GS, PV, GAMB, NATvdM. Data analysis was performed by NATvdM with input from PV, GAMB. PV wrote the first draft of the manuscript and all co-authors contributed to subsequent drafts. All authors read and approved the final manuscript.

## DECLARATION OF INTEREST

None of the authors received payment or service from a third part at any time, nor does anyone have a financial relationship with entities in the bio-medical arena. None of the authors have any patents relevant to the work.

## DATA AVAILABILITY STATEMENT

All of the individual participant data collected during the trial, after de-identification will be available for individual participant data meta-analysis immediately following publication and ending 10 years following article publication, to investigators whose proposed use of the data has been approved by an independent review committee. Proposals should be directed to nicoline.van.der.maas@rivm.nl; to gain access, data requestors will need to sign a data access agreement.

## ACKNOWLEDGEMENTS

This study is entirely funded by the Dutch government.

## REFERENCES


1. Mattoo, S. and J.D. Cherry, Molecular pathogenesis, epidemiology, and clinical manifestations of respiratory infections due to Bordetella pertussis and other Bordetella subspecies. Clin Microbiol Rev, 2005. 18(2): p. 326-382, DOI: 10.1128/cmr.18.2.326-382.2005.
2. Cherry, J.D., Pertussis in Young Infants Throughout the World. Clin Infect Dis, 2016. 63(suppl 4): p. S119-S122. doi: 10.1093/cid/ciw550.
3. Kandeil, W., et al., The burden of pertussis in older adults: what is the role of vaccination? A systematic literature review. Expert Rev Vaccines, 2019. 18(5): p. 439-455. doi: 10.1080/14760584.2019.1588727.
4. Schurink-van 't Klooster, T. and H. de Melker, The National Immunisation Programme in the Netherlands : Surveillance and developments in 2019-2020. 2020, DOI: 10.21945/rivm-2020-0077, Rijksinstituut voor Volksgezondheid en Milieu RIVM.
5. David, S., P.E. Vermeer-de Bondt, and N.A.T. van der Maas, Reactogenicity of infant whole cell pertussis combination vaccine compared with acellular pertussis vaccines with or without simultaneous pneumococcal vaccine in the Netherlands. Vaccine, 2008. 26(46): p. 5883-5887, DOI: 10.1016/j.vaccine.2008.07.105.
6. Health Council of the Netherlands, Vaccination against pertussis. Vol. 2004. publication no. 2004/04, The Hague: Health Council of the Netherlands, 2004.
7. Sheridan, S.L., R.S. Ware, K. Grimwood, and S.B. Lambert, Number and order of whole cell pertussis vaccines in infancy and disease protection. Jama, 2012. 308(5): p. 454-456. doi: 10.1001/jama.2012.6364.
8. Quinn, H.E., et al., The seroepidemiology of pertussis in NSW: fluctuating immunity profiles related to changes in vaccination schedules. N S W Public Health Bull, 2011. 22(11-12): p. 224-229, DOI: 10.1071/nb11023.
9. de Melker, H.E. and M.A. Conyn-van Spaendonck, Immunosurveillance and the evaluation of national immunization programmes: a population-based approach. Epidemiol Infect, 1998. 121(3): p. 637-643, DOI: 10.1017/s0950268898001587.
10. van der Klis, F.R., et al., Second national serum bank for population-based seroprevalence studies in the Netherlands. Neth J Med, 2009. 67(7): p. 301-8.
11. de Greeff, S.C., et al., Seroprevalence of pertussis in The Netherlands: evidence for increased circulation of Bordetella pertussis. PLoS One, 2010. 5(12): p. e14183, DOI: 10.1371/journal.pone.0014183.
12. Ruijs, W.L., et al., Religious subgroups influencing vaccination coverage in the Dutch Bible belt: an ecological study. BMC Public Health, 2011. 11: p. 102, DOI: 10.1186/1471-2458-11-102.
13. Verberk, J.D.M., et al., Third national biobank for population-based seroprevalence studies in the Netherlands, including the Caribbean Netherlands. BMC Infect Dis, 2019. 19(1): p. 470, DOI: 10.1186/s12879-019-4019-y.
14. Hijnen, M., et al., The Bordetella pertussis virulence factor P.69 pertactin retains its immunological properties after overproduction in Escherichia coli. Protein Expr Purif, 2005. 41(1): p. 106-112, DOI: 10.1016/j.pep.2005.01.014.
15. van Gageldonk, P.G.M., F.G. van Schaijk, F.R. van der Klis, and G.A.M. Berbers, Development and validation of a multiplex immunoassay for the simultaneous determination of serum antibodies to Bordetella pertussis, diphtheria and tetanus. J Immunol Methods, 2008. 335(1-2): p. 79-89, DOI: 10.1016/j.jim.2008.02.018.

**16.** van der Lee, S., et al., Enhanced Bordetella pertussis acquisition rate in adolescents during the 2012 epidemic in the Netherlands and evidence for prolonged antibody persistence after infection. Euro Surveillance, 2017. 22(47): p. 17-00011. doi: 10.2807/1560-7917.ES.2017.22.47.17-00011.

**17.** PERISCOPE Consortium, PERISCOPE: road towards effective control of pertussis. Lancet Infect Dis, 2019. 19(5): p. e179-e186, DOI: 10.1016/s1473-3099(18)30646-7.

**18.** Mollers M., R.W.L.M. Kinkhoest richtlijn. 2018 23 July 2020 [cited 2020 19-8-2020].

**19.** Guiso, N., et al., What to do and what not to do in serological diagnosis of pertussis: recommendations from EU reference laboratories. Eur J Clin Microbiol Infect Dis, 2011. 30(3): p. 307-312, DOI: 10.1007/s10096-010-1104-y.

**20.** Witt, M.A., P.H. Katz, and D.J. Witt, Unexpectedly limited durability of immunity following acellular pertussis vaccination in preadolescents in a North American outbreak. Clin Infect Dis, 2012. 54(12): p. 1730-1735, DOI: 10.1093/cid/cis287.

**21.** van der Lee, S., et al., Whole-Cell or Acellular Pertussis Primary Immunizations in Infancy Determines Adolescent Cellular Immune Profiles. Front Immunol, 2018. 9: p. 51. doi: 10.3389/fimmu.2018.00051.

**22.** Dalby, T., J.W. Petersen, Z.B. Harboe, and K.A. Krogfelt, 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(Pt 9): p. 1029-1036, DOI: 10.1099/jmm.0.020826-0.

**23.** Barkoff, A.M., et al., Pertactin-deficient Bordetella pertussis isolates: evidence of increased circulation in Europe, 1998 to 2015. Euro Surveill, 2019. 24(7): p. 1700832. doi: 10.2807/1560-7917.Es.2019.24.7.1700832.

**24.** Schure, R.M., et al., Differential T- and B-cell responses to pertussis in acellular vaccine-primed versus whole-cell vaccine-primed children 2 years after preschool acellular booster vaccination. Clin Vaccine Immunol, 2013. 20(9): p. 1388-1395. doi: 10.1128/cvi.00270-13.

**25.** ECDC. Pertussis: Recommended vaccinations. 2020 [cited 2020 16 September]; Available from: https://vaccine-schedule.ecdc.europa.eu/Scheduler/ByDisease?SelectedDiseaseId=3&SelectedCountryIdByDisease=-1.

**26.** ECDC. Disease data from ECDC Surveillance Atlas for pertussis. 2019 [cited 2019 20 September]; Available from: http://ecdc.europa.eu/en/pertussis/surveillance-and-disease-data/atlas].

**27.** Warfel, J.M., L.I. Zimmerman, and T.J. Merkel, Acellular pertussis vaccines protect against disease but fail to prevent infection and transmission in a nonhuman primate model. Proc Natl Acad Sci U S A, 2014. 111(2): p. 787-792, DOI: 10.1073/pnas.1314688110.

**28.** Mossong, J., et al., Social contacts and mixing patterns relevant to the spread of infectious diseases. PLoS Med, 2008. 5(3): p. e74, DOI: 10.1371/journal.pmed.0050074.

**29.** De Serres, G., et al., Morbidity of pertussis in adolescents and adults. J Infect Dis, 2000. 182(1): p. 174-179, DOI: 10.1086/315648.

**30.** Prince, H.E., J.M. Lieberman, and J.D. Cherry, Age-related differences in patterns of increased Bordetella pertussis antibodies. Clin Vaccine Immunol, 2012. 19(4): p. 545-550, DOI: 10.1128/cvi.05725-11.

**31.** van den Hof, S., G.A.M. Berbers, H.E. de Melker, and M.A. Conyn-van Spaendonck, Sero-epidemiology of measles antibodies in the Netherlands, a cross-sectional study in a national sample and in communities with low vaccine coverage. Vaccine, 1999. 18(9-10): p. 931-940, DOI: 10.1016/s0264-410x(99)00348-5.

**32.** van der Maas, N.A.T., et al., Immunity against poliomyelitis in the Netherlands, assessed in 2006 to 2007: the importance of completing a vaccination series. Euro Surveillance, 2014. 19(7): p. 20705, DOI: 10.2807/1560-7917.es2014.19.7.20705.

**33.** Barkoff, A.M. and Q. He, Molecular Epidemiology of Bordetella pertussis. Adv Exp Med Biol, 2019. 1183: p. 19-33, DOI: 10.1007/5584_2019_402.

**34.** Kilgore, P.E., A.M. Salim, M.J. Zervos, and H.J. Schmitt, Pertussis: Microbiology, Disease, Treatment, and Prevention. Clin Microbiol Rev, 2016. 29(3): p. 449-486, DOI: 10.1128/cmr.00083-15.

**35.** Narkeviciute, I., E. Kavaliunaite, G. Bernatoniene, and R. Eidukevicius, Clinical presentation of pertussis in fully immunized children in Lithuania. BMC Infect Dis, 2005. 5: p. 40, DOI: 10.1186/1471-2334-5-40.

**36.** Pool, V., et al., Humoral immunity 10years after booster immunization with an adolescent and adult formulation combined tetanus, diphtheria, and 5-component acellular pertussis vaccine in the USA. Vaccine, 2018. 36(17): p. 2282-2287. doi: 10.1016/j.vaccine.2018.03.029.

**37.** Sheikh, S., et al., A report on the status of vaccination in Europe. Vaccine, 2018. 36(33): p. 4979-4992, DOI: 10.1016/j.vaccine.2018.06.044.

**38.** Gkentzi, D., et al., Maternal vaccination against pertussis: a systematic review of the recent literature. Arch Dis Child Fetal Neonatal Ed, 2017. 102(5): p. F456-F463, DOI: 10.1136/archdischild-2016-312341.

**39.** Kirkwood, B.R. and J.A.C. Sterne, Chapter 29: Regression modelling, in Essential Medical Statistics, F. Goodgame, Editor. 2003, Blackwell Publishing: Malden, Massachusetts, USA. p. 315-342.

## SUPPLEMENTARY MATERIALS

### Supplementary Text 1: Nederlandse samenvatting

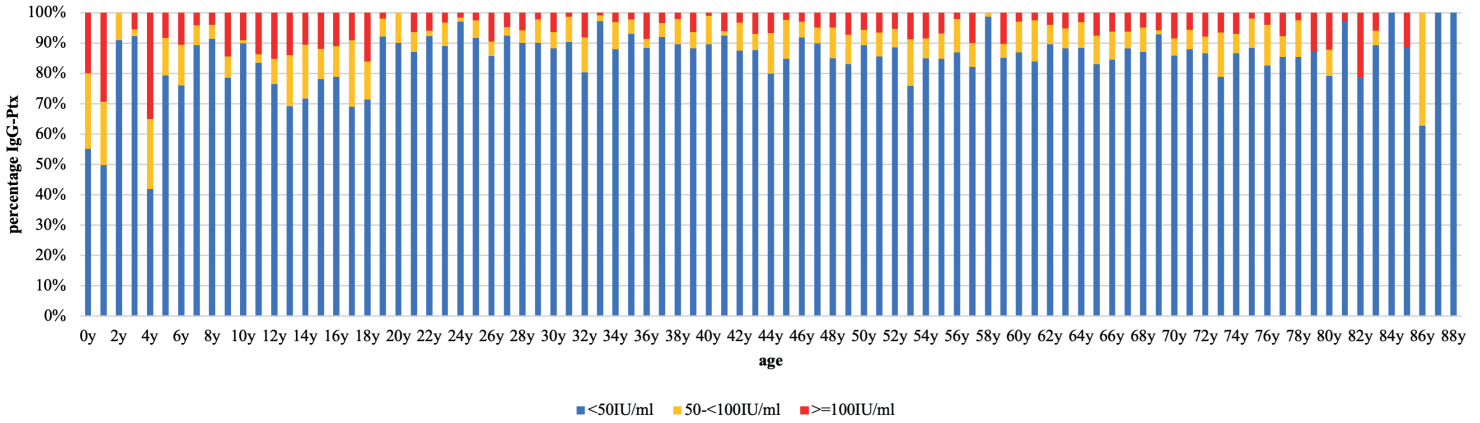
**Achtergrond:** Pertussis (kinkhoest) is een luchtweginfectie die ondanks een hoge vaccinatiegraad nog steeds endemisch is. In het Nederlandse rijksvaccinatieprogramma werden in 2005 cellulaire kinkhoest vaccins voor zuigelingen vervangen door acellulaire kinkhoest vaccins. Serosurveillance geeft de mogelijkheid om in de Nederlandse populatie de effecten van veranderingen in het rijksvaccinatieprogramma op de infectieprevalentie en vaccinrespons in de tijd objectief te monitoren.

**Methoden:** Voor deze cross-sectionele serosurveillance van de Nederlandse populatie is in 2016/2017 een representatieve steekproef van Nederlanders (0-89 jaar) getrokken. De primaire uitkomstmaat was het percentage deelnemers met pertussis toxine-specifieke antilichaamconcentraties ≥ 100 IU/ml, dat geldt als indicator voor een recente infectie en om groepen te identificeren die mogelijk kwetsbaarder zijn voor een pertussisinfectie. Percentages zijn vergeleken met eerdere resultaten uit de serosurveillance van 2006/2007.

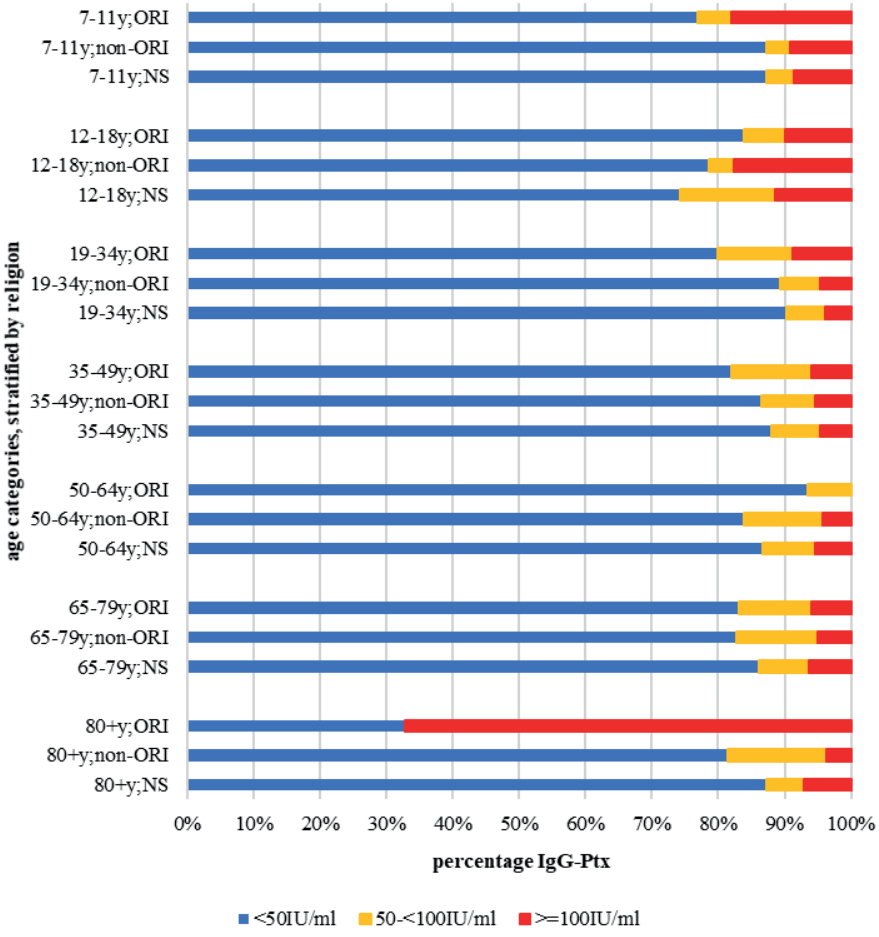
**Bevindingen:** In totaal werden 7621 personen in de analyse betrokken. Bij de bevolking van 7 jaar en ouder (n=6013) werd in 2016/2017 een toename van recente infecties gevonden van 3.5% naar 5.9% ten opzichte van 2006/2007. De meest opmerkelijke toename werd gezien bij 12-18-jarigen die als zuigeling cellulaire kinkhoestvaccins hebben gehad en additioneel een acellulaire voorschoolse booster toegediend hebben gekregen.

**Interpretatie:** De pertussis infectieprevalentie in Nederland neemt nog steeds toe, wat leidt tot een risico op kinkhoest bij kwetsbare (leeftijds)groepen. Het uitstellen van de voorschoolse booster kan de beschermingsperiode tijdens de basisschool verlengen en daardoor mogelijke jongere broers en zussen beschermen. Extra boosters kunnen overwogen worden voor risicopopulaties zoals ouderen en mensen met (pulmonale) comorbiditeiten, omdat zij een grotere kans hebben op complicaties en ziekenhuisopname.

**Financiering:** Ministerie van Volksgezondheid, Welzijn en Sport.




**Supplementary Figure 1.** Proportion of IgG-Ptx in the national sample.




**Supplementary Figure 2.** Pertussis infection prevalence in the national sample compared to low vaccination coverage areas. ORI: orthodox reformed individuals within low vaccination coverage areas; non-ORI: non-orthodox reformed individuals within low vaccination coverage areas; NS: national sample.

**Supplementary Table 1.** *B. Pertussis* vaccine schedules and vaccines in the Netherlands.

| Year | Scheme | Vaccine composition | Change | Pertussis vaccine name (Company) |
|---|---|---|---|---|
| before | | | | No vaccine available |
| 1954 | | DTwP | Combination vaccine became available | DTwP (RIVM) |
| 1957 | 3,4,5,11 m | DTwP | Start of NIP | DTwP (RIVM) |
| 1962 | 3,4,5,11 m | DTwP-IPV | Addition of IPV | DTwP-IPV (RIVM) |
| 1997 | 3,4,5,11 m | DTwP-IPV + Hib | Addition of Hib tot schedule | DTwP-IPV (RIVM) |
| 1999 | 2,3,4,11 m | DTwP-IPV + Hib | Accelerated vaccination | DTwP-IPV (RIVM) |
| 2001 | 2,3,4,11 m<br>4y | DTwP-IPV + Hib<br>DT-IPV + aP3 | Introduction aP booster, 3 Bp components | DTwP-IPV (RIVM)<br>monovalent aP (GSK) |
| 2003 | 2,3,4,11 m<br>4y | DTwP-IPV-Hib<br>DT-IPV + aP3 | Combination vaccine | DTwP-IPV/Hib (NVI)<br>monovalent aP (GSK) |
| 2005 | 2,3,4,11 m<br>4y | DTaP3-IPV-Hib<br>DT-IPV + aP3 | Introduction aP priming, 3 Bp components | Infanrix IPV + Hib (GSK)<br>monovalent aP (GSK) |
| 2006 (Jan) | 2,3,4,11 m<br>4y | DTaP5-IPV-Hib<br>DT-IPV + aP3 | 5 Bp components | Pediacel (SP MSD)<br>monovalent aP (GSK) |
| 2006 (Jun) | 2,3,4,11 m<br>4y | DTaP5-IPV-Hib + Pneu<br>DT-IPV + aP3 | Addition of Pneu to schedule; | Pediacel (SP MSD)<br>monovalent aP (GSK) |
| 2006 (Jul) | 2,3,4,11 m<br>4y | DTaP5-IPV-Hib + Pneu<br>DTaP5-IPV | Combination vaccine, 5 Bp components | Pediacel (SP MSD)<br>Triaxis Polio (SP MSD) |
| 2008 (Feb) | 2,3,4,11 m<br>4y | DTaP5P-IPV-Hib + Pneu<br>DTaP3-IPV | 3 Bp components | Pediacel (SP MSD)<br>Infanrix-IPV (GSK) |
| 2008 (Jul) | 2,3,4,11 m<br>4y | DTaP3-IPV-Hib + Pneu<br>DTaP3-IPV | 3 Bp components | Infanrix-IPV + Hib (GSK)<br>Infanrix-IPV (GSK) |
| 2009 | 2,3,4,11 m<br>4y | DTaP3/5-IPV-Hib + Pneu<br>DTaP3-IPV | 3 or 5 Bp components | Pediacel (SP MSD)/Infanrix-IPV + Hib (GSK)<br>Infanrix-IPV (GSK) |
| 2010 | 2,3,4,11 m<br>4y | DTaP5-IPV-Hib + Pneu<br>DTaP3-IPV | 5 Bp components | Pediacel (SP MSD)<br>Infanrix-IPV (GSK) |
| 2011 (Oct) | 2,3,4,11 m<br>4y | DT**aP3**-IPV-Hib-HepB + Pneu<br>DT**aP3**-IPV | Addition of HepB to schedule, 3 Bp components | Infanrix hexa (GSK)<br>Infanrix-IPV (GSK) |

| Year | Scheme | Vaccine composition | Change | Pertussis vaccine name (Company) |
|---|---|---|---|---|
| 2017 (Jan) | 2,3,4,11 m<br>4y | DT**aP3**-IPV-Hib-HepB + Pneu<br>Td**ap3**-IPV | <br>Reduced dose Bp components | Infanrix hexa (GSK)<br>Boostrix-IPV (GSK) |
| 2018 (Dec) | 2,3,4,11 m<br>4y | DT**aP5**-IPV-Hib-HepB + Pneu<br>Td**ap3**-IPV | 5 Bp components | Vaxelis (SP MSD)<br>Boostrix-IPV (GSK) |
| 2019 (Dec) | Maternal (from 22 weeks gestational age) | Tdap3 | Introduction ap booster for pregnant women, 3 reduced dose Bp components | Boostrix (GSK) |
| 2020 | 3, 5, 11m<br>4y | DT**aP3**-IPV-Hib-HepB + Pneu<br>Td**ap3**-IPV | Revised priming schedule | Vaxelis (SP MSD)<br>Boostrix-IPV (GSK) |

m=months; y= years; D=diphtheria; T=tetanus; wP: whole cell pertussis; aP: acellular pertussis; IPV: inactivated poliovirus; Pneu: pneumococcal; Hib: Haemophilus influenzae type B; HepB: Hepatitis B; Bp: *Bordetella pertussis*; NVI: Netherlands Vaccine Institute; GSK: GlaxoSmithKline; SP: Sanofi Pasteur; MSD: Merck Sharp & Dohme.

# Chapter 3

# Responses to an acellular pertussis booster vaccination in children, adolescents, and young and older adults: a collaborative study in Finland, the Netherlands, and the United Kingdom

P. Versteegen, M. Valente Pinto, A. M. Barkoff, P. G. M. van Gageldonk, J. van de Kassteele, M. A. van Houten, E. A.M. Sanders, R. de Groot, D. A. Diavatopoulos, S. Bibi, R. Luoto, Q. He, A. M. Buisman, D. F. Kelly, J. Mertsola, and G. A..M. Berbers.

EBioMedicine 65 (Feb 26 2021): 103247.

## ABSTRACT

**Background:** Pertussis can lead to serious disease and even death in infants. Older adults are more vulnerable to complications as well. In high-income countries, acellular pertussis vaccines are used for priming vaccination. In the administration of booster vaccinations to different age groups and target populations there is a substantial between-country variation. We investigated the effect of age on the response to acellular pertussis booster vaccination in three European countries.

**Methods:** This phase IV longitudinal intervention study performed in Finland, the Netherlands and the United Kingdom between October 2017 and January 2019 compared the vaccine responses between healthy participants of four age groups: children (7–10y), adolescents (11–15y), young adults (20–34y), and older adults (60–70y). All participants received a three-component acellular pertussis vaccine. Serum IgG and IgA antibody concentrations to pertussis antigens at day 0, 28, and 1 year were measured with a multiplex immunoassay, using pertussis toxin concentrations at day 28 as primary outcome. This trial is registered with ClinicalTrialsRegister.eu (2016–003,678–42).

**Findings:** Children (n = 109), adolescents (n = 121), young adults (n = 74), and older adults (n = 75) showed high IgG antibody concentrations to pertussis toxin at day 28 with GMCs of 147 (95% CI 120–181), 161 (95% CI 132–196), 103 (95% CI 80–133), and 121 IU/ml (95% CI 94–155), respectively. A significant increase in GMCs for vaccine antigens in all age groups by 28 days was found which had decreased by 1 year. Differences in patterns of IgG GMCs at 28 days and 1 year post-vaccination did not have a consistent relationship to age. In contrast, IgA antibodies for all antigens increased with age at all timepoints.

**Interpretation:** Acellular pertussis booster vaccination induces significant serum IgG responses to pertussis antigens across the age range which are not uniformly less in older adults. Acellular boosters could be considered for older adults to reduce the health and economic burden of pertussis.

## RESEARCH IN CONTEXT

**Evidence before this study:** Pertussis is a severe and life-threatening respiratory disease caused by *Bordetella pertussis*. Unvaccinated or incomplete vaccinated infants are most vulnerable to serious complications of pertussis, but older adults have a higher chance of complications and hospitalisation as well. In most European countries the acellular pertussis vaccine is used with different infant priming vaccination schedules and booster vaccinations at different ages. Despite a high vaccination coverage in European countries, notification rates are increasing indicating an increasing circulation of *Bordetella pertussis*. We searched PubMed for clinical trials and reviews up to August 14, 2017, with no language restrictions, using the search terms (older adults OR elderly) AND pertussis AND (vaccination OR vaccine OR Tdap) in the title and/or abstract. This search revealed that older adults do show a response to an acellular pertussis booster vaccination but did not yield any clinical trials that investigated possible differences in immunogenicity between older adults and other age groups who regularly received booster vaccinations, like school aged children, adolescents, and young adults. Therefore, we performed this prospective clinical trial, comparing four different age groups (children 7–10 years old, adolescents 11–15 years old, young adults 20–34 years old, and older adults 60–70 years old) in three different countries (Finland, the Netherlands, and the United Kingdom) with different epidemiological and vaccination background.

**Added value of this study:** This phase IV longitudinal intervention study is conducted as a multicentre trial in Finland, the Netherlands, and the UK and compares the effect of an acellular pertussis booster vaccination in four different age groups and three European countries. Here we present the IgG and IgA immune responses directed against the pertussis vaccine components of a Tdap-IPV booster vaccination administered to children, adolescents, and young and older adults with a follow-up to one year. Our study shows that IgG responses to the pertussis vaccine antigens do not have a consistent relationship to age up to one year post-vaccination and thus that the influence of age, and vaccination and epidemiological background on the IgG responses is limited. The IgA responses on the other hand seem to be higher at baseline in older adults, who also show greater increase upon vaccination and thus age influences the IgA responses.

**Implications of all the available evidence:** This study emphasizes that despite considerable differences in age, country-specific vaccination schedules and history, and epidemiological background, the IgG responses on the three-component acellular pertussis vaccine in older adults is not uniformly less compared to children, adolescents, and young adults. The widespread circulation of *B. pertussis* in high-income countries underscores the need for vigilant surveillance of whooping cough. Maternal immunisation to prevent pertussis in young infants at high risk for serious disease and death has been implemented in many countries and has been found highly effective from birth until the primary vaccinations.

Besides the young infants also older adults are at increased risk of complications and hospitalisation due to whooping cough. This study shows that a booster vaccination could be considered for older adults in order to prolong their protection and reduce the epidemiological pressure on the circulation of pertussis in the population.

## INTRODUCTION

Pertussis is an acute respiratory disease caused by the gram-negative bacterium *Bordetella pertussis* [1]. It can lead to serious disease and even death, especially in infants [2]. Complications due to pertussis are also associated with patients with chronic diseases and older age [3]. The incidence of clinical pertussis cases dropped with the introduction of whole cell pertussis (wP) vaccines in the 1940/ 1950s [4]. However, due to reported high reactogenicity rates and consequent decrease in vaccine coverage, acellular pertussis (aP) vaccines were developed in the late 1970/1980s. These vaccines proved to be less reactogenic and the short-term efficacy seemed to be similar to wP vaccines [5, 6]. However, long-term efficacy of aP vaccines appeared to be inferior compared with wP vaccines and natural infection although whole cell vaccines were reported to have a broad range of efficacy, some had significantly lower efficacy than aP vaccines [6-8]. While natural infection, acellular and whole cell vaccination all three induce antibodies and protect against disease, the ability of aP vaccines to protect against colonisation in comparison with wP vaccines or natural infection seems limited. In the baboon model it has been shown that natural infection and wP vaccination also protect against newly acquired colonisation and consequent transmission of *B. pertussis*. Therefore aP vaccines may be less effective than wP vaccines in generating herd immunity [9].



The switch from wP to aP vaccines was made since 1990 in many developed countries [6, 10-13]. Several aP vaccines are available: two-component aP (aP2) vaccines contain pertussis toxin (Ptx) and filamentous haemagglutinin (FHA), three-component aP (aP3) vaccines also contain pertactin (Prn) and five-component aP (aP5) vaccines additionally contain Fimbriae 2 and 3 (Fim2/3). The aP vaccines have proven to induce high IgG antibody levels against the various vaccine antigens that play an important role in protection against pertussis [14, 15]. Specific antibody levels against pertussis vary per vaccine antigen and the lack of an internationally established correlate of protection makes the interpretation of the data challenging [16-19].

Despite high vaccination coverage of wP/aP vaccines, pertussis has re-emerged worldwide, usually with cyclic outbreaks [20]. These cyclic outbreaks of pertussis occurred every three to four years in Finland before the onset of aP vaccinations in 2005 [21]. Since 1996 a similar pattern was seen in the Netherlands with cyclic outbreaks every three to five years. Introduction of aP vaccines seemed to disrupt this cyclic pattern in Finland, but also after the introduction of aP vaccines in 2005, the Netherlands still faced outbreaks in 2008, 2012, and 2014 (**Figure 1**) [22-24]. In the United Kingdom (UK), a cyclic pattern was observed in the wP era despite low incidence, and following the switch to aP in 2004, there was a major outbreak in 2012 and since then a cyclical pattern of outbreaks every three to four years [25, 26]. Notification rates are dependent on the use of diagnostics, reporting and ascertainment per country and therefore do not reflect the real incidence numbers.

In response to continued pertussis disease, many countries have adopted an accelerated schedule of primary series vaccinations and all western countries have implemented booster vaccinations in childhood [27, 28]. In addition, some countries introduced extra aP boosters for adolescents and adult target populations, including pregnant women, military conscripts, healthcare workers, and older adults [29-34].

From the pertussis incidence data in Europe, it can be deduced that the proportion of the observed pertussis cases in young children is rather constant over the years, while that of the adolescents is declining and the proportion of cases in adults 30 years and older is rising [24]. These European trends in pertussis incidences are also seen for the three individual countries involved in this study. In the study countries, the pertussis vaccination coverage at around age one in 2017 was 94% in the Netherlands and the UK, and 99% in Finland [35].

The aim of this study was to investigate the effect of age on aP booster vaccination when administered in school aged children, adolescents, and young and older adults. The study was performed in three European countries with differences in vaccination schedules and pertussis epidemiology which allowed additional investigation of possible country-specific effects.

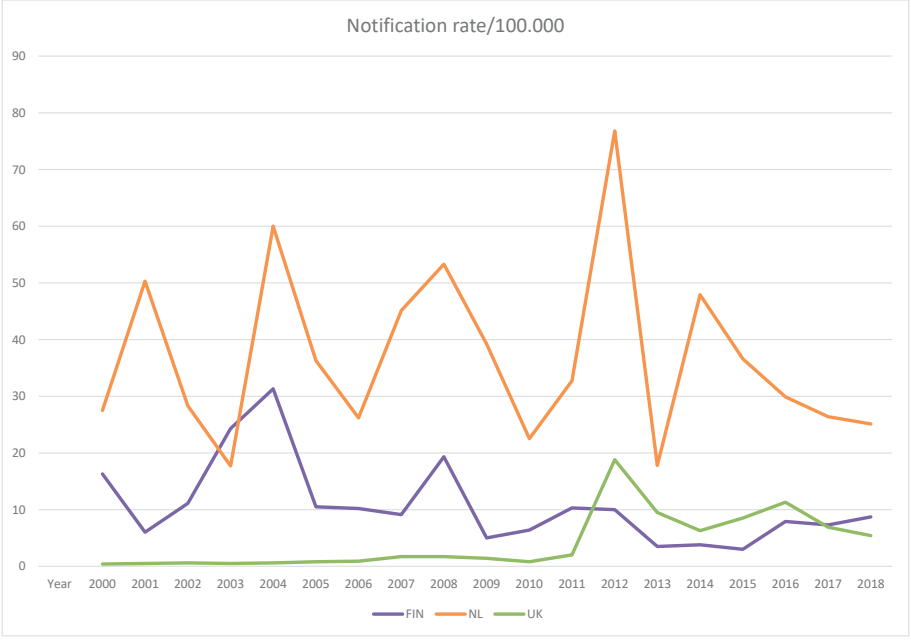



**Figure 1.** Notification rates from the ECDC surveillance atlas. Disease data from ECDC Surveillance Atlas for pertussis 2019 [24].

## METHODS

### Study design and participants

This phase IV longitudinal intervention study was conducted as a multicentre trial in Finland, the Netherlands, and the UK. In all three countries, children aged 7-10 years, adolescents aged 11-15 years, young adults aged 20-34 years, and older adults aged 60-70 years old were recruited. Finnish children were informed at local schools in Turku after permission was received from the school authorities. Adult participants were recruited by information in the newspapers and by University of Turku and Turku University Hospital web pages. The Finnish arm of this study was conducted by the Department of Microbiology and Immunology (University of Turku, Turku, Finland) in Turku University Hospital. Dutch participants were recruited by mail-outs to a sex balanced sample in the region of Hoofddorp, attained via the Municipal Administration (adolescents, young adults, and older adults) or through the NIP (children). The Dutch arm of the study was conducted by the Spaarne Academy (Spaarne Hospital, Hoofddorp, the Netherlands). In the UK, age appropriate participants in the Oxford region, identified via National Health Service databases, received mail-outs, also posters and media advertisings were used. The UK arm of the study was conducted by the Oxford Vaccine Group (University of Oxford, Oxford, UK). All participants should have been healthy and vaccinated according to the country-specific NIPs, which are presented in **Supplementary Table 1**. Most important exclusion criteria were the presence of serious immune modulating illnesses, previous administration of serum products, occurrence of serious adverse events after previous vaccinations, pregnancy, and for adults administration of pertussis containing vaccines in the last five years. A full list of inclusion and exclusion criteria can be found in the **Supplementary Panel**. All participants received a Tdap3-IPV vaccine (Boostrix™-IPV - GlaxoSmithKline (GSK), Wavre, Belgium) at baseline. Serum samples were collected at day 0 and 28 (± 4 days), and 1 year (± 4 weeks) post-vaccination and subsequently stored at -20 °C until analysis. As part of a broader set of immunological studies not reported here additional samples were drawn at day 7 and day 14 in all groups and day 1 in the 11-15year age group. All laboratory analyses were performed at a single laboratory in Bilthoven the Netherlands. This study was initiated by the IMI2 PERISCOPE Consortium [36].

### Ethics statement

This human clinical study was designed and conducted in accordance with the provisions of the Declaration of Helsinki (1996) and the International Conference on Harmonisation Guidelines for Good Clinical Practice. The trial was registered at the EU Clinical Trial database (EudraCT number 2016-003678-42) and was approved by the Medical Research Ethics Committees United (MEC-U, NL60807.100.17-R17.039) in the Netherlands, the South Central - Hampshire B Research Ethics Committee (REC, 19/SC/0368) in the UK, and the MREC UTU (ETMK Dnro: 129/1800/2017) in Finland. Written informed consent was

obtained from all adult participants, and parents or legal guardian of minors, at the start of the study.

### Serological analysis

Serum IgG concentrations against Ptx (GSK), FHA (SP), Prn (GSK), and Fim2/3 (SP) were quantified in independent duplicate using the fluorescent-bead-based multiplex immunoassay (MIA) as previously described [37-39], and serum IgA concentrations for the pertussis antigens were measured. In brief, the conjugated fluorescent microbeads were incubated with serum samples in two dilutions (200 and 4000), a reference serum in a dilution series and control sera, on each plate. The measurement of the IgG and IgA antibody levels was performed with a Bioplex LX200 in combination with BioPlex Manager 6.2 (Bio-Rad Laboratories). For IgG an in-house standard, calibrated on the Pertussis Antiserum (human) 1st WHO International Standard (IS) was used to express IgG antibody concentrations in IU/ml. For IgA the Pertussis Antiserum (human) 1st WHO IS itself was used. For each analyte, the MFI was converted to IU/ml by interpolation from a five-parameter logistic standard curve. The in-house standard reference for IgG-Fim2/3 was calibrated against U.S. reference pertussis antiserum (human) lot 3 and arbitrarily set at 100 AU/ml as previously described [38], and for IgA-Fim2/3 the Pertussis Antiserum (human) 1st WHO IS was arbitrarily set at 100 AU/ml. The lower limits of quantification (LLOQs) for IgG were 0.214 IU/ml for Ptx and 0.092 AU/ml for Fim2/3. The LLOQs for IgA were 0.032 IU/ml for Ptx and FHA, 0.021 IU/ml for Prn, and 0.049 AU/ml for Fim2/3. For IgG-Ptx, 20 IU/ml was defined as arbitrary cut-off of protection against clinical disease [40-42], and 100 IU/ml was defined to be indicative for a recent infection in absence of a vaccination in the last few years [39]. All the Luminex antibody data have been deposited in the central database of the PERISCOPE Consortium and can be accessed by a request to the PERISCOPE management team.

### Outcomes

The primary outcome of the study is the specific Ptx-IgG serological antibody level at 28 days after vaccination in the four age groups of the three countries. Secondary outcomes are IgG levels at 0 and 28 days, and one year of Ptx (only day 0 and one year), FHA, Prn, and Fim2/3, and pertussis-specific IgA levels at those timepoints for the same antigens.

### Statistical analysis

In each of the three countries the study aimed to include 36 children aged 7-10 years, 36 adolescents aged 11-15 years, 25 young adults aged 20-34 years, and 25 older adults aged 60-70 years, with equal distribution between aP and wP primed adolescents in early infancy in the Netherlands and Finland. The large number in the school children and

adolescent groups also allowed sufficient additional samples to be collected at day 1, day 7 or day 14, for immunological end-points not reported here. Unlike adult participants not each child could be bled at all timepoints. A sample size of 108 per child cohort, across all countries was estimated to give 80% power to detect a standardised difference in log-anti-Ptx-IgG at one month post booster of 0.42 IU/ml between age cohorts, allowing 15% loss to follow-up or sample loss. The data obtained from the participants in each country will be combined and analysed per age cohort. From all participants successful blood samples at day 0 and 28 should have been taken, otherwise they could be replaced.

Concentrations below the LLOQ were replaced by LLOQ/2. For each antibody and antigen combination a linear mixed model was fitted to the log-transformed concentrations. A linear mixed model can be considered as a generalisation of a paired t-test [43]. This model describes the log geometric mean concentrations (GMCs) while accounting for the longitudinal structure of the measurements. Timepoint of blood sampling and age group were included in the model as a two-way interaction as fixed effects. Participant ID was included as a random intercept in the model and by the random intercept the baseline concentration of each participant was taken into account. An additional analysis with country included in the model was performed as a three-way interaction as fixed effects, to reveal differences within age groups between countries.



To explore the effect of previous vaccinations with or without Prn and/or Fim2/3, children and adolescents were subdivided in a group exclusively vaccinated with aP2 vaccines (without Prn and Fim2/3) (n = 10), a group exclusively vaccinated with aP3 vaccines (with Prn, without Fim2/3) (n = 7), a group at least three times vaccinated with aP5 vaccines (with Prn and Fim2/3)(n = 65), and a group primed at least three times with a wP vaccine (n = 41). A similar model, where age group was replaced by vaccination group, was used to analyse the differences between aP and wP priming background, and between participants who previously received only aP2, only aP3, at least three times aP5 or at least three times wP vaccines.

Overall significance of the fixed effect terms was assessed by a type III ANOVA. GMCs and their corresponding 95% confidence intervals (95% CI), as well as their mutual GMC ratios, corresponding 95% CI and p-values were obtained by post hoc analysis using Satterthwaite's method [44, 45]. P-values were adjusted by applying the Benjamini-Hochberg procedure for multiple comparisons, controlling the false discovery rate [46]. Non-relevant comparisons were excluded.

To investigate whether seasonal, diurnal or sex effects were present, month (12-level categorical variable), hour (ten-level categorical variable, based on quantiles), and sex (two-level categorical variable) were included as fixed effects in the model as well. These, however, were not significant and were excluded in further analyses.

All statistical analysis were done in R [47], using the lme4 package [48], and lmerTest package [49].

### Role of the funding source

The EFPIA partners from the PERISCOPE Consortium had some input on the study design, e.g. the determination of the age of the four groups of participants. They had no role in the data collection, analysis, interpretation of the data, nor in the writing of the report. The corresponding authors had full access to all data from the study, and final responsibility for the decision to submit for publication was by consensus of all co-authors.

## RESULTS

### Participant and data overview

In Finland, participants were enrolled in the study between August 2018 and January 2019. In total 123 study participants completed the study, 36 children, 37 adolescents, 25 young adults, and 25 older adults (**Figure 2**). In the Netherlands, participants were enrolled in the study between October 2017 and March 2018. Since the distribution between aP and wP primed adolescents was distorted, 12 extra participants were enrolled in October 2018. A total of 134 participants completed the study subdivided in 36 children, 48 adolescents, 25 young adults, and 25 older adults (**Figure 2**). In the UK participants were enrolled in the study between April 2018 and January 2019. A total of 117 participants completed the study, 36 children, 35 adolescents, 22 young adults, and 24 older adults (**Figure 2**). Characteristics of all 379 participants (53% female) from whom serological data were available and used for analyses, are shown in **Table 1**.

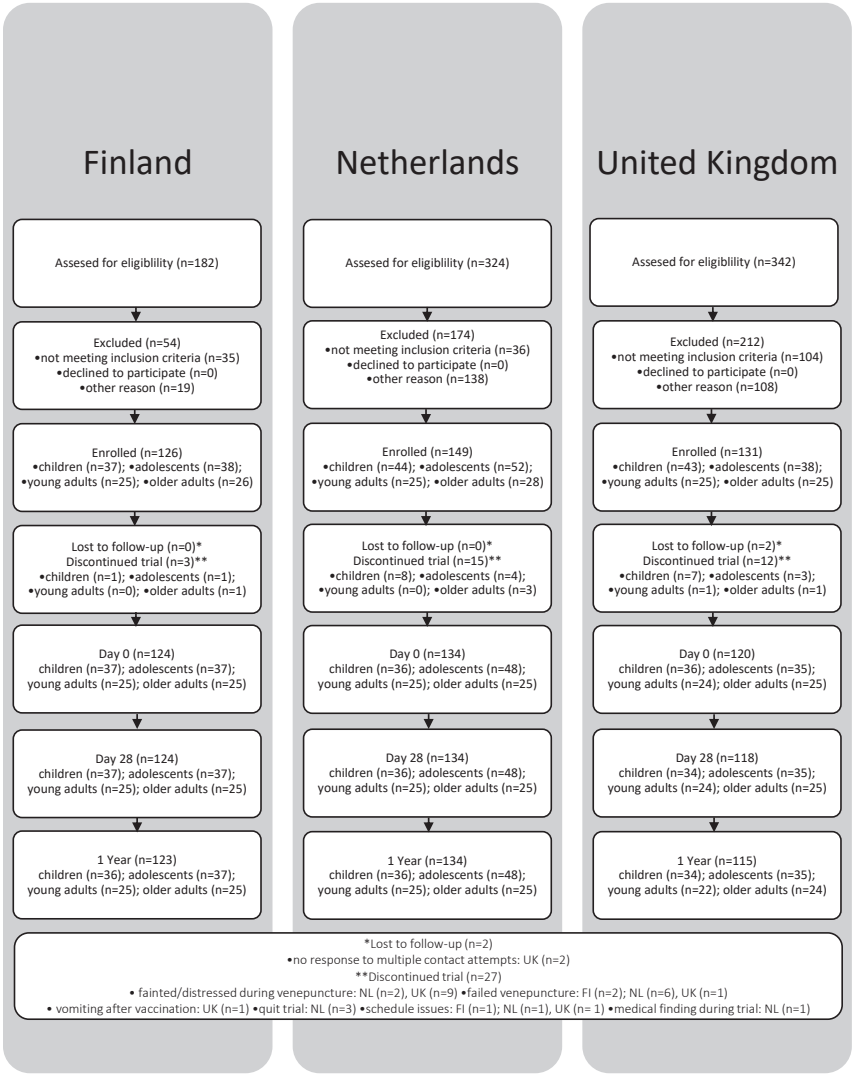



**Figure 2.** Flow diagram BERT-study.

**Table 1.** Participant characteristics.

| Characteristic | | Country | No. of children aged 7-10 yrs [%] | No. of adolescents aged 11-15 yrs [%] | No. of young adults aged 20-34 yrs [%] | No. of older adults aged 60-70 yrs [%] |
|---|---|---|---|---|---|---|
| No. of participants | Total | FI | 37 | 37 | 25 | 25 |
| | | NL | 36 | 48 | 25 | 25 |
| | | UK | 36 | 36 | 24 | 25 |
| Age in years | Mean (95% CI) | FI | 9.0 (8.7-9.3) | 13.7 (13.2-14.2) | 30.2 (28.7-31.7) | 64.2 (63.2-65.2) |
| | | NL | 8.5 (8.4-8.6) | 13.6 (13.2-13.9) | 28.6 (27.0-30.2) | 65.9 (64.8-67.0) |
| | | UK | 9.2 (8.9-9.6) | 12.8 (12.5-13.1) | 26.1 (24.2-28.0) | 65.7 (64.5-67.0) |
| Gender | Female | FI | 18 [48.6] | 19 [51.4] | 21 [84.0] | 21 [84.0] |
| | | NL | 18 [50.0] | 17 [35.4] | 10 [40.0] | 14 [56.0] |
| | | UK | 16 [44.4] | 18 [50.0] | 16 [66.7] | 13 [52.0] |
| Pertussis priming background | aP | FI | 37 [100.0] | 19 [51.4] | 0 [0.0] | 0 [0.0] |
| | | NL | 36 [100.0] | 25 [52.1] | 0 [0.0] | 0 [0.0] |
| | | UK | 36 [100.0] | 36 [100.0] | 0 [0.0] | 0 [0.0] |
| | wP / unknown | FI | 0 [0.0] | 18 [48.6] | 25 [100.0] | 25 [100.0] |
| | | NL | 0 [0.0] | 23 [47.9] | 25 [100.0] | 25 [100.0] |
| | | UK | 0 [0.0] | 0 [0.0] | 24 [100.0] | 25 [100.0] |

FI: Finland; NL: Netherlands; UK: United Kingdom. CI: confidence interval.

### Pertussis-specific IgG concentrations per age at day 0

The baseline GMCs were low, though several significant differences between age groups were observed as summarised in **Table 2**. The baseline GMCs of IgG-Ptx antibodies in all age groups were below 20 IU/ml, though six participants from the Netherlands, three from Finland, and one from the UK showed antibody concentrations above 100 IU/ml suggestive of a recent infection (**Figure 3**). Baseline IgG-Ptx and FHA GMCs in young adults (n = 74) were significantly lower than in children (n = 109), adolescents (n = 120), and older adults (n = 75), while this group had higher GMCs for Fim2/3 compared to the three other age groups. Older adults showed a lower GMC for IgG-Ptx compared to adolescents, a lower GMC for IgG-Prn but a higher GMC for IgG-Fim2/3 compared to children.

**Table 2.** IgG GMCs per age group.

| Antigen | Timepoint | Children | Adolescents | Young adults | Older adults |
|---|---|---|---|---|---|
| Ptx GMC (CI) in IU/ml | Day 0 | 11 (9-14)[a] | 14 (12-18)[a,b] | 4 (3-5)[b,c,d] | 9 (7-12)[a,d] |
| | Day 28 | 147 (120-181)[e] | 161 (132-196)[e] | 103 (80-133)[f,g] | 121 (94-155) |
| | 1 year | 35 (28-43)[h] | 49 (40-59)[i,j] | 26 (20-34)[h,k] | 43 (34-56)[j] |
| FHA GMC (CI) in IU/ml | Day 0 | 28 (24-32)[a] | 35 (30-40)[a] | 20 (17-24)[b,c,d] | 31 (25-37)[a] |
| | Day 28 | 290 (248-340) | 313 (269-364) | 299 (247-361) | 255 (211-308) |
| | 1 year | 88 (76-104)[h] | 121 (104-141)[i] | 113 (93-137) | 109 (90-132) |
| Prn GMC (CI) in IU/ml | Day 0 | 16 (13-22)[b] | 12 (10-16) | 13 (9-18) | 8 (6-11)[c] |
| | Day 28 | 293 (223-386)[l] | 318 (245-414)[l] | 331 (237-463)[l] | 171 (123-239) [e,f,g] |
| | 1 year | 85 (64-111)[j] | 114 (88-149) | 151 (108-212)[i,k] | 78 (56-109)[j] |
| Fim2/3 GMC (CI) in AU/ml | Day 0 | 2.1 (1.5-2.9)[a,b] | 3.3 (2.4-4.4)[a] | 8.0 (5.4-12.0)[b,c,d] | 4.0 (2.7-5.9)[a,c] |
| | Day 28 | 3.5 (2.5-4.8)[e] | 4.4 (3.2-6.0)[e] | 9.0 (6.0-13.4)[f,g,l] | 4.2 (2.8-6.2)[e] |
| | 1 year | 2.6 (1.9-3.6)[j] | 3.4 (2.5-4.6)[j] | 6.3 (4.2-9.4)[h,j] | 4.0 (2.7-5.9) |

Ptx: pertussis toxin; FHA: filamentous haemagglutinin; Prn: pertactin; Fim2/3: fimbriae 2 and 3; GMC: geometric mean concentration; CI: confidence interval; IU/ml: international units per millilitre; AU/ml: arbitrary units per millilitre. Significance per antigen has been tested between age groups within a timepoint; $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$. Significantly different from **a** young adults at day 0; **b** older adults at day 0; **c** children at day 0; **d** adolescents at day 0; **e** young adults at day 28; **f** children at day 28; **g** adolescents at day 28; **h** adolescents at 1 year; **i** children at 1 year; **j** young adults at 1 year; **k** older adults at 1 year; **l** older adults at day 28.

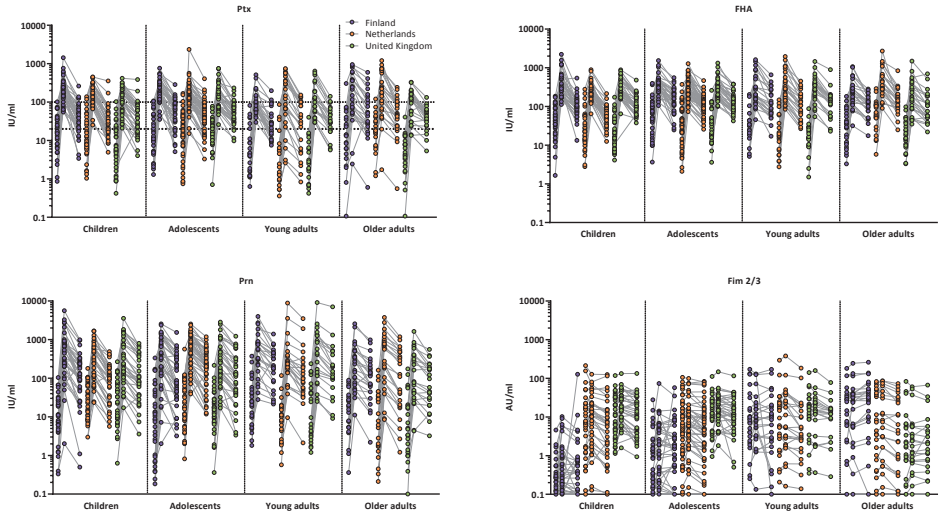



**Figure 3.** Individual IgG responses per age and country. Ptx: pertussis toxin; FHA: filamentous haemagglutinin; Prn: pertactin; Fim2/3: Fimbriae 2 and 3; IU/ml: international units per millilitre. Ptx 20 IU/ml: arbitrary cut-off of protection; Ptx 100 IU/ml: indication of recent infection in absence of a recent vaccination; Values below LLOQ are shown as 0.1 IU/ml or AU/ml. Concentrations from day 0, day 28 and 1 year connected per individual.

### Vaccine-specific IgG concentrations per age at day 28

The pertussis antigen specific GMCs 28 days after the Tdap-IPV booster vaccination increased significantly in all age groups and for all antigens except for Fim2/3 which antigen was not in the vaccine (**Table 2**). For IgG-Ptx, the young adults (n = 74) still had the lowest GMC (103 IU/ml), significantly lower than children (n = 107) and adolescents (n = 120) (147 and 161 IU/ml, respectively). In contrast young adults had the highest GMC (331 IU/ml) for Prn which along with the children and adolescents (293, 318 IU/ml respectively) was significantly higher than the older adults (n = 75) (171 IU/ml).

### Vaccine-specific IgG concentrations per age at one year

All pertussis antibody levels decreased significantly one year after vaccination compared with levels at day 28, except for Fim2/3 (**Table 2**), but had remained significantly above baseline levels. The group of young adults (n = 71) still had the lowest GMC for IgG-Ptx, significantly lower than both paediatric groups. By comparison we observed the highest GMC for IgG Prn in the young adults group, significantly higher compared with the children (n = 106) and older adults (n = 74). For both Ptx and FHA, children showed a lower antibody concentration compared with the adolescents (n = 120).

### Pre- and post-vaccination IgA concentrations

In contrast to IgG antibodies the IgA antibodies to all pertussis antigens tended to be higher with increasing age at day 0, day 28, and at 1 year (**Table 3**). For IgA-Ptx and IgA-Prn, the fold increase from day 0 to day 28 was also clearly age dependant (varying from 4- fold for Ptx and Prn in children to 12-fold for Ptx and 21-fold for Prn in older adults). The fold change decrease from day 28 to 1 year was also slightly age dependant (varying from 1.3-fold for Ptx in children to 2.3-fold for Prn in older adults). For FHA such a pattern could not be observed.

**Table 3.** IgA GMCs per age group.

| Antigen | Timepoint | Children | Adolescents | Young adults | Older adults |
|---|---|---|---|---|---|
| Ptx GMC (CI) in IU/ml | Day 0 | 0.28 (0.23-0.35)[a,b,c] | 0.46 (0.37-0.57)[c,d] | 0.6 (0.46-0.78)[c,d] | 1.01 (0.77-1.31)[a,b,d] |
| | Day 28 | 1.05 (0.84-1.31)[e,f,g] | 1.93 (1.56-2.38)[g,h] | 2.6 (1.98-3.4)[g,h] | 12.16 (9.3-15.91)[e,f,h] |
| | 1 year | 0.78 (0.63-0.97)[i,j,k] | 1.29 (1.05-1.6)[k,l] | 1.71 (1.3-2.25)[k,l] | 5.43 (4.15-7.11)[i,j,l] |
| FHA GMC (CI) in IU/ml | Day 0 | 0.12 (0.08-0.19)[a,b,c] | 0.45 (0.3-0.68)[c,d] | 0.41 (0.24-0.7)[c,d] | 3.3 (1.96-5.57)[a,b,d] |
| | Day 28 | 2.17 (1.41-3.35)[e,f,g] | 5.49 (3.63-8.3)[f,g,h] | 10.89 (6.44-18.42)[e,g,h] | 98.18 (58.18-165.69)[e,f,h] |
| | 1 year | 0.25 (0.16-0.39)[i,j,k] | 1.29 (0.85-1.96)[k,l] | 2.45 (1.44-4.16)[k,l] | 28.55 (16.89-48.28)[i,j,l] |
| Prn GMC (CI) in IU/ml | Day 0 | 1.15 (0.84-1.6)[b,c] | 1.78 (1.3-2.42)[b,c] | 4.14 (2.79-6.14)[a,d] | 6.19 (4.17-9.17)[a,d] |
| | Day 28 | 4.62 (3.34-6.39)[e,f,g] | 8.77 (6.43-11.95)[f,g,h] | 45.55 (30.69-67.61)[e,g,h] | 131.96 (89.02-195.62)[e,f,h] |
| | 1 year | 4.31 (3.13-5.95)[i,j,k] | 6.82 (4.99-9.32)[j,k,l] | 20.92 (14.04-31.15)[j,k,l] | 57.21 (38.55-84.89)[i,j] |
| Fim2/3 GMC (CI) in AU/ml | Day 0 | 1.05 (0.73-1.51)[a,b,c] | 2.25 (1.59-3.18)[b,c,d] | 7.3 (4.67-11.41)[a,d] | 10.14 (6.49-15.84)[a,d] |
| | Day 28 | 1.79 (1.24-2.57)[f,g] | 2.78 (1.97-3.93)[f,g] | 8.64 (5.52-13.51)[e,h] | 13.17 (8.43-20.58)[e,h] |
| | 1 year | 1.99 (1.39-2.86)[i,j,k] | 3.68 (2.59-5.21)[j,k,l] | 8.19 (5.22-12.84)[j,l] | 14.21 (9.09-22.22)[j,l] |

Ptx: pertussis toxin; FHA: filamentous haemagglutinin; Prn: pertactin; Fim2/3: fimbriae 2 and 3; GMC: geometric mean concentration; CI: confidence interval; IU/ml: international units per millilitre; AU/ml: arbitrary units per millilitre. Significance per antigen has been tested between age groups within a timepoint; $p \leq 0.05$, *$p \leq 0.01$*, $\underline{p \leq 0.001}$. Significantly different from **a** adolescents at day 0; **b** young adults at day 0; **c** older adults at day 0; **d** children at day 0; **e** adolescents at day 28; **f** young adults at day 28; **g** older adults at day 28; **h** children at day 28; **i** adolescents at 1 year; **j** young adults at 1 year; **k** older adults at 1 year; **l** children at 1 year.

### Vaccine-specific antibody concentration differences between countries

At baseline several country-specific differences could be observed (**Supplementary Table 2**). Notable was the higher baseline IgG-FHA GMC in Dutch older adults (n = 25) compared with the older adults from Finland (n = 25) and the UK (n = 25). Also, IgG-Ptx is higher at baseline in the Dutch older adults compared with their peers from the UK. Children and adolescents in the Finnish groups showed significantly higher baseline IgG-FHA GMCs compared with those groups from the Netherlands and the UK but showed lower baseline IgG-Prn and IgG-Fim2/3 levels.

At day 28 post-vaccination, country-specific differences were observed in Dutch older adults who still showed a higher GMC for IgG-FHA (425 IU/ml) than their peers in Finland

and the UK (225 and 174 IU/ml, respectively) (**Supplementary Table 2**). The UK young adults (n = 24) had a higher GMC for IgG-Prn compared with their older adults. IgG-Ptx antibody concentrations below the arbitrary cut-off of protection of 20 IU/ml were observed in two older adults per country. This was also the case in four Dutch young adults and one Dutch adolescent (**Figure 3**).

One year post-vaccination most country-specific differences had disappeared (**Supplementary Table 2**). In all age groups and in all three countries 3 to 13 participants per group had antibody concentrations below the arbitrary protective cut-off of 20 IU/ml (**Figure 3**).

IgA concentrations in older adults were generally highest in the Netherlands, followed by Finland, and lowest in the UK at all three timepoints and for all antigens (**Figure 4** and **Supplementary Table 3**).

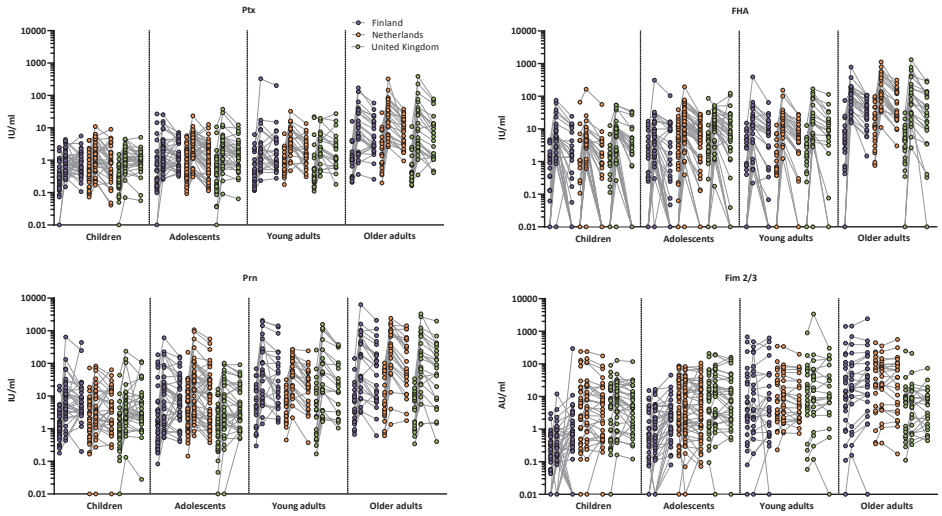



**Figure 4.** Individual IgA responses per age and country. Ptx: pertussis toxin; FHA: filamentous haemagglutinin; Prn: pertactin; Fim2/3: Fimbriae 2 and 3; IU/ml: international units per millilitre. values below LLOQs for are shown as 0.01 IU/ml or AU/ml. Concentrations from day 0, day 28 and 1 year connected per individual.

## Pertussis antibody concentrations in children and adolescents in relation to vaccination background

When the results from adolescents from Finland and the Netherlands were divided in two groups, one group with wP (n = 41) and the other with aP (n= 44) priming vaccine history (**Figures 5 and 6**), no pre- or post-vaccination differences between the aP and

wP group were observed against Ptx, FHA, and Prn. The observed differences between Dutch and Finnish aP primed adolescents for IgG-Fim2/3 and Prn were in line with the different vaccination history regarding antigens included in the different aP vaccines. The subanalyses between the different aP groups showed that the aP2 group (n = 10) had lower IgG-Prn GMCs at all timepoints compared with children and adolescents previously vaccinated with aP3 (n= 7) and wP vaccines (n= 41) (**Supplementary Table 4**). For Fim2/3 a significant difference between aP3, aP5 (n = 65), and wP was observed at all timepoints, moreover, the aP3 group did not show a significant increase and subsequent decrease, where the aP5 group did show this pattern (**Supplementary Table 5**).

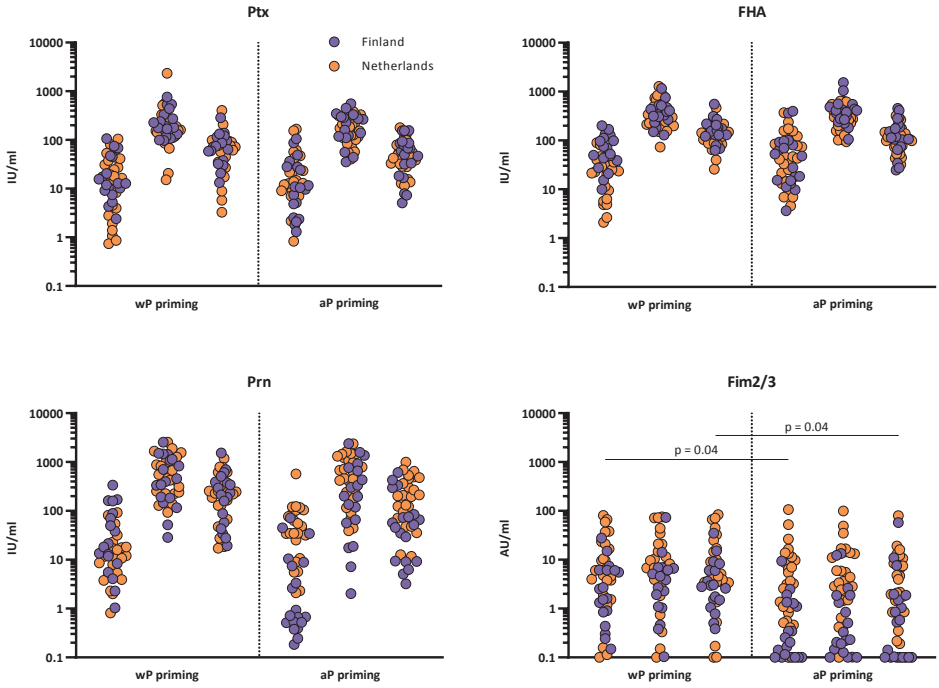



**Figure 5.** IgG concentrations of pertussis vaccine components per wP and aP background. Ptx: pertussis toxin; FHA: filamentous haemagglutinin; Prn: pertactin; Fim2/3: Fimbriae 2 and 3; IU/ml: international units per millilitre; aP priming: participants received exclusively acellular pertussis components containing vaccines in the primary series; wP priming: participants received exclusively whole cell pertussis containing vaccines in the primary series. values below LLOQ are shown as 0.1 IU/ml or AU/ml. Significance has been tested at day 0, day 28 and 1 year between wP and aP priming.

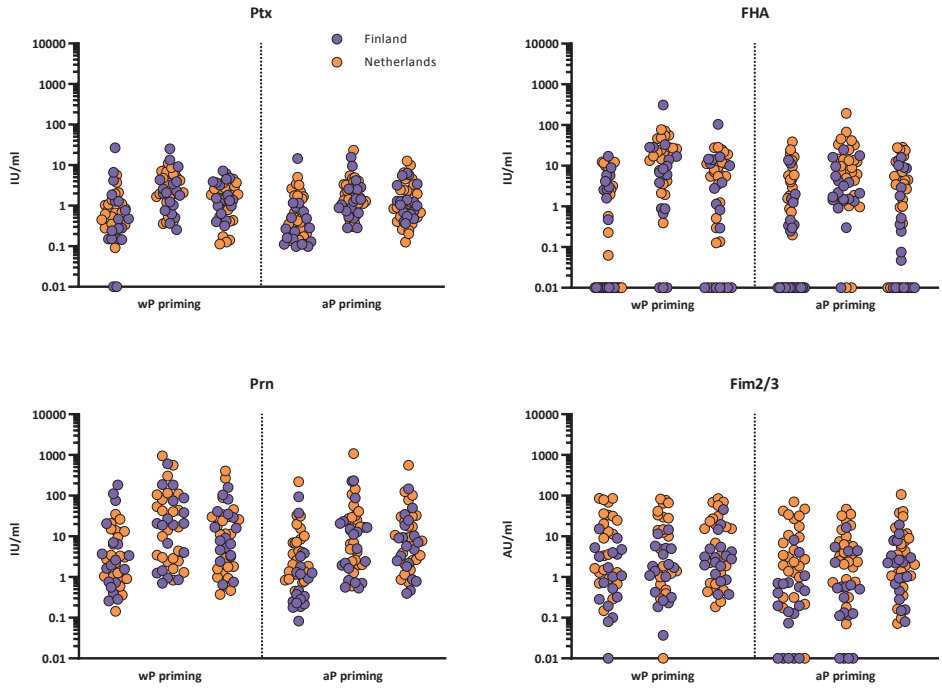



**Figure 6.** IgA concentrations of pertussis vaccine components per wP and aP background. Ptx: pertussis toxin; FHA: filamentous haemagglutinin; Prn: pertactin; Fim2/3: Fimbriae 2 and 3; IU/ml: international units per millilitre; aP priming: participants received exclusively acellular pertussis components containing vaccines in the primary series; wP priming: participants received exclusively whole cell pertussis containing vaccines in the primary series. values below LLOQ are shown as 0.1 IU/ml or AU/ml. Significance has been tested at day 0, day 28 and 1 year between aP and wP priming.

## DISCUSSION

Here we present the serological data after a Tdap-IPV booster vaccination in four age groups of three European countries. IgG concentrations for all antigens demonstrated a booster pattern with considerable increases of pertussis-specific antibodies observed in all age groups after the first month. Although there were significant decreases at one year the GMCs remained at least 3-fold higher than baseline for all antigens and age groups. There were some significant differences between age groups which varied by antigen but antibody responses were not consistently worse with increasing age. These data emphasise that at least in terms of antibody quantity, responses are not uniformly less in older adults compared with the other age groups. When analysed with the countries separately, the IgG antibody concentrations were overall not significantly different in 90% (81/90 at 28 days) and 97% (87/90 at 1 year) of the comparisons. For IgA increases for all antigens were relatively smaller and decreases varied in significance. Moreover, the IgA antibody

concentrations showed pronounced increase with age for all antigens and timepoints especially notable in older adults.

Age seemed to influence the specific IgA responses to vaccination. It is already known that pre-vaccination IgA concentrations increase with age [50-52]. It is also previously described that greater IgA-antibody increase due to vaccination is positively correlated to high pre-vaccination concentrations [53]. In this study we see a similar pattern of higher IgA-antibody concentrations at baseline with higher age. One month post-vaccination a clear age dependant increase for IgA-Ptx and Prn is observed. Interestingly the antibody decay of these IgA antibodies was also age dependant, with greater fold decrease in older age groups. IgG responses on the other hand did not show a consistent relationship with age. In contrast a booster vaccine study in the USA previously described higher post-vaccination IgG concentrations for Ptx in adolescents compared with adults until three years post-vaccination. They hypothesised that this was either due to greater immune responsiveness at adolescent age or due to a shorter time interval since the paediatric vaccinations in the adolescents compared to the adults [54]. The post-vaccination GMCs for IgG-Ptx of young adults in our study was lower compared with the adolescents, but the older adults on the other hand, had a significantly higher GMC compared with young adults one year post-vaccination. Therefore, with respect to IgG, we did not observe less or better immune responsiveness, or a positive or negative effect with more recent vaccinations. Although the fold increase and subsequent fold decrease in IgG responses was greater than in IgA, the degree of change in IgG responses did not seem to be influenced by age. Differences in antibody kinetics between specific IgG and IgA responses can possibly be explained by the route of primary activation. Usually specific IgA responses result after the person encounters *B. pertussis* in life [55], and IgA production is therefore considered activated via the mucosal route, while IgG production has mostly been parenterally activated after vaccination in early infancy. It is plausible to assume that these two routes lead to different kinetics [56]. Both IgG and IgA responses will be boosted by revaccination and reinfection. It is highly likely that constant circulation of *B. pertussis* in the population makes that the number of encounters increase with age. Therefore, our results imply that IgA antibody responses are more influenced by this constant circulation than the IgG responses. This is supported by the Dutch data, given that the circulation of *B. pertussis* is higher in the Netherlands and the Dutch older adults had highest IgA concentrations for all antigens at all timepoints. The difference in IgA concentrations between young and older adults might not only be explained by the amount of encounters, but also the route of primary and secondary activation is likely to influence the IgA response since the older adults will not all have been primed by vaccination but by natural infection and also later the natural boosting might have had a more pronounced effect to IgA mediated immunity.

Based on the reported cases with pertussis the circulation of *B. pertussis* might be different between the three countries participating in this study. The notification rate (N/100,000, all ages) in the last decade was highest in the Netherlands, followed by Finland, and lowest numbers in the UK (**Figure 1**) [24]. These differences in circulation between the countries appear to be reflected in some findings in this study. The Dutch older adults had a significantly higher IgG and IgA baseline GMC for Ptx, and FHA compared with UK older adults, while the GMC of older Finnish adults were in between these two. For FHA this difference still exists one month post-vaccination. The effect of pertussis circulation is probably best reflected in older adults, since their vaccination history is comparable between the countries and previous vaccinations were administered long ago or not at all. Additionally, in this study the number of recent pertussis infections defined as ≥ 100 IU/ml for IgG-Ptx at baseline [39], were most observed in the Dutch cohort, followed by the Finnish cohort, and then the UK, which is also in line with the national notification rates. Nevertheless, we should bear in mind that the notification rates in the European countries as illustrated on the ECDC website are fluctuating heavily per country and are strongly dependant on the use of diagnostic methods (PCR and/or serology) and the accuracy of the pertussis surveillance system in each country. Thus the reports might therefore not reflect the real incidence numbers. Seroepidemiological studies could provide more information about the seroprevalence of pertussis antibodies in the population and thereby on the degree of circulation of the pathogen, but these studies have been performed in only a few European countries [57, 58].

Differences due to vaccination background, especially between wP and aP priming were anticipated since higher responses in aP primed 4-year-olds compared with their wP primed peers have been described [38]. Two studies in children of respectively 9 years of age and 11 and 12 years of age, on the other hand, described better responses in wP primed individuals [59, 60]. In this study however, the two priming backgrounds did not reveal any differences in IgG levels against the vaccine antigens pre- or post-vaccination, which might be explained by the limited number of participants for the comparison between wP and aP priming. Participants previously vaccinated with aP2 vaccines (and thus lacking Prn) show a significantly lower baseline and a lower Prn antibody increase upon 'booster' vaccination, reflecting a more primary response to Prn compared with participants previously aP3 or wP vaccinated who show a real booster response (**Supplementary Table 4**) [61]. Participants previously vaccinated with aP3 vaccines (and thus lacking Fim2/3) had lower Fim2/3 concentrations at baseline and did not show a significant increase where aP5 and wP primed participants did show a significant increase although Fim2/3 was not even in the vaccine, indicating polyclonal activation of non-vaccine antigens (**Supplementary Table 5**). Polyclonal activation of unrelated antigens to which the donor was previously immunised has been observed before [62].

It is questionable if a booster vaccination induces sufficient pertussis antibody levels on the long term (i.e. five years) for protection considering the decrease in pertussis antibody levels one year after the booster vaccination found in this study. However Ward et al. found a vaccine efficacy of 92% in adolescents and adults for 22 months, Zoldi et al. found a decrease in pertussis notifications in young adults due to military conscripts vaccination, and Liu et al. found a protective effect in older adults even after five years [29, 63, 64]. The study from Taranger et al. found a correlation between the height of the post-vaccination antibody concentrations and protection against pertussis in toddlers up to 33 months postvaccination [65]. The one month post-vaccination GMCs from our groups correspond most with the GMCs of the toddler group that developed mild pertussis disease within 33 months indicating that our study population might be susceptible to contract mild pertussis within three years after the booster vaccination. The implementation of additional pertussis boosters in the (older) adults should be discussed not only in the context of the persistence of antibody levels on the long term but also on the epidemiological pertussis situation in each individual country. Moreover, a systematic literature review from Kandeil et al. states that the pertussis disease burden is considerably underestimated in older adults. Older adults are more likely to have (pulmonary) comorbidities and are therefore more prone to serious complications and hospitalisation. With the ageing population, health and economic burden of pertussis is expected to rise [3].



A strength of the study is that serological antibody results of all participants could reliably be compared between the three countries and the four age groups, since all antibody measurements were performed in the same lab with a highly standardised and validated multiplex assay. In addition, 375 participants in total completed this pertussis booster vaccination study based on inclusion of 22-48 participants per age group per country.

A limitation of the study is that the vaccination background of the participants appeared to be quite divergent between countries and even within countries, thereby making analysis based on vaccination background quickly underpowered.

In conclusion, the participants from all four age groups in the three countries responded well to the aP booster vaccination as measured after one month and one year. The influence of age and epidemiological background of pertussis in the countries seems limited on the IgG vaccine responses, however increasing age does seem to have a positive effect on IgA responses. Therefore, acellular pertussis booster vaccination might also be considered for older adults and individuals with pulmonary morbidities in order to reduce the health and economic burden of pertussis in the population.

## DECLARATION OF COMPETING INTEREST

None of the authors received payment or service from a third part at any time, nor does anyone have a financial relationship with entities in the bio-medical arena. None of the authors have any patents relevant to the work. MVP is a member of the Portuguese National Immunisation Technical Advisory Group (Comissão Técnica de Vacinação da Direcção Geral de Saúde).

## ACKNOWLEDGEMENTS

We thank all participants of the study and we acknowledge the work of all individuals at the Oxford Vaccine Group, the Turku University group, the Dutch Spaarne Hospital and RIVM who have undertaken the work of the BERT study including the administrative staff, play assistants, clinical research nurses, clinical research fellows, laboratory staff and members of the IT team. In particular, we acknowledge Jacqueline Zonneveld and Greetje van Asselts at the Spaarne Gasthuis Hospital, Hoofddorp, the Netherlands for their help in the management of clinical data and performing home visits. We are also grateful to Inge Pronk and Eleonora Lambert, who both work at the Centre for Infectious Disease Control, RIVM for their assistance in clinical trial management. PERISCOPE has received funding from the Innovative Medicines Initiative 2 Joint Undertaking under grant agreement No 115910. This Joint Undertaking receives support from the European Union's Horizon 2020 research and innovation programme and EFPIA and BMGF. DFK receives salary for support from the NIHR Oxford Biomedical Research Centre.

## DATA SHARING STATEMENT

Individual participant data that underlie the results reported in this article, have been de-identified and deposited in the central database of the PERISCOPE Consortium and can be accessed by a request to the PERISCOPE management team.

## CONTRIBUTORS

GAMB, DFK, and JM designed the trial with input from QH, EAMS, RdG, DAD, A-MB, PV, MVP, and AMB. Trial coordination, clinical trial management and clinical data collection was performed by PV, MVP, AMB, MAvH, RL, and SB. Samples were processed by PGMvG, SB, MVP, AMB and PV. Luminex data were generated by PGMvG and PV. Underlying data were verified by PV, PGMvG, GAMB, DFK, JM. Data analysis was performed by JvdK with input from PV, GAMB, DFK, and JM. PV and GAMB wrote the first draft of the manuscript and all co-authors contributed to subsequent drafts. All authors read and approved the final manuscript.

## REFERENCES


1. Mattoo, S. and J.D. Cherry, Molecular pathogenesis, epidemiology, and clinical manifestations of respiratory infections due to Bordetella pertussis and other Bordetella subspecies. Clin Microbiol Rev, 2005. 18(2): p. 326-382, DOI: 10.1128/cmr.18.2.326-382.2005.
2. Cherry, J.D., Pertussis in Young Infants Throughout the World. Clin Infect Dis, 2016. 63(suppl 4): p. S119-S122. doi: 10.1093/cid/ciw550.
3. Kandeil, W., et al., The burden of pertussis in older adults: what is the role of vaccination? A systematic literature review. Expert Rev Vaccines, 2019. 18(5): p. 439-455. doi: 10.1080/14760584.2019.1588727.
4. Cherry, J.D., Historical review of pertussis and the classical vaccine. J Infect Dis, 1996. 174 Suppl 3: p. S259-63.
5. David, S., P.E. Vermeer-de Bondt, and N.A.T. van der Maas, Reactogenicity of infant whole cell pertussis combination vaccine compared with acellular pertussis vaccines with or without simultaneous pneumococcal vaccine in the Netherlands. Vaccine, 2008. 26(46): p. 5883-5887, DOI: 10.1016/j.vaccine.2008.07.105.
6. Lambert, L.C., Pertussis vaccine trials in the 1990s. J Infect Dis, 2014. 209 Suppl 1(Suppl 1): p. S4-9.
7. Sheridan, S.L., R.S. Ware, K. Grimwood, and S.B. Lambert, Number and order of whole cell pertussis vaccines in infancy and disease protection. Jama, 2012. 308(5): p. 454-456. doi: 10.1001/jama.2012.6364.
8. Klein, N.P., et al., Waning Protection after Fifth Dose of Acellular Pertussis Vaccine in Children. http://dx.doi.org/10.1056/NEJMoa1200850, 2012.
9. Warfel, J.M., L.I. Zimmerman, and T.J. Merkel, Acellular pertussis vaccines protect against disease but fail to prevent infection and transmission in a nonhuman primate model. Proc Natl Acad Sci U S A, 2014. 111(2): p. 787-792, DOI: 10.1073/pnas.1314688110.
10. Campbell, H., et al., Accelerating control of pertussis in England and Wales. Emerg Infect Dis, 2012. 18(1): p. 38-47.
11. van der Maas, N.A.T., S. David, J.M. Kemmeren, and P.E. Vermeer-de Bondt, Safety surveillance in the National Vaccination Programme; fewer adverse events with the DTP-IPV-Hib vaccine after the transition to an acellular pertussis component in 2005. Ned Tijdschr Geneeskd, 2007. 151(49): p. 2732-7.
12. Elomaa, A., Q. He, N.N. Minh, and J. Mertsola, Pertussis before and after the introduction of acellular pertussis vaccines in Finland. Vaccine, 2009. 27(40): p. 5443-9.
13. Berbers, G.A.M., S.C. de Greeff, and F.R. Mooi, Improving pertussis vaccination. Hum Vaccin, 2009. 5(7): p. 497-503.
14. Kapil, P. and T.J. Merkel, Pertussis vaccines and protective immunity. Curr Opin Immunol, 2019. 59: p. 72-78.
15. Marcellini, V., et al., Protection against Pertussis in Humans Correlates to Elevated Serum Antibodies and Memory B Cells. Front Immunol, 2017. 8: p. 1158.
16. Amirthalingam, G., et al., Effectiveness of maternal pertussis vaccination in England: an observational study. Lancet, 2014. 384(9953): p. 1521-8.
17. Cherry, J.D., J. Gornbein, U. Heininger, and K. Stehr, A search for serologic correlates of immunity to Bordetella pertussis cough illnesses. Vaccine, 1998. 16(20): p. 1901-6.

**18.** Dabrera, G., et al., A case-control study to estimate the effectiveness of maternal pertussis vaccination in protecting newborn infants in England and Wales, 2012-2013. Clin Infect Dis, 2015. 60(3): p. 333-7.

**19.** Storsaeter, J., H.O. Hallander, L. Gustafsson, and P. Olin, Levels of anti-pertussis antibodies related to protection after household exposure to Bordetella pertussis. Vaccine, 1998. 16(20): p. 1907-16.

**20.** Broutin, H., et al., Impact of vaccination and birth rate on the epidemiology of pertussis: a comparative study in 64 countries. Proc Biol Sci, 2010. 277(1698): p. 3239-45.

**21.** Elomaa, A., et al., Strain variation among Bordetella pertussis isolates in finland, where the whole-cell pertussis vaccine has been used for 50 years. J Clin Microbiol, 2005. 43(8): p. 3681-7.

**22.** van der Maas, N.A.T., et al., Pertussis in the Netherlands, is the current vaccination strategy sufficient to reduce disease burden in young infants? Vaccine, 2013. 31(41): p. 4541-7.

**23.** Schurink-van't Klooster, T.M. and H.E. de Melker, The National Immunisation Programme in the Netherlands: Surveillance and developments in 2018-2019. 2019.

**24.** ECDC. Disease data from ECDC Surveillance Atlas for pertussis. 2019 [cited 2019 20 September]; Available from: http://ecdc.europa.eu/en/pertussis/surveillance-and-disease-data/atlas].

**25.** Carvalho, C.F.A., et al., National Outbreak of Pertussis in England, 2011-2012: A Case-control Study Comparing 3-Component and 5-Component Acellular Vaccines With Whole-cell Pertussis Vaccines. Clin Infect Dis, 2020. 70(2): p. 200-207.

**26.** Public Health England, Laboratory confirmed cases of pertussis reported to the enhanced pertussis surveillance programme in England: annual report for 2012. GOV.UK; Health Protection Report 2013, 2013. 7(14-17): p. 26-28.

**27.** Ramsay, M.E., et al., Antibody response to accelerated immunisation with diphtheria, tetanus, pertussis vaccine. Lancet, 1993. 342(8865): p. 203-5.

**28.** Forsyth, K.D., et al., Recommendations to control pertussis prioritized relative to economies: A Global Pertussis Initiative update. Vaccine, 2018. 36(48): p. 7270-7275.

**29.** Zoldi, V., et al., Decreased incidence of pertussis in young adults after the introduction of booster vaccine in military conscripts: Epidemiological analyses of pertussis in Finland, 1995-2015. Vaccine, 2017. 35(39): p. 5249-5255.

**30.** Barug, D., et al., Maternal pertussis vaccination and its effects on the immune response of infants aged up to 12 months in the Netherlands: an open-label, parallel, randomised controlled trial. Lancet Infect Dis, 2019. 19(4): p. 392-401.

**31.** Sandmann, F., et al., Infant hospitalisations and fatalities averted by the maternal pertussis vaccination programme in England, 2012-2017: Post-implementation economic evaluation. Clin Infect Dis, 2020.

**32.** Cassimos, D.C., et al., Vaccination Programs for Adults in Europe, 2019. Vaccines (Basel), 2020. 8(1):34(1).

**33.** Australian Government, Australian Immunisation Handbook, D.o. Health, Editor. 2019, Australian Government.

**34.** Haviari, S., et al., Vaccination of healthcare workers: A review. Hum Vaccin Immunother, 2015. 11(11): p. 2522-37.

**35.** OECD. Child vaccination rates (indicator). on 21 August 2020)]; Available from: doi: 10.1787/b23c7d13-en

**36.** PERISCOPE Consortium, PERISCOPE: road towards effective control of pertussis. Lancet Infect Dis, 2019. 19(5): p. e179-e186, DOI: 10.1016/s1473-3099(18)30646-7.

**37.** van Gageldonk, P.G.M., F.G. van Schaijk, F.R. van der Klis, and G.A.M. Berbers, Development and validation of a multiplex immunoassay for the simultaneous determination of serum antibodies to Bordetella pertussis, diphtheria and tetanus. J Immunol Methods, 2008. 335(1-2): p. 79-89, DOI: 10.1016/j.jim.2008.02.018.

**38.** Hendrikx, L.H., et al., IgG responses after booster vaccination with different pertussis vaccines in Dutch children 4 years of age: effect of vaccine antigen content. Vaccine, 2009. 27(47): p. 6530-6.

**39.** van der Lee, S., et al., Enhanced Bordetella pertussis acquisition rate in adolescents during the 2012 epidemic in the Netherlands and evidence for prolonged antibody persistence after infection. Euro Surveillance, 2017. 22(47): p. 17-00011. doi: 10.2807/1560-7917.ES.2017.22.47.17-00011.

**40.** Long, S.S., C.J. Welkon, and J.L. Clark, Widespread silent transmission of pertussis in families: antibody correlates of infection and symptomatology. J Infect Dis, 1990. 161(3): p. 480-6.

**41.** Sato, Y. and H. Sato, Anti-pertussis toxin IgG and anti-filamentous hemagglutinin IgG production in children immunized with pertussis acellular vaccine and comparison of these titers with the sera of pertussis convalescent children. Dev Biol Stand, 1985. 61: p. 367-72.

**42.** Olin, P., et al., How to make sense of pertussis immunogenicity data. Clin Infect Dis, 2001. 33 Suppl 4: p. S288-91.

**43.** Guo, B. and Y. Yuan, A comparative review of methods for comparing means using partially paired data. Stat Methods Med Res, 2017. 26(3): p. 1323-1340. doi: 10.1177/0962280215577111.

**44.** Giesbrecht, F.G. and J.C. Burns, Two-Stage Analysis Based on a Mixed Model: Large-Sample Asymptotic Theory and Small-Sample Simulation Results. Biometrics, 1985. 41(2): p. 477-486. doi: 10.2307/2530872.

**45.** Hrong-Tai Fai, A. and P.L. Cornelius, Approximate F-tests of multiple degree of freedom hypotheses in generalized least squares analyses of unbalanced split-plot experiments. Journal of Statistical Computation and Simulation, 1996. 54(4): p. 363-378. doi: 10.1080/00949659608811740.

**46.** Benjamini, Y. and Y. Hochberg, Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. Journal of the Royal Statistical Society. Series B (Methodological), 1995. 57(1): p. 289-300.

**47.** R Core Team, R: A language and environment for statistical computing. R Foundation for Statistical Computing. 2021: Vienna, Austria.

**48.** Bates, D., M. Mächler, B. Bolker, and S. Walker, Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software; Vol 1, Issue 1 (2015), 2015: p. 1-48. doi: 10.18637/jss.v067.i01.

**49.** Kuznetsova, A., P.B. Brockhoff, and R.H.B. Christensen, lmerTest Package: Tests in Linear Mixed Effects Models. Journal of Statistical Software; Vol 1, Issue 13 (2017), 2017.

**50.** Fumimoto, R., et al., Seroprevalence of IgA and IgM antibodies to Bordetella pertussis in healthy Japanese donors: Assessment for the serological diagnosis of pertussis. PLoS One, 2019. 14(7): p. e0219255.

**51.** May, M.L., et al., Pertussis toxin IgA testing over-diagnoses recent pertussis infection. Pathology, 2017. 49(7): p. 770-775.

**52.** Prince, H.E., J.M. Lieberman, and J.D. Cherry, Age-related differences in patterns of increased Bordetella pertussis antibodies. Clin Vaccine Immunol, 2012. 19(4): p. 545-550, DOI: 10.1128/cvi.05725-11.

**53.** Le, T., et al., Immune responses and antibody decay after immunization of adolescents and adults with an acellular pertussis vaccine: the APERT Study. J Infect Dis, 2004. 190(3): p. 535-44.

**54.** Pool, V., et al., Humoral immunity 10years after booster immunization with an adolescent and adult formulation combined tetanus, diphtheria, and 5-component acellular pertussis vaccine in the USA. Vaccine, 2018. 36(17): p. 2282-2287. doi: 10.1016/j.vaccine.2018.03.029.

**55.** Hendrikx, L.H., et al., Serum IgA responses against pertussis proteins in infected and Dutch wP or aP vaccinated children: an additional role in pertussis diagnostics. PLoS One, 2011. 6(11): p. e27681.

**56.** Engels, N. and J. Wienands, Memory control by the B cell antigen receptor. Immunol Rev, 2018. 283(1): p. 150-160.

**57.** de Greeff, S.C., et al., Seroprevalence of pertussis in The Netherlands: evidence for increased circulation of Bordetella pertussis. PLoS One, 2010. 5(12): p. e14183, DOI: 10.1371/journal.pone.0014183.

**58.** Barkoff, A.M., K. Gröndahl-Yli-Hannuksela, and Q. He, Seroprevalence studies of pertussis: what have we learned from different immunized populations. Pathog Dis, 2015. 73(7).

**59.** van der Lee, S., et al., Whole-Cell or Acellular Pertussis Primary Immunizations in Infancy Determines Adolescent Cellular Immune Profiles. Front Immunol, 2018. 9: p. 51. doi: 10.3389/fimmu.2018.00051.

**60.** Decker, M.D., D.P. Greenberg, D.R. Johnson, and V. Pool, Randomized study of immune responses to two Tdap vaccines among adolescents primed with DTaP and comparison with results among adolescents primed with DTwP. Vaccine, 2019. 37(35): p. 5003-5008.

**61.** Nicholson, L.B., The immune system. Essays Biochem, 2016. 60(3): p. 275-301.

**62.** Bernasconi, N.L., E. Traggiai, and A. Lanzavecchia, Maintenance of serological memory by polyclonal activation of human memory B cells. Science, 2002. 298(5601): p. 2199-202.

**63.** Ward, J.I., et al., Efficacy of an acellular pertussis vaccine among adolescents and adults. N Engl J Med, 2005. 353(15): p. 1555-63.

**64.** Liu, B., et al., Effectiveness of acellular pertussis vaccine in older adults: nested matched case-control study. Clin Infect Dis, 2019.

**65.** Taranger, J., et al., Correlation between pertussis toxin IgG antibodies in postvaccination sera and subsequent protection against pertussis. J Infect Dis, 2000. 181(3): p. 1010-3.

## SUPPLEMENTARY MATERIALS

**Supplementary Table 1a.** *B. Pertussis* vaccine schedules and vaccines in Finland.

| Year | Scheme | Vaccine composition | Change | Pertussis vaccine name (Company) | Study cohort | Vaccination coverage [35] |
|---|---|---|---|---|---|---|
| before | | | | No vaccine available | Older adults | |
| 1952 | | DTwP | First only DwP, T added in 1957<br>1962 change to DTwP (Fim2,3 strain)<br>Additional Fim2 strain added in 1976 | Diphteria-Pertussis Forte (Orion)<br>PDT (Orion)<br>DTwP (KTL) | Older and young adults | 92-99% |
| 2003 | 3,4,5, 20-24 m<br>6y | DTwP + IPV + Hib<br>Tdap3 | <br>First ap booster vaccine in NIP | DTwP (KTL)<br>Boostrix (GSK) | Adolescents<br>Adults | 98% |
| 2005 | 3,5,12 m<br>4y<br>14-15y | DTaP2-IPV-Hib<br>DTaP2-IPV<br>Tdap3 | Introduction aP priming, 2 Bp components<br><br>Also catch-up for 11-13y | Pentavac (Sanofi)<br>Tetravac (Sanofi)<br>Boostrix (GSK) | Adolescents and children<br><br>Young adults | 97-99% |
| 2009 | 3,5,12 m<br>4y<br>14-15y | DTaP3-IPV-Hib<br>DTaP2-IPV<br>Tdap3 | 3 Bp components | Infanrix (GSK)<br>Tetravac (Sanofi)<br>Boostrix (GSK) | Children<br>Adolescents<br>Young adults | 99% |
| 2012 | Military | Tdap3 | Addition of pertussis vaccination in the military, 18-25y (estimation) | Boostrix (GSK) | Young adults | 89-99% |
| 2018 | 25y | Tdap3 | Introduction of adult booster | Boostrix (GSK) | Young adults | 91% |
| 2019 (Sep) | 3,5,12 m | DTaP2-IPV-Hib | 2 Bp components | Pentavac (Sanofi) | | |

**Supplementary Table 1b.** *B. Pertussis* vaccine schedules and vaccines in the Netherlands.

| Year | Scheme | Vaccine composition | Change | Pertussis vaccine name (Company) | Study cohort | Vaccination coverage [35] |
|---|---|---|---|---|---|---|
| before | | | | No vaccine available | Older adults | |
| 1954 | | DT**wP** | Combination vaccine became available | DTwP (RIVM) | Older adults | |
| 1957 | 3,4,5,11 m | DT**wP** | Start of NIP | DTwP (RIVM) | Older and young adults | 96-97% |
| 1999 | 2,3,4,11 m | DT**wP**-IPV + Hib | Accelerated vaccination | DTwP (RIVM) | | 97.0-97.2% |
| 2001 | 2,3,4,11 m<br>4y | DT**wP**-IPV + Hib<br>DT-IPV + **aP3** | Introduction aP booster, 3 Bp components | DTwP (RIVM)<br>monovalent aP (GSK) | Adolescents | 97.2-97.8% |
| 2005 | 2,3,4,11 m<br>4y | DT**aP3**-IPV-Hib<br>DT-IPV + **aP3** | Introduction aP priming, 3 Bp components | Infanrix (GSK)<br>monovalent aP (GSK) | Adolescents | 96.0% |
| 2006 (Jan) | 2,3,4,11 m<br>4y | DT**aP5**-IPV-Hib<br>DT-IPV + **aP3** | 5 Bp components | Pediacel (SP MSD)<br>monovalent aP (GSK) | Adolescents | 96.3-96.9% |
| 2011 (Oct) | 2,3,4,11 m<br>4y | DT**aP3**-IPV-Hib-HepB + Pneu<br>DT**aP3**-IPV | 3 Bp components | Infanrix (GSK)<br>Infanrix (GSK) | | 94.8-96.7% |
| 2017 (Jan) | 2,3,4,11 m<br>4y | DT**aP3**-IPV-Hib-HepB + Pneu<br>Td**ap3**-IPV | Reduced dose of 3 Bp components | Infanrix (GSK)<br>Boostrix (GSK) | | 93.9% |
| 2018 (Dec) | 2,3,4,11 m<br>4y | DT**aP5**-IPV-Hib-HepB + Pneu<br>Td**ap3**-IPV | 5 Bp components | Vaxelis (SP MSD)<br>Boostrix (GSK) | | 93.5% |
| 2019 (Dec) | Pregnant women (from 22 w of gestational age) | Td**ap3** | Introduction ap booster for pregnant women, reduced dose of 3 Bp components | Boostrix (GSK) | | |

**Supplementary Table 1c.** *B. Pertussis* vaccine schedules and vaccines in the UK.

| Year | Scheme | Vaccine composition | Change | Pertussis vaccine name (Company) | Study cohort | Vaccination coverage [35] |
|---|---|---|---|---|---|---|
| before | | | | No vaccine available | Older adults | |
| 1957 | 3, 4.5-5, 8.5-11 m | DT**wP** | Start of NIP | DTwP | Older adults | 40.0-78.0% |
| 1990 (Jun) | 2,3,4 m | DT**wP**-IPV + Hib | Accelerated schedule | DTwP-IPV-Hib | Young adults | 85.0-96.0% |
| 2000-2001 | 2,3,4 m | DT**wP**-IPV + Hib / DT-IPV + **aP3** | Shortness of wP -use of a mix aP/wP | DTwP-IPV-Hib | | 91.0% |
| 2001 (Nov) | 2,3,4 m<br>3y 4m | DT**wP**-IPV-Hib<br>DT-IPV + **aP3** | Introduction aP booster | DTwP-IPV/Hib | | 91.0% |
| 2004 (Aug) | 2,3,4 m<br>3y 4m | DT**aP5**-IPV-Hib<br>DT-IPV + **aP5** | Introduction aP priming, 5 Bp components | Pediacel (SP MSD)<br>Repevax (MSD) | Adolescents | 92% |
| 2005 (Jan) | 2,3,4 m<br>3y 4m | DT**aP5**-IPV-Hib / DT**aP3**-IPV-Hib<br>DT-IPV + **aP5 /** DT-IPV + **aP3** | Availability of 3 or 5 components | Pediacel (SP MSD) / Infanrix (GSK)<br>Repevax (MSD) / Infanrix (GSK) | Adolescents and children | 91.0-95.0% |
| 2012 (Sep) | Pregnant woman (28-32w of gestational age) | DT-IPV + **aP5** | Introduction ap booster for pregnant women , reduced dose of 5 Bp components | Repevax (MSD) | | 94.0-95.0% |
| 2016 | Pregnant woman (from 16 weeks of gestational age) | DT-IPV + **aP5 /** DT-IPV + **aP3** | changed timing of vaccination, availability of 3 or 5 reduced Bp components | Repevax (MSD) / Boostrix (GSK) | | |
| 2019 (Dec) | Pregnant women (from 22 weeks gestational age) | Td**ap3** | changed timing of vaccination, discontinuation of use of Repevax | Boostrix (GSK) | | |

m=months; y= years; D=diphtheria; T=tetanus; wP: whole cell pertussis; aP: acellular pertussis; IPV: inactivated poliovirus; Pneu: pneumococcal; Hib: *Haemophilus influenzae* type B; HepB: Hepatitis B; Bp: *Bordetella pertussis*; NVI: Netherlands Vaccine Institute; GSK: GlaxoSmithKline; SP: Sanofi Pasteur; MSD: Merck Sharp & Dohme.

**Supplementary Table 2.** IgG GMCs per age and country.

| Antigen | timepoint | Country | Children | Adolescents | Young adults | Older adults |
|---|---|---|---|---|---|---|
| Ptx GMC (CI) in IU/ml | Day 0 | FI | 16 (11-23)[a,b,c,d] | 13 (9-18)[a,e,f] | 5 (3-8)[g,h,i] | 10 (7-16)[j,k,l] |
| | | NL | 12 (8-17)[m,n,o] | 14 (10-19)[m,p,q] | 3 (2-5)[r,s,t,u,v] | 15 (10-24)[j,m,w,x] |
| | | UK | 8 (5-11)[g,y,z,aa,bb] | 16 (11-23)[b,j,z,cc,dd] | 3 (2-5)[b,y,ee,ff] | 5 (3-7)[t,y,gg,hh,ii] |
| | Day 28 | FI | 197 (138-282)[d,g,h] | 202 (141-288)[f,h,jj] | 99 (64-153)[a,c,e,i] | 132 (85-204)[l,gg] |
| | | NL | 147 (103-211)[o,r] | 150 (109-205)[q,s] | 99 (64-152)[m,v] | 156 (101-240)[t,x] |
| | | UK | 110 (77-157)[b,bb] | 137 (96-196)[y,dd] | 112 (72-174)[z,ff] | 86 (56-133)[ii] |
| | 1 year | FI | 39 (27-55)[c,g] | 54 (38-77)[e,i,jj] | 27 (17-42)[a,f,h] | 51 (33-78)[k,gg] |
| | | NL | 30 (21-43)[n,r] | 44 (32-60)[p,s,v] | 22 (14-34)[m,q,u] | 43 (28-66)[t,w] |
| | | UK | 36 (25-51)[b,aa] | 48 (34-70)[y,cc] | 30 (19-47)[z,ee] | 38 (25-59)[j,hh] |
| FHA GMC (CI) in IU/ml | Day 0 | FI | 41 (31-54)[b,c,d,r] | 47 (36-62)[e,f,s,y,gg] | 30 (21-41)[h,i,z] | 27 (20-38)[k,l,t,jj] |
| | | NL | 25 (19-33)[n,o,g,t] | 30 (24-38)[m,p,q,t,jj] | 18 (13-25)[s,t,u,v] | 59 (42-81)[j,m,r,s,w,x,gg] |
| | | UK | 20 (15-26)[g,aa,bb] | 29 (22-38)[z,cc,dd,jj] | 15 (11-21)[a,y,ee,ff] | 18 (13-25)[t,hh,ii] |
| | Day 28 | FI | 363 (277-476)[d,g] | 369 (282-484)[f,k,jj] | 281 (202-390)[a,i] | 225 (162-312)[e,l,w,gg] |
| | | NL | 234 (178-308)[o,r,w] | 291 (230-369)[q,s] | 330 (237-458)[m,v] | 425 (306-590)[k,n,t,x,hh] |
| | | UK | 288 (218-380)[b,bb,hh] | 285 (216-375)[y,dd,hh] | 287 (205-401)[z,ff] | 174 (125-241)[j,w,aa,cc,ii] |
| | 1 year | FI | 102 (78-135)[c,g] | 139 (106-182)[e,jj] | 117 (84-162)[a,h] | 104 (75-144)[k,gg] |
| | | NL | 60 (45-79)[n,r] | 107 (85-136)[p,s] | 103 (74-143)[m,u] | 140 (101-194)[t,w] |
| | | UK | 113 (87-148)[b,aa] | 119 (89-157)[y,cc] | 119 (84-169)[z,ee] | 88 (63-123)[j,hh] |
| Prn GMC (CI) in IU/ml | Day 0 | FI | 9 (6-15)[c,d,r] | 7 (4-10)[o,e,f,s,y] | 22 (12-39)[h,i,m,jj] | 11 (6-19)[k,l] |
| | | NL | 24 (15-39)[g,m, n,o,t] | 20 (13-30)[m,t,p,q,jj] | 8 (5-15)[a,r,s,u,v] | 8 (5-15)[r,s,w,x] |
| | | UK | 20 (13-32)[j,aa,bb] | 15 (9-24)[cc,dd,jj] | 12 (7-23)[ee,ff] | 6 (3-11)[b,hh,ii] |
| | Day 28 | FI | 391 (244-629)[d,g] | 256 (159-411)[f,jj] | 413 (232-735)[a,i] | 195 (109-347)[l,gg] |
| | | NL | 259 (160-419)[o,r] | 467 (308-708)[q,s] | 237 (133-421)[m,v] | 232 (130-412)[t,x] |
| | | UK | 248 (154-397)[b,bb] | 270 (169-431)[y,dd] | 371 (206-668)[z,ff,hh] | 111 (63-198)[j,ee,ii] |
| | 1 year | FI | 95 (59-152)[c,g] | 85 (53-137)[e,k,jj] | 193 (108-343)[a,h] | 90 (50-159)[k,gg] |
| | | NL | 71 (44-116)[n,q,r] | 170 (112-258)[o,p,s] | 107 (60-190)[m,u] | 81 (46-145)[t,w] |
| | | UK | 89 (57-141)[b,aa] | 103 (64-166)[y,cc] | 168 (92-308)[z,ee] | 65 (37-117)[j,hh] |
| Fim2/3 GMC (CI) in AU/ml | Day 0 | FI | 0.2 (0.1-0.3)[a,b,c,d,r,jj,gg] | 0.7 (0.4-1.3)[a,e,f,g,s,y,gg] | 7.4 (3.7-14.7)[g,jj] | 10.1 (5.1-20.2)[g,j,jj] |
| | | NL | 5.4 (3.0-9.5)[g,n] | 4.3 (2.6-7.1)[y,jj] | 6.6 (3.3-13.2) | 4.0 (2.0-7.9) |
| | | UK | 10.3 (6.1-17.5)[g,j,qq] | 11.1 (6.5-18.8)[j,s,jj] | 10.6 (5.2-21.4)[j] | 1.5 (0.8-3.1)[b,y,z,gg] |
| | Day 28 | FI | 0.3 (0.2-0.6) [c,e,g,h,k,n,aa] | 1.1 (0.6-1.9)[c,h,k,p,cc,jj] | 8.8 (4.4-17.5)[c,e,f,i] | 10.1 (5.1-20.1)[c,e,f,hh] |
| | | NL | 7.6 (4.3-13.5) [o,r] | 5.2 (3.2-8.6)[e,q,cc] | 7.7 (3.9-15.4)[v] | 4.3 (2.2-8.6)[x] |
| | | UK | 17 (10.0-28.9)[b,c,bb,hh] | 14.9 (8.8-25.3)[e,p,dd,hh] | 10.7 (5.3-21.7)[hh] | 1.7 (0.8-3.3)[k,aa,cc,ee] |
| | 1 year | FI | 0.3 (0.2-0.6) [d,f,g,i,l,o,bb,ii] | 1.1 (0.6-1.9)[d,q,i,l,dd,ii,jj] | 5.7 (2.9-11.4)[d,h,ii] | 11.6 (5.8-23.0)[d,x,bb,dd,ff,ii] |
| | | NL | 4.7 (2.6-8.3) [n,bb] | 3.5 (2.1-5.7)[f,p,dd] | 5.0 (2.5-10.0)[u] | 2.9 (1.5-5.8)[l,w] |
| | | UK | 11.8 (7.0-19.7)[d,o,aa] | 10.5 (6.1-18.0)[f,q,cc] | 8.8 (4.3-17.9) | 1.9 (0.9-3.7)[l] |

Ptx: pertussis toxin; FHA: filamentous haemagglutinin; Prn: pertactin; Fim2/3: fimbriae 2 and 3; GMC: geometric mean concentration; CI: confidence interval; IU/ml: international units per millilitre; AU/ml: arbitrary units per millilitre; FI: Finland; NL: Netherlands; UK: United Kingdom. Significance per antigen has been tested between timepoints of an age group, age groups within a country and between countries within an age group, p ≤ 0.05, *p ≤ 0.01*, **p ≤ 0.001**. Significantly different from **a** young adults FI at day 0; **b** children UK at day 0; **c** children FI at day 28; **d** children FI at 1 year; **e** adolescents FI at day 28; **f** adolescents FI at1 year; **g** children FI at day 0; **h** young adults FI at day 28; **i** young adults FI at 1 year; **j** older adults UK at day 0; **k** older adults FI at day 28; **l** older adults FI at 1 year; **m** young adults NL at day 0; **n** children NL at day 28; **o** children NL at 1 year; **p** adolescents NL at day 28; **q** adolescents NL at 1 year; **r** children NL at day 0; **s** adolescents NL at day 0; **t** older adults NL at day 0; **u** young adults NL at day 28; **v** young adults NL at 1 year; **w** older adults NL at day 28; **x** older adults NL at 1 year; **y** adolescents UK at day 0; **z** young adults UK at day 0; **aa** children UK at day 28; **bb** children UK at 1 year; **cc** adolescents UK at day 28; **dd** adolescents UK at 1 year; **ee** young adults UK at day 28; **ff** young adults UK at 1 year; **gg** older adults FI at day 0; **hh** older adults UK at day 28; **ii** older adults UK at 1 year; **jj** adolescents FI at day 0.

**Supplementary Table 3.** IgA GMCs per age and country.

| Antigen | timepoint | Country | Children | Adolescents | Young adults | Older adults |
|---|---|---|---|---|---|---|
| Ptx GMC (CI) in IU/ml | Day 0 | FI | 0.26 (0.18-0.38)[a,b,c] | 0.39 (0.26-0.57)[a,d,e] | 0.49 (0.30-0.77)[f,g] | 0.86 (0.54-1.37)[h,i,j,k,l] |
| | | NL | 0.38 (0.26-0.56)[j,m,n,o,p] | 0.68 (0.49-0.95)[j,q,r,s,t] | 0.77 (0.48-1.23)[j,q,u,u,v] | 1.88 (1.18-2.99)[a,m,n,q,w,x,y] |
| | | UK | 0.23 (0.16-0.34)[w,z,aa,bb] | 0.36 (0.25-0.53)[m,cc,dd] | 0.57 (0.35-0.91)[ee,ff,gg] | 0.63 (0.39-1.00)[j,ee,hh,ii] |
| | Day 28 | FI | 0.82 (0.56-1.20)[d,f,h,k] | 1.66 (1.14-2.44)[b,i,k] | 2.07 (1.30-3.29)[b,k,jj] | 8.2 (5.2-13.1)[a,b,d,f,l] |
| | | NL | 1.32 (0.89-1.94)[p,q,u,x] | 2.15 (1.53-3.00)[m,t,x] | 3.63 (2.28-5.78)[n,o,v,x] | 19.5 (12.3-31.1)[j,o,s,u,y] |
| | | UK | 1.06 (0.72-1.56)[cc,ee,ff,hh] | 2.01 (1.37-2.94)[r,aa,dd,hh] | 2.33 (1.46-3.73)[z,aa,hh] | 11.2 (7.0-17.9)[w,aa,cc,ff,ii] |
| | 1 year | FI | 0.79 (0.54-1.16)[g,h,l] | 1.45 (0.99-2.12)[i,l] | 1.73 (1.09-2.75)[c,l,jj] | 4.78 (3.00-7.61)[a,c,e,g,k] |
| | | NL | 0.71 (0.48-1.05)[o,q,v,y] | 1.11 (0.79-1.55)[m,s,y] | 1.84 (1.15-2.92)[n,p,u,y] | 7.1 (4.5-11.3)[j,p,t,v,x] |
| | | UK | 0.85 (0.59-1.23)[ee,ii] | 1.36 (0.92-2.01)[r,cc,ii] | 1.58 (0.97-2.56)[z,ii] | 4.71 (2.95-7.53)[w,bb,dd,gg,hh] |
| FHA GMC (CI) in IU/ml | Day 0 | FI | 0.14 (0.07-0.30)[a,b] | 0.22 (0.10-0.45)[a,d,m] | 0.32 (0.13-0.80)[a,f,g] | 1.76 (0.71-4.35)[h,i,j,k,l,jj] |
| | | NL | 0.11 (0.05-0.23)[j,m,n,o] | 0.74 (0.38-1.41)[i,j,q,s,t] | 0.46 (0.19-1.14)[j,q,u,u,v] | 10.5 (4.2-25.9)[a,m,n,q,w,x,y] |
| | | UK | 0.12 (0.06-0.26)[r,w,z,aa,bb] | 0.58 (0.28-1.23)[cc,dd,ee] | 0.47 (0.19-1.19)[ee,ff,gg] | 1.96 (0.79-4.84)[j,ee,hh,ii] |
| | Day 28 | FI | 1.65 (0.78-3.48)[f,h,k,c] | 2.56 (1.22-5.39)[e,i,k,s] | 8.9 (3.6-22.03)[b,g,k,jj] | 84 (34-209)[a,b,d,f,l] |
| | | NL | 1.79 (0.84-3.80)[p,q,s,u,x] | 10.1 (5.2-19.3)[d,m,o,t,x] | 14.9 (6.0-37.0)[n,o,v,x] | 148 (59-366)[j,o,s,u,y,hh] |
| | | UK | 3.47 (1.64-7.38)[bb,ee,hh] | 6.4 (3.0-13.6)[r,dd,hh] | 9.7 (3.9-24.3)[z,gg,hh] | 76 (31-188)[w,x,aa,cc,ff,ii] |
| | 1 year | FI | 0.25 (0.12-0.52)[b,g,l] | 0.40 (0.19-0.83)[d,g,l,t,dd] | 1.69 (0.68-4.20)[c,e,f,l,jj] | 29.8 (12.0-73.7)[a,c,e,g,k] |
| | | NL | 0.18 (0.09-0.39)[o,dd,gg,ii] | 2.75 (1.43-5.30)[e,m,s,y,bb] | 3.23 (1.30-7.99)[n,u,y,bb] | 42 (17-105)[j,t,v,bb] |
| | | UK | 0.35 (0.17-0.74)[aa,dd,ee,gg,ii] | 1.98 (0.92-4.26)[e,r,bb,cc,ii] | 2.68 (1.04-6.92)[z,bb,ff,ii] | 18.5 (7.4-46.3)[w,bb,dd,gg,hh] |
| Prn GMC (CI) in IU/ml | Day 0 | FI | 1.25 (0.71-2.18)[a,b,c,jj] | 1.67 (0.95-2.92)[a,d,e,jj] | 4.50 (2.27-8.90)[f,g,h,i] | 6.2 (3.1-12.3)[h,i,k,l] |
| | | NL | 1.45 (0.82-2.56)[j,m,n,o,p] | 3.55 (2.17-5.80)[q,r,s,t] | 4.24 (2.14-8.39)[q,u,u,v] | 8.4 (4.2-16.6)[q,x,y] |
| | | UK | 0.85 (0.49-1.48)[w,z,aa,bb] | 0.95 (0.54-1.64)[m,w,z,cc,dd] | 3.71 (1.87-7.39)[r,ee,ff,gg] | 4.55 (2.3-8.99)[r,ee,hh,ii] |
| | Day 28 | FI | 7.0 (4.0-12.3)[f,h,k] | 9.2 (5.3-16.2)[f,i,k] | 55 (28-108)[b,d,g,jj] | 97 (49-192)[a,b,d,l] |
| | | NL | 3.49 (1.98-6.15)[q,s,u,x] | 15.3 (9.3-25.0)[m,o,t,u,x,cc] | 46.7 (23.6-92.3)[n,o,s,v,x] | 230 (116-455)[j,o,s,u,y] |
| | | UK | 4.03 (2.31-7.03)[ee,ff,hh] | 4.78 (2.75-8.31)[r,s,ff,hh] | 37.1 (18.7-73.9)[z,aa,cc,gg] | 103 (52-203)[w,aa,cc,ii] |
| | 1 year | FI | 6.6 (3.8-11.6)[g,h,l] | 7.4 (4.2-13.0)[g,i,l] | 28.9 (14.6-57.2)[c,e,f,jj] | 45.8 (23.2-90.6)[a,c,e,k] |
| | | NL | 3.38 (1.92-5.97)[q,t,v,y] | 9.1 (5.6-15.0)[m,p,s,y] | 17.0 (8.6-33.7)[u,n,p,y] | 89 (45-177)[j,p,t,v,x] |
| | | UK | 3.58 (2.09-6.14)[ee,gg,ii] | 4.67 (2.65-8.22)[r,gg,ii] | 18.5 (9.2-37.6)[z,bb,dd,ff] | 45.8 (23.0-91.0)[w,bb,dd,hh] |
| Fim2/3 GMC (CI) in AU/ml | Day 0 | FI | 0.14 (0.08-0.27)[a,b,c,q,i,ee,jj] | 0.45 (0.24-0.86)[a,e,m,h,r,jj] | 4.8 (2.2-10.3)[h,i] | 11.8 (5.4-25.6)[h,i,l] |
| | | NL | 2.61 (1.37-4.96)[h,j] | 4.41 (2.52-7.70)[i,j] | 8.2 (3.8-17.8) | 25.7 (11.8-55.6)[q,m,w] |
| | | UK | 3.09 (1.69-5.66)[h,aa] | 5.7 (3.1-10.4)[i] | 9.9 (4.6-21.6) | 3.44 (1.59-7.46)[j] |
| | Day 28 | FI | 0.28 (0.15-0.53)[c,f,h,k,o,aa] | 0.66 (0.35-1.25)[e,f,k,s,cc] | 6.1 (2.8-13.2)[b,d] | 18.6 (8.6-40.3)[b,d] |
| | | NL | 3.50 (1.84-6.67)[b,x] | 4.11 (2.35-7.18)[d,x] | 9.2 (4.2-19.8) | 24.5 (11.3-53.1)[o,s,hh] |
| | | UK | 5.8 (3.2-10.8)[b,ee] | 7.9 (4.3-14.5)[d] | 11.5 (5.3-25.1) | 5.0 (2.3-10.8)[x] |
| | 1 year | FI | 0.56 (0.30-1.06)[b,e,g,h,l,p,bb] | 1.96 (1.04-3.71)[c,d,i,l,dd] | 6.3 (2.9-13.6)[c,l] | 24.8 (11.4-53.7)[a,c,e,g,ii] |
| | | NL | 3.14 (1.65-5.98)[c,y] | 3.87 (2.22-6.77)[y] | 8.0 (3.7-17.4) | 23.1 (10.4-50.0)[p,t,ii] |
| | | UK | 4.51 (2.49-8.14)[c] | 6.5 (3.5-12.1)[e] | 10.9 (5.0-24.1) | 5.0 (2.3-10.9)[l,y] |

Ptx: pertussis toxin; FHA: filamentous haemagglutinin; Prn: pertactin; Fim2/3: fimbriae 2 and 3; GMC: geometric mean concentration; CI: confidence interval; IU/ml: international units per millilitre; AU/ml: arbitrary units per millilitre; FI: Finland; NL: Netherlands; UK: United Kingdom. Significance per antigen has been tested between timepoints of an age group, age groups within a country and between countries within an age group, $p \leq 0.05$, *$p \leq 0.01$*, **$p \leq 0.001$**. Significantly different from **a** older adults, FI at day 0; **b** children, FI at day 28; **c** children, FI at 1 year; **d** adolescents, FI at day 28; **e** adolescents, FI at 1 year; **f** young adults, FI at day 28; **g** young adults, FI at 1 year; **h** children, FI at day 0; **i** adolescents, FI at day 0; **j** older adults, NL at day 0; **k** older adults, FI at day 28; **l** older adults, FI at 1 year; **m** adolescents, NL at day 0; **n** young adults, NL at day 0; **o** children, NL at day 28; **p** children, NL at 1 year; **q** children, NL at day 0; **r** adolescents, UK at day 0; **s** adolescents, NL at day 28; **t** adolescents, NL at 1 year; **u** young adults, NL at day 28; **v** young adults, NL at 1 year; **w** older adults, UK at day 0; **x** older adults, NL at day 28; **y** older adults, NL at 1 year; **z** young adults, UK at day 0; **aa** children, UK at day 28; **bb** children, UK at 1 year; **cc** adolescents, UK at day 28; **dd** adolescents, UK at 1 year; **ee** children, UK at day 0; **ff** young adults, UK at day 28; **gg** young adults, UK at 1 year; **hh** older adults, UK at day 28; **ii** older adults, UK at 1 year; **jj** young adults, FI at day 0.

**Supplementary Table 4.** GMCs of pertactin per vaccination background.

| Ig class | Timepoint | aP2 (n=10) | aP3 (n=7) | wP (n=41) |
|---|---|---|---|---|
| IgG GMC (CI) in IU/ml | Day 0 | 0.9 (0.4-2.2)[a,b,c,d] | 24.7 (9.0-67.7)[e,f,g] | 14.9 (9.8-22.6)[e,h,i] |
| | Day 28 | 62 (26-143)[d,e,f,h] | 652 (238-1788)[a,c] | 451 (297-683)[b,c,i] |
| | 1 Year | 11 (5-26)[c,e,g,i] | 263 (96-720)[a,d] | 175 (115-265)[b,d,h] |
| IgA GMC (CI) in IU/ml | Day 0 | 0.6 (0.2-1.9)[b,c,d] | 4.0 (1.0-16.3)[f,g] | 3.2 (1.8-5.8)[e,h,i] |
| | Day 28 | 5 (2-16)[e] | 29 (7-119)[a] | 17 (9-30)[b,i] |
| | 1 Year | 10 (3-33)[e] | 18 (4-74)[a] | 9 (5-16)[b,h] |

aP2: participants prior to study exclusively vaccinated with 2 acellular pertussis components containing vaccines; aP3: participants prior to study exclusively vaccinated with 3 acellular pertussis components containing vaccines; wP: participants prior to study at least 3 times vaccinated with a whole cell pertussis containing vaccine; GMC: geometric mean concentration; CI: confidence interval; IU/ml: international units per millilitre. Significance per Ig class has been tested between timepoints within a vaccine group and between vaccine groups within a timepoint, $p \leq 0.05$, *$p \leq 0.01$*, **$p \leq 0.001$**. Significantly different from **a** aP3 at day 0; **b** wP at day 0; **c** aP2 at day 28; **d** aP2 at 1 year; **e** aP2 at day 0, **f** aP3 at day 28; **g** aP3 at 1 year; **h** wP at day 28, **i** wP at 1 year.

**Supplementary Table 5.** GMCs of Fim2/3 per vaccination background.

| Ig class | Timepoint | aP3 (n=7) | aP5 (n=65) | wP (n=41) |
|---|---|---|---|---|
| IgG GMC (CI) in AU/ml | Day 0 | 0.2 (0.1-0.8)[a,b] | 7.4 (5.1-10.8)[b,c,d,e] | 3.5 (2.2-5.7)[a,c,f] |
| | Day 28 | 0.4 (0.1-1.2)[d,f] | 10.1 (6.9-14.7)[a,e,f,g] | 4.6 (2.8-7.4)[b,g,d] |
| | 1 Year | 0.3 (0.1-1.1)[e,h] | 6.0 (4.1-8.7)[a,d,i] | 3.9 (2.4-6.3)[i] |
| IgA GMC (CI) in AU/ml | Day 0 | 0.6 (0.1-2.5) | 3.6 (2.2-5.9) | 2.7 (1.4-4.9) |
| | Day 28 | 1.0 (0.2-4.4) | 4.7 (2.9-7.6) | 2.7 (1.5-5.1) |
| | 1 Year | 0.9 (0.2-3.8) | 4.2 (2.6-6.8) | 3.9 (2.1-7.1) |

aP3: participants prior to study exclusively vaccinated with 3 acellular pertussis components containing vaccines; aP5: participants prior to study at least 3 timed vaccinated with 5 acellular pertussis components containing vaccines; wP: participants prior to study at least 3 timed vaccinated with a whole cell pertussis containing vaccine; GMC: geometric mean concentration; CI: confidence interval; IU/ml: international units per millilitre. IgG and IgA GMCs and confidence intervals (CI). Significance per Ig class has been tested between timepoints within a vaccine group and between vaccine groups within a timepoint, $p \leq 0.05$, *$p \leq 0.01$*, **$p \leq 0.001$**. Significantly different from **a** aP5 at day 0; **b** wP at day 0; **c** aP3 at day 0; **d** aP5 at day 28; **e** aP5 at 1 year; **f** wP at day 28, **g** aP3 at day 28; **h** wP at 1 year, **i** aP3 at 1 year.

**Supplementary Panel.** List of in- and exclusion criteria

Inclusion criteria

In order to be eligible to participate in this study, participants must meet all of the following criteria:

- normal general health;
- within the right age group for the cohort;
- received all regular vaccines for their age group according to the Dutch NIP in the Netherlands, UK NIP in the United Kingdom, or Finnish NIP in Finland; a copy of the vaccination booklet will be included in the participant's documents. If booklet is not available for cohorts A, B and C, vaccination status will be checked with regulatory agencies / GP. For cohort C and D this booklet might not be available due to their age;
- provision of written informed consent from the adult participants and parents or legal guardians of minors;
- willing to adhere to the protocol and be available during the study period.



Exclusion criteria

- present evidence of serious disease(s) within the last 3 months before inclusion requiring immunosuppressive or immune modulating medical treatment, such as systemic corticosteroids, that might interfere with the results of the study;
- chronic infection;
- known or suspected immune deficiency;
- history of any neurologic disorder, including epilepsy;
- previous administration of serum products (including immunoglobulins) within 6 months before vaccination and blood sampling;
- known or suspected allergy to any of the vaccine components (by medical history);
- occurrence of serious adverse events (SAEs) after primary DTwP-IPV vaccination, DTaP-IPV vaccination or any other vaccination (by medical history);
- vaccination with any pertussis containing vaccine other than those described in the inclusion criteria (i.e. only according to NIP);
- adult pertussis vaccination according to the NIP in the last 5 years (i.e. maternal vaccination);
- children in cohort B in Finland who already received the Tdap booster at 14-15 years of age;
- vaccination with any other diphtheria, tetanus or polio containing vaccine in the last 5 years, other than described in the NIP;
- children between 8 and 10 years of age eligible for cohort A in the Netherlands who have already received the dT-IPV booster vaccination according to the Dutch NIP around 9 years of age;
- children in cohort B in the United Kingdom who already received the dT-IPV booster vaccination according to the UK NIP around 14 years of age;
- mixed wP and aP priming within a participant;
- pregnancy.

# Chapter 4

# Pertussis toxin neutralizing antibody response after an acellular booster vaccination in Dutch and Finnish participants of different age groups

A. Knuutila, P. Versteegen, A. M. Barkoff, P. G. M. van Gageldonk,
J. Mertsola, G. A. M. Berbers, and Q. He.

Emerging Microbes & Infections 11 (Mar 30 2022): 956-63.

## ABSTRACT

Pertussis incidence has increased in many countries and the disease occurs among all age groups, suggesting the need for booster immunizations through life. In addition to determining the concentration of anti-pertussis toxin (PT) antibodies, the ability of PT neutralizing antibodies (PTNAs) could be used to assess vaccine responses.

Altogether 258 participants [7–10-year-old (N = 73), 11–15-year-old (N = 85), 20–35-year-old (N = 50) and 60–70-yearold (N = 50)] were included. Sera were collected before, one month, and one year after a single dose of a three pertussis component containing acellular pertussis vaccine. The adolescents were primed in childhood either by acellular or whole-cell vaccination. PTNA titres were determined by a Chinese hamster ovary cell assay and anti-PT IgG/IgA antibody concentrations by multiplex immunoassay.

In all age groups, a significant increase in levels of PTNAs and anti-PT IgG was observed one month after vaccination and remained at least two-fold higher one year post-booster, in comparison to pre-booster. Young adults had the lowest response. The strongest increase in PTNAs was observed in participants who had ≥10 IU/mL concentration of anti-PT IgG antibodies pre-booster. At pre-booster, whole-cell-primed adolescents had higher PTNAs than acellular-primed peers ($p = 0.047$). One year post-booster, the Finnish whole-cell-primed adolescents had a higher level of PTNAs than acellular primed adolescents ($p = 0.049$), however, this was not observed in Dutch adolescents. In conclusion, PTNAs increased after vaccination in all age groups, and the strongest increase was related to the presence of high pre-booster antibodies.

## INTRODUCTION

Pertussis is an acute respiratory infection in humans, caused by the Gram-negative bacterium *Bordetella pertussis*. Since the introduction of whole-cell pertussis vaccines (wPv) in the 1940s, widespread vaccination of young children has reduced the incidence of pertussis. However, due to the high reactogenicity of wPvs, acellular pertussis vaccines (aPv) were developed in the early 1980s. Current aPvs contain a combination of purified proteins from *B. pertussis*: (detoxified) pertussis toxin (PT), filamentous hemagglutinin, pertactin and fimbriae 2/3. In 2005, aPvs were nationally implemented in the Netherlands and Finland. Afterward, the aPvs' composition, and their priming and boosting schedules have often changed in both countries (**Supplementary Figure 1**). However, despite high vaccination coverage (>95%), a rise in notifications of pertussis in the Netherlands and many other European countries has been seen, with regular epidemic peaks observed every two to four years [1-3]. In particular, the pertussis incidence in the last decennium has increased in adolescents and adults. In contrast, in Finland, there have been considerably fewer notifications in the last 15 years since the implementation of aPvs [4] (**Figure 1**). One explanation for this might be the use of aPv boosters in adolescents and young adults in Finland.



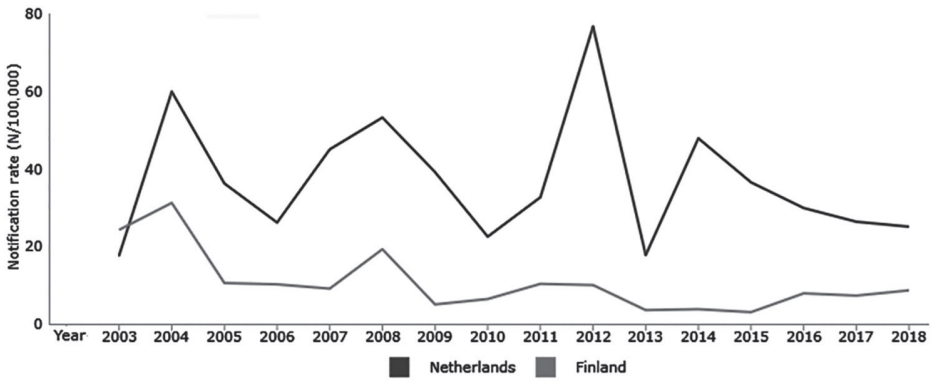



**Figure 1.** Laboratory confirmed pertussis notification rates in Finland and the Netherlands [5]. The study participants for this study were recruited from late 2017 to early 2019.

Waning immunity to wPvs and especially to aPvs following primary immunization is thought to contribute to the sustained transmission of *B. pertussis* in the population. Protection against the disease was shown to wane soon after a fifth booster vaccination at 4–6 years of age, however, children primed with wPvs seemed to be better protected than aPv primed children [2, 6-8]. Booster studies in aPv/wPv-primed school children in the Netherlands corroborate this finding [9], resulting in higher antibody levels in wPv-primed

children one year after booster vaccination. Although antibody levels are usually higher after aPv than after wPv priming [10], their concentration decreases relatively fast during the first years after vaccination [10-12]. *B. pertussis* antigen-specific antibodies could be related to protection against pertussis [13-15], but the presence of antibodies alone does not always confer protection against infection [11, 12, 16]. In addition, in the baboon model, aPv protected against the disease, but could not prevent bacterial transmission. In contrast, vaccination with wPv or previous infection with *B. pertussis* conferred protection to infection in baboons [17].

Many aPv efficacy studies are mainly based on detecting anti-PT IgG antibody concentrations. The neutralization activity of antibodies has been studied as the quantity of neutralizing antibodies to PT (PTNA), which induces the inhibition of clustering of the Chinese hamster ovary (CHO) cells [18]. Essentially, aPvs have demonstrated high post-vaccination PTNAs [19, 20], whereas wPvs induce considerably lower levels of PTNAs [21, 22]. This could be attributed to the overall magnitude of the induced concentration of anti-PT IgG antibodies and to the amount of PT in the vaccines [19, 23, 24]. Generally, a fourfold increase at one month post-vaccination or post-infection was considered as a significant increase [25], and despite a rapid decrease post-vaccination, the induced PTNAs were still detectable after two to five years [20, 26].

The majority of CHO-cell-based studies have shown a clear correlation between the concentration of anti-PT IgG antibodies and PTNA titres [24], concluding that basic ELISA measurements generally demonstrate the neutralization capacity of antibodies [23, 27, 28]. Thereafter, as CHO cell-based assays are laborious, prone to subjective result analysis and less sensitive in comparison to ELISAs [29], the majority of studies in the last few decades have left CHO-cell assays out of laboratory practice. However, the role of PTNAs may remain essential in more thorough analysis models in large clinical studies with multiple serological and humoral immunological parameters aiming for the search of potential biomarkers of immune responses to *B. pertussis* and its waning immunity. This study aimed to characterize the PTNA response to an aPv booster in children, adolescents, young adults and older adults, with different priming vaccination backgrounds in two countries with different epidemiological backgrounds for pertussis incidence.

## MATERIALS AND METHODS

### Study approval

This “Booster against pertussis” (BERT) study trial was registered at the EU Clinical Trial database (EudraCT number 2016-003678-42) and was approved by the Medical Research Ethics Committees United (MEC-U, NL60807.100.17-R17.039) in the Netherlands and by the MREC UTU (ETMK Dnro: 129/1800/2017) in Finland [30]. Written informed consent

was obtained from all adult and adolescent participants and parents or legal guardians of children at the start of the study.

### Study design

Participants from the BERT study cohort [30] (N = 258) **(Table 1**) were included in 2017–2019 in Finland and the Netherlands, and received a booster dose of a Tdap3-IPV vaccine (Boostrix™-IPV - GlaxoSmithKline (GSK), Wavre, Belgium). Samples for this study were analyzed from pre-booster, one month after and one year after booster vaccination. All sera in this study were stored at −20 °C, and their anti-PT IgG and IgA antibodies were measured previously with a fluorescent-bead-based multiplex immunoassay at the National Institute for Public Health and the Environment, The Netherlands [30, 31].

**Table 1.** Study cohorts.

| | Country | Age (Mean yr) | No. of study participants | No. of Female/ Male | Primary vaccination* | Booster vaccinations*, age |
|---|---|---|---|---|---|---|
| Children | FI | 9.0 | 37 | 18/19 | aPv | 4 years, aPv |
| | NL | 8.5 | 36 | 18/18 | | |
| Adolescents (aP) | FI | 12.5 | 19 | 7/12 | aPv | 4 years, aPv |
| | NL | 12.4 | 25 | 17/8 | | |
| Adolescents (wP) | FI | 15.0 | 18 | 11/7 | wPv | 2 and 6 years, aPv** |
| | NL | 14.8 | 23 | 14/9 | | 4 years, aPv |
| Young adults | FI | 30.2 | 25 | 21/4 | wPv | n/A |
| | NL | 28.6 | 25 | 10/15 | | |
| Older adults | FI | 64.2 | 25 | 21/4 | wPv | n/A |
| | NL | 65.9 | 25 | 14/11 | | |

* Detailed vaccine compositions and schedules in **Supplementary Table 1**.

** One participant was boosted by wPv at 2 years of age.

### CHO cell assay

Titres of PTNAs were determined by a CHO cell assay at the University of Turku, Finland, and at the Capital Medical University, Beijing, China, as previously described [32, 33]. In brief: 5,000 or 10,000 CHO cells were mixed with 0.84 ng/mL of native PT (GlaxoSmithKline, Rixensart, Belgium), and with a two-step dilution series of serum (1:8–1:4096). The wells were evaluated visually after 24 h either by microscopy or by Incucyte or by IncucyteZoom (Essen Bioscience, Michigan, USA) instruments. The neutralizing titre was reported as the serum dilution in the last well without clusters. Each 96-well plate included three controls: 1) only PT and cells; 2) only testing sera and cells and 3) only cells.

### Statistics

The PTNA results were analyzed as neutralization titres and as the proportion of neutralization divided by anti-PT IgG concentration (IU/mL) [34]. Samples with titres below the assay cut-off "1:8" were arbitrarily attributed to "1:4" for analyses. For this study, a proportion value of 2.0 for a high proportion. Cases of >2.0 PTNAs per anti-PT IgG may be slightly overrepresented in pre-booster samples due to very low anti-PT IgG concentrations (less than 1 IU/mL) extrapolating these values. Data were analyzed using IBM SPSS statistics 25.0 software for Windows (IBM Corp., Armonk, NY, USA). The differences in distributions between the groups were tested with Mann–Whitney U-tests with Bonferroni corrections, and two-sided p-values less than 0.05 were considered statistically significant. The correlations of PTNA responses to the overall anti-PT IgG and IgA concentrations were calculated with the Pearson correlation coefficient. Wilcoxon signed rank test was used for the comparison of median titres between the pre- and post-booster time points.

## RESULTS

PTNA titres increased in all age groups during one month and remained higher than baseline after one year (Wilcoxon $p < 0.05$) (**Table 2**). Participants with a higher anti-PT IgG concentration pre-booster, regardless of study group or country, had a significantly higher anti-PT IgG and PTNA response after one month and after one year of vaccination ($p < 0.05$) (**Table 3**). The lowest pre-booster anti-PT IgG concentration to demonstrate this effect was 10 IU/ mL, which was determined comparing adjacent ordinal variable values over a range of two-fold IU/mL intervals, starting from 5 IU /ml. This effect was not observed with PTNA per IgG proportions. In 120 out of 123 (97.6%) Finnish and 119 out of 134 (88.8%) Dutch participants at least two-fold higher PTNAs were observed one month post-booster in comparison to pre-booster. The PTNA titres remained at least two-fold higher in 85.4% of Finnish and 50.0% of Dutch participants one year post-booster. In comparison, sole anti-PT IgG concentrations increased two-fold at one month post-booster in 95.9% of Finnish and 96.3% Dutch participants. One year post-booster 72.4% of Finnish and 67.2% of Dutch participants had at least two-fold higher anti-PT IgG concentrations compared to pre-booster, respectively. Of note, the participants without a two-fold increase in PTNAs or anti-PT IgGs were present in all age groups, and the majority of these participants had very high (>50 IU) anti-PT IgG or high PTNA titres (≥32) pre-booster.

**Table 2.** Geometric mean values of anti-PT IgG concentrations (IU/mL), PTNA titers, and PTNA per anti-PT IgG ratios before the booster and one month (1M) and one year (1Y) post-booster.

| | | PTNAs | | | anti-PT IgG | | | PTNAs per anti-PT IgG | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Cohort** | **Country** | **Pre** | **1M** | **1Y** | **Pre** | **1M** | **1Y** | **Pre** | **1M** | **1Y** |
| All | FI | 14* | 139* | 35 | 11 | 159 | 42 | 1.28* | 0.85* | 0.95 |
| | NL | 22* | 171* | 37 | 10 | 139 | 35 | 2.12* | 1.24* | 1.19 |
| Children | FI | 18 | 198 | 47* | 16 | 197 | 39 | 1.13 | 1.00* | 1.22* |
| | NL | 20 | 210 | 29* | 12 | 147 | 30 | 1.72 | 1.43* | 0.96* |
| Adolescents (aP) | FI | 12* | 154 | 36 | 10 | 187 | 43 | 1.11 | 0.82 | 0.82 |
| | NL | 28* | 203 | 53 | 17 | 140 | 41 | 1.61 | 1.47 | 1.29 |
| Adolescents (wP) | FI | 19* | 181* | 69 | 16 | 219 | 68 | 1.16* | 0.83* | 1.02 |
| | NL | 43* | 272* | 57 | 11 | 161 | 48 | 3.81* | 1.69* | 1.19 |
| Young adults | FI | 8 | 68 | 20 | 5 | 99 | 27 | 1.49* | 0.68 | 0.78 |
| | NL | 11 | 68 | 29 | 3 | 99 | 22 | 3.39* | 0.68 | 1.33 |
| Older adults | FI | 16 | 118 | 43 | 10 | 132 | 51 | 1.58 | 0.89 | 0.86 |
| | NL | 21 | 179 | 56 | 15 | 156 | 43 | 1.36 | 1.15 | 1.31 |

*Significant difference between the countries, Mann-Whitney U-test, p<0.05.



**Table 3.** Comparison of anti-PT IgG concentrations (IU/mL) and PTNA titers before and after booster vaccination in study participants with lower or higher pre-booster anti-PT IgG concentrations. Statistical significant differences (Mann-Whitney U-test, p<0.05) between the pre-booster anti-PT IgG classifications were observed at all time points and both variables in all age groups.

| | **Country** | **Pre-booster anti-PT IgG** | **Number of participants** | **anti-PT IgG pre** | **1M** | **1Y** | **PTNA pre** | **1M** | **1Y** |
|---|---|---|---|---|---|---|---|---|---|
| Children | FI | ≥10 IU/mL | 27 | 27 | 231 | 49 | 25 | 208 | 49 |
| | | <10 IU/mL | 10 | 4 | 128 | 20 | 7 | 169 | 30 |
| | NL | ≥10 IU/mL | 18 | 32 | 211 | 56 | 42 | 287 | 44 |
| | | <10 IU/mL | 18 | 4 | 109 | 19 | 16 | 105 | 7 |
| Adolescents | FI | ≥10 IU/mL | 23 | 27 | 230 | 91 | 24 | 201 | 74 |
| | | <10 IU/mL | 14 | 4 | 163 | 23 | 7 | 122 | 25 |
| | NL | ≥10 IU/mL | 32 | 31 | 181 | 59 | 63 | 299 | 67 |
| | | <10 IU/mL | 16 | 3 | 100 | 20 | 9 | 163 | 22 |
| Young adults | FI | ≥10 IU/mL | 7 | 33 | 185 | 67 | 43 | 141 | 64 |
| | | <10 IU/mL | 18 | 3 | 78 | 19 | 4 | 51 | 13 |
| | NL | ≥10 IU/mL | 4 | 38 | 487 | 105 | 91 | 362 | 91 |
| | | <10 IU/mL | 21 | 2 | 73 | 17 | 7 | 49 | 16 |
| Older adults | FI | ≥10 IU/mL | 13 | 33 | 185 | 67 | 43 | 141 | 64 |
| | | <10 IU/mL | 12 | 3 | 78 | 19 | 4 | 51 | 13 |
| | NL | ≥10 IU/mL | 18 | 26 | 227 | 60 | 27 | 228 | 27* |
| | | <10 IU/mL | 7 | 4 | 59 | 17 | 11 | 95 | 29* |

*The only pair without statistically significant difference (p<0.05).

In terms of PTNA per anti-PT IgG ratio, the ratios decreased significantly in both countries from prebooster to one month and one year post-booster (Wilcoxon $p < 0.05$), and the ratios remained at the same level from one month to one year after vaccination (Wilcoxon $p = 0.84$) (**Figure 2**, **Table 2**). Both PTNAs, as well as PTNA per anti-PT IgG proportions pre- and one month post-booster, were significantly higher in the Netherlands compared to Finland ($p < 0.006$). The number of individuals possessing overall low PTNA per anti-PT IgG proportions at post-booster increased from pre-booster (**Table 4**) in both countries, however, the magnitude of change was higher in the Dutch participants. On the contrary, the number of individuals possessing overall high PTNA per anti-PT IgG proportions at post-booster decreased from pre-booster. After one month postbooster, 29.3% of Finnish and 32.1% of Dutch participants had an improved PTNA per anti-PT IgG ratio in comparison to pre-booster. The ratio remained higher after one year in 33.9% of Finnish and 26.1% of Dutch participants. Particularly, Dutch children and adolescents had declining PTNA per anti-PT IgG titres after vaccination, whereas Finnish children and adolescents had an increasing or constant level of neutralization between one month and one year post-booster (**Table 2**).

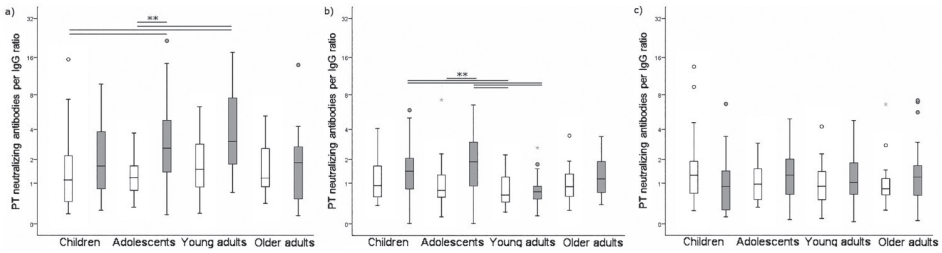



**Figure 2.** PTNA per anti-PT IgG ratio distribution at a) pre-booster, b) one month c) one year after vaccination. Light boxplots = Finland, Grey = Netherlands. The box plots demonstrate the median, quartile range, and 1.5 times the quartile range of PTNA per anti-PT IgG ratios. Mann-Whitney *U*-test $^{**}p < 0.01$.

**Table 4.** Comparison of PTNA per anti-PT IgG proportions in participants from Finland and the Netherlands.

| | | Number of participants (%) | | |
|---|---|---|---|---|
| **PTNA per anti-PT IgG** | **Country** | **Pre** | **One month** | **One year** |
| <0.5 | FI | 19 (15.4%) | 23 (18.5%) | 24 (19.7%) |
| | NL | 8 (6.0%) | 12 (9.1%) | 28 (21.1%) |
| 0.5–2.0 | FI | 69 (56.1%) | 84 (67.7%) | 82 (67.2%) |
| | NL | 53 (39.8%) | 80 (60.6%) | 79 (59.4%) |
| >2.0 | FI | 35 (28.5%) | 17 (13.7%) | 16 (13.1%) |
| | NL | 72 (53.7%) | 40 (30.3%) | 26 (19.5%) |

The PTNA titres were found to correlate significantly with overall antibody concentrations of anti-PT IgG antibodies (1:1 ratio, Pearson R = 0.829) (**Figure 3**). However, there was no correlation between age and PTNA titres. In the older adults, a high correlation between anti-PT IgA antibodies and PTNAs at one month was noted (R = 0.755), as well as at one year post-booster (R = 0.576), implying a possible role of anti-PT IgA in neutralization. Correlations in the other age cohorts were all below 0.5 at all time points. Consequently, it could be interpreted that, particularly for older adults, the calculated PTNA per anti-PT Ig ratios would slightly decrease with the implementation of IgA to overall IgG concentrations (**Table 2**).

The adolescents with wPv background had significantly higher pre-booster PTNA per anti-PT IgG ratios in comparison to aPv background adolescents (p = 0.047) when data from both countries were combined. One year after the booster, Finnish wPv-primed adolescents had significantly higher PTNAs than aPv primed adolescents (p = 0.049), however, this was not observed in Dutch adolescents. Of note, the Dutch adolescents had significantly higher PTNAs pre-booster and one month after vaccination in comparison to Finnish adolescents (p < 0.01) (**Table 2**). Consequently, when data were analyzed individually per country, no consistent differences were noticed between the vaccination backgrounds at any time points with either PTNAs or PTNA per anti-PT IgG proportions.



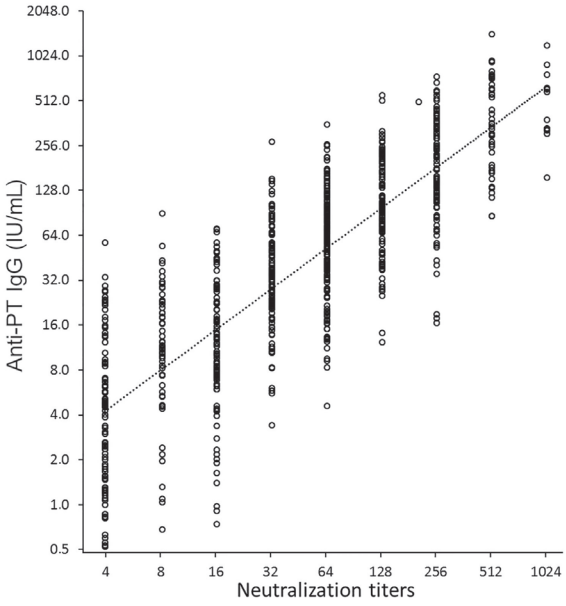



**Figure 3.** The correlation between overall anti-PT IgG concentration and PT neutralizing antibody titres was high (Pearson R = 0.829). All study samples at all three time points are presented (N = 769).

## DISCUSSION

Vaccination-induced antibodies play an important role in the protection against infection with *B. pertussis*. Antibodies can provide protection in many distinct ways. In this study, the functionality of vaccination-induced antibodies was assessed through the ability to neutralize PT in different age groups. All study groups in the Netherlands had higher PTNA titres and PTNA per anti-PT IgG ratios in pre-booster samples in comparison to Finland. Based on the reported incidence (**Figure 1**) and recent serosurveillance data [2, 3, 35, 36], pertussis is indeed more prevalent in the Netherlands than in Finland. Additionally, the pertussis primary vaccination coverage in 2017 was 94% in the Netherlands and 99% in Finland, and the children receive different amounts of vaccinations (**Supplementary Figure 1**). However, the epidemiological differences of pertussis between both countries were not reflected in the level of anti-PT IgG concentrations in pre-booster samples. Although in general, not all pertussis patients or infected individuals develop high PTNAs or anti-PT IgG concentrations [25, 33], high pre-booster PTNAs, particularly noticed in the Dutch adolescents, may be an indication of natural infection.

Long-lasting memory from previous exposure to *B. pertussis* or vaccination may present a challenge for the evaluation of vaccine responses. Although PTNAs increased significantly in all age groups after vaccination, the induced PTNA titres were significantly higher in participants with a pre-booster concentration of at least 10 IU/mL anti-PT IgG antibodies. A similar phenomenon was reported earlier with an aPv booster in Finnish adolescents [37]. Interestingly, if only subjects who had less than 10 anti-PT IgG IU/ml pre-booster (**Table 3**) were compared, the anti-PT IgG responses were close to equal in all age groups in both countries one year post vaccination. After one month, the children and adolescents had a more vigorous response in comparison to the adults. Conversely, if subjects had more than 10 anti-PT IgG IU/ml pre-booster, the differences between anti-PT IgGs could be over two fold after one year. Thereafter it could be deduced that the individuals with high pre-booster anti-PT IgG greatly impact upon the variety and magnitude in the observed vaccination responses between the age cohorts. Only 11/50 participants in the young adult group had more than 10 IU/mL anti-PT IgG, suggesting less frequent exposure or completely waned immunological memory to PT (**Supplementary Figure 1**). This may shed some insight into why the young adult cohort responded poorly to the vaccine in comparison to the other age groups in this study [30]. A similar effect of pre-booster antibodies was observed with the same study participants when anti-PT memory and plasma B-cells were determined by ELISPOT assays (Versteegen, Barkoff, Pinto, unpublished work). Also, post-booster mucosal antibody response was found significantly higher in participants with evidence of prior infection (van Schuppen, unpublished work) in the Dutch aP primed adolescents.

This phenomenon was not observed in the proportions of PTNAs per anti-PT IgG one month or one year post-booster. In addition, the model of PTNA per anti-PT IgG implied that the proportion of vaccine-induced PTNAs remains the same from one month to one year after vaccination, which indicates that merely the quantity of PTNAs changes over time. Based on a recent study by Zhang et al. [33], the ratio of PTNAs to anti-PT IgG was slightly above a ratio of 1.0 in young pertussis patients with recent infection, and who had received at least three vaccine doses. Although the number of participants with a relatively low proportion of PTNAs per total anti-PT IgG was increased after boosting, the post-booster proportions (**Table 2**) were equally high as post-infection [33]. In this light, acellular booster vaccination produced sufficiently high proportions of PTNAs in all age groups. The high proportion of PTNAs per anti-PT IgG in pre-booster antibodies could be possibly related to the importance of maintaining these antibodies in blood circulation. It is uncertain whether a low PTNA per anti-PT IgG proportion is reflected on the quality or function of antibodies through epitope-specificity and affinity of the induced antibodies [24], or whether sera with low PTNAs per anti-PT IgG contain in return a lot of other antibody classes or antibodies which excel in other protective functions, such as opsonization. Thus far, anti-PT IgA PTNAs have mostly been speculated to play a minor role in neutralization overall in comparison to anti-PT IgG since aPvs induce hardly any anti-PT IgA response in comparison to anti-PT IgG [6, 25, 27, 30, 38]. In this study, a high correlation between anti-PT IgA to PTNA titres was noted in older adults, suggesting that IgA antibodies possess the function to neutralize PT.

Although differences were observed between different priming vaccination backgrounds in the adolescent cohort pre-booster, it was not possible to further demonstrate the effect of age or priming background on booster response consistently in both study countries. Of note, the wPv group in Finland had received five doses in childhood, whereas the aPv group had received only four (**Supplementary Figure 1**). However, in previous studies, no differences were noticed in PTNAs between children who received four or three doses of vaccines in childhood, either at one or at five years of age [26, 39]. Also, in the Dutch cohort, the pre-school aPv booster in the wPv group was administered two years earlier compared to their aPv background peers, whereas in Finland the time difference from the latest vaccination was close to six months. These factors may influence the outcome of aPv/wPv comparisons. Although the research setting for the evaluation of vaccination history was simple, the vaccination background in the adolescent age group appeared to be quite divergent, thereby making the results hard to interpret. Further, standardization and comparison of the PTNA results with earlier studies are challenging, since standards are rarely used in CHO-cell assays, and they have been found to vary up to four-fold between laboratories [40]. Despite the significant correlation between antibody concentrations and CHO-cell assays, strongly diverging results between anti-PT IgG concentration and PTNAs occur. Thereafter, a result obtained by one method could not be used to predict a

concentration for the other method with accuracy in an individual serum sample [23, 27, 28, 33]. This is often remarked as an inconvenience for assay comparisons, but on the other hand, may as well highlight an alternative landmark for evaluating a successful vaccination response, which may have a significant impact at an individual level.

It can be concluded that the epidemiological differences between the two countries and inter-individual differences in pre-existing memory to pertussis have a significant influence on anti-PT IgG and PTNAs after vaccination. Our finding would suggest that a pre-booster anti-PT IgG concentration as low as 10 IU/mL works as an excellent predictor of successful vaccine response in terms of a high quantity as well as a good quality of anti-PT IgG response. Conversely, it could be considered that individuals with less than 10 IU/ mL PT antibodies would have required either more frequent boosting, or that a single booster dose for those individuals was not enough to reactivate immunological memory. Pre-existing antibodies from an earlier infection or previous vaccination may very well be influenced by priming background and thereafter to the likelihood of being infected by *B. pertussis*. This was reflected particularly in the young adult study cohort of both countries, in which the participants had very low pre-booster anti-PT antibodies and in return, responded, at least in terms of quantity of PTNAs, weaker towards the vaccination. However, the relative proportion of PTNAs among overall post-booster antibodies between the different study groups did not differ significantly. On the other hand, based on PTNA titres, adolescents in the Netherlands were likely to be more frequently exposed to *B. pertussis*. In addition to PTNAs, other functional antibodies to PT e.g. antibodies responsible for bacteria killing and opsonophagocytosis as well as antibody avidity and B-cell memory should be considered. Altogether, our results stress the importance to determine PT neutralizing antibodies for the assessment of functional antibodies after aPv vaccinations. Additionally, there is a clear indication for future studies to present and analyze data more explicitly in regards subjects' pre-antibody levels in order to visualize the effect of subject background on reported vaccination responses.

## ACKNOWLEDGMENTS

Elisa Knuutila, Institute of Biomedicine, University of Turku, Turku, Finland, and Yuxiao Zhang, Department of Medical Microbiology, Capital Medical University, Beijing, China, are acknowledged for their indispensable technical assistance in performing the CHO cell assays. Tuula Rantasalo, Elisa Knuutila, Johanna Teräsjärvi, Elina Tenhu, Kaisu Kaistinen, and Raakel Luoto, Institute of Biomedicine, University of Turku, Turku, Finland, are acknowledged for their contribution in the sample collection and work at the clinic during the BERT study. Members involved with the overall BERT study are acknowledged as earlier described [30].

The purified PT antigen was kindly provided by GlaxoSmithKline, Belgium.

## FUNDING

This work was supported by the PERISCOPE (pertussis correlates of protection Europe) project. The PERISCOPE project has received funding from the Innovative Medicines Initiative 2 Joint Undertaking under grant agreement number 115910. This Joint Undertaking receives support from the European Union's Horizon 2020 research and innovation programme; the European Federation of Pharmaceutical Industries and Associations; and Bill & Melinda Gates Foundation.

## DATA SHARING STATEMENT

Individual participant data that underlie the results reported in this article, have been de-identified and deposited in the central database of the PERISCOPE Consortium and can be accessed by a request to the PERISCOPE management team.

## DISCLOSURE STATEMENT

No potential conflict of interest was reported by the author(s).

## REFERENCES


1. de Melker, H.E., et al., Pertussis in The Netherlands: an outbreak despite high levels of immunization with whole-cell vaccine. Emerg Infect Dis, 1997. 3(2): p. 175-8.
2. de Greeff, S.C., et al., Seroprevalence of pertussis in The Netherlands: evidence for increased circulation of Bordetella pertussis. PLoS One, 2010. 5(12): p. e14183, DOI: 10.1371/journal.pone.0014183.
3. Versteegen, P., et al., More than 10 years after introduction of an acellular pertussis vaccine in infancy: a cross-sectional serosurvey of pertussis in the Netherlands. The Lancet Regional Health – Europe, 2021. 10: p. 100196.
4. Elomaa, A., et al., Pertussis before and after the introduction of acellular pertussis vaccines in Finland. Vaccine, 2009. 27(40): p. 5443-9.
5. ECDC. Disease data from ECDC Surveillance Atlas for pertussis. 2019 [cited 2019 20 September]; Available from: http://ecdc.europa.eu/en/pertussis/surveillance-and-disease-data/atlas].
6. Le, T., et al., Immune responses and antibody decay after immunization of adolescents and adults with an acellular pertussis vaccine: the APERT Study. J Infect Dis, 2004. 190(3): p. 535-44.
7. Klein, N.P., et al., Waning Protection after Fifth Dose of Acellular Pertussis Vaccine in Children. http://dx.doi.org/10.1056/NEJMoa1200850, 2012.
8. Sheridan, S.L., et al., Number and order of whole cell pertussis vaccines in infancy and disease protection. Jama, 2012. 308(5): p. 454-456. doi: 10.1001/jama.2012.6364.
9. Hendrikx, L.H., et al., Enhanced memory B-cell immune responses after a second acellular pertussis booster vaccination in children 9 years of age. Vaccine, 2011. 30(1): p. 51-8.
10. Hendrikx, L.H., et al., Different IgG-subclass distributions after whole-cell and acellular pertussis infant primary vaccinations in healthy and pertussis infected children. Vaccine, 2011. 29(40): p. 6874-80.
11. Hallander, H.O., et al., Pertussis antitoxin decay after vaccination with DTPa. Response to a first booster dose 3 1/2-6 1/2 years after the third vaccine dose. Vaccine, 2005. 23(46-47): p. 5359-64.
12. Riffelmann, M., et al., Antibody decay after immunisation of health-care workers with an acellular pertussis vaccine. Eur J Clin Microbiol Infect Dis, 2009. 28(3): p. 275-9.
13. Cherry, J.D., et al., A search for serologic correlates of immunity to Bordetella pertussis cough illnesses. Vaccine, 1998. 16(20): p. 1901-6.
14. Storsaeter, J., et al., Levels of anti-pertussis antibodies related to protection after household exposure to Bordetella pertussis. Vaccine, 1998. 16(20): p. 1907-16.
15. Hewlett, E.L. and S.A. Halperin, Serological correlates of immunity to Bordetella pertussis, in Vaccine. 1998: Netherlands. p. 1899-900.
16. Mills, K.H., Immunity to Bordetella pertussis. Microbes Infect, 2001. 3(8): p. 655-77.
17. Warfel, J.M., L.I. Zimmerman, and T.J. Merkel, Acellular pertussis vaccines protect against disease but fail to prevent infection and transmission in a nonhuman primate model. Proc Natl Acad Sci U S A, 2014. 111(2): p. 787-792, DOI: 10.1073/pnas.1314688110.
18. Hewlett, E.L., et al., Induction of a novel morphological response in Chinese hamster ovary cells by pertussis toxin. Infect Immun, 1983. 40(3): p. 1198-203.

**19.** Vaccines, A.H.G.f.t.S.o.P., Placebo-controlled trial of two acellular pertussis vaccines in Sweden--protective efficacy and adverse events. Ad Hoc Group for the Study of Pertussis Vaccines. Lancet, 1988. 1(8592): p. 955-60.

**20.** Blennow, M. and M. Granström, Long term serologic follow-up after pertussis immunization. Pediatr Infect Dis J, 1990. 9(1): p. 21-6.

**21.** Pichichero, M.E., et al., Safety and immunogenicity of an acellular pertussis vaccine booster in 15- to 20-month-old children previously immunized with acellular or whole-cell pertussis vaccine as infants. Pediatrics, 1993. 91(4): p. 756-60.

**22.** Edwards, K.M., et al., Comparison of 13 acellular pertussis vaccines: overview and serologic response. Pediatrics, 1995. 96(3 Pt 2): p. 548-57.

**23.** Meade, B.D., et al., Relationships between functional assays and enzyme immunoassays as measurements of responses to acellular and whole-cell pertussis vaccines. Pediatrics, 1995. 96(3 Pt 2): p. 595-600.

**24.** Barkoff, A.M., et al., Evaluation of Anti-PT Antibody Response after Pertussis Vaccination and Infection: The Importance of Both Quantity and Quality. Toxins (Basel), 2021. 13(8).

**25.** Trollfors, B., et al., Toxin-neutralizing antibodies in patients with pertussis, as determined by an assay using Chinese hamster ovary cells. J Infect Dis, 1988. 158(5): p. 991-5.

**26.** Carlsson, R.M., et al., Antibody persistence in five-year-old children who received a pentavalent combination vaccine in infancy. Pediatr Infect Dis J, 2002. 21(6): p. 535-41.

**27.** Dalby, T., et al., Pertussis serology: assessment of IgG anti-PT ELISA for replacement of the CHO cell assay. Apmis, 2010. 118(12): p. 968-72.

**28.** Isacson, J., et al., Comparison of a toxin neutralization assay and ELISA for determination of pertussis toxin antibodies. Serodiagnosis and Immunotherapy in Infectious Disease, 1997. 8(3): p. 163-167.

**29.** Storsaeter, J., et al., Secondary analyses of the efficacy of two acellular pertussis vaccines evaluated in a Swedish phase III trial. Vaccine, 1990. 8(5): p. 457-61.

**30.** Versteegen, P., et al., Responses to an acellular pertussis booster vaccination in children, adolescents, and young and older adults: A collaborative study in Finland, the Netherlands, and the United Kingdom. EBioMedicine, 2021. 65: p. 103247.

**31.** van Gageldonk, P.G.M., et al., Development and validation of a multiplex immunoassay for the simultaneous determination of serum antibodies to Bordetella pertussis, diphtheria and tetanus. J Immunol Methods, 2008. 335(1-2): p. 79-89, DOI: 10.1016/j.jim.2008.02.018.

**32.** Gillenius, P., et al., The standardization of an assay for pertussis toxin and antitoxin in microplate culture of Chinese hamster ovary cells. J Biol Stand, 1985. 13(1): p. 61-6.

**33.** Zhang, Y., et al., Determination of serum neutralizing antibodies reveals important difference in quality of antibodies against pertussis toxin in children after infection. Vaccine, 2021. 39(13): p. 1826-1830.

**34.** Moriuchi, T., et al., A high seroprevalence of antibodies to pertussis toxin among Japanese adults: Qualitative and quantitative analyses. PLoS One, 2017. 12(7): p. e0181181.

**35.** Barkoff, A.M., K. Gröndahl-Yli-Hannuksela, and Q. He, Seroprevalence studies of pertussis: what have we learned from different immunized populations. Pathog Dis, 2015. 73(7).

**36.** Berbers, G., et al., Circulation of pertussis and poor protection against diphtheria among middle-aged adults in 18 European countries. Nat Commun, 2021. 12(1): p. 2871.

**37.** Tran Minh, N.N., et al., Acellular vaccines containing reduced quantities of pertussis antigens as a booster in adolescents. Pediatrics, 1999. 104(6): p. e70. doi: 10.1542/peds.104.6.e70.

**38.** Carlsson, R.M., et al., Safety and immunogenicity of a combined diphtheria-tetanus-acellular pertussis-inactivated polio vaccine-Haemophilus influenzae type b vaccine administered at 2-4-6-13 or 3-5-12 months of age. Pediatr Infect Dis J, 1998. 17(11): p. 1026-33.

**39.** Xing, D., et al., Characterization of reference materials for human antiserum to pertussis antigens by an international collaborative study. Clin Vaccine Immunol, 2009. 16(3): p. 303-11.

**40.** Granström, M., et al., Acellular pertussis vaccine in adults: adverse reactions and immune response. Eur J Clin Microbiol, 1987. 6(1): p. 18-21.

## SUPPLEMENTARY MATERIALS

**Supplementary Figure 1.** Pertussis vaccine schedules and vaccines in Finland and the Netherlands. Modified from Versteegen et al. [30].

| Year | Scheme | Vaccine composition | Change | Pertussis vaccine name (Company) | Study cohort | Vaccination coverage [1] |
|---|---|---|---|---|---|---|
| Finland 1952 | | DTwP | First only DwP, T added in 1957<br>1962 change to DTwP (Fim2,3 strain)<br>Additional Fim2 strain added in 1976 | Diphteria-Pertussis Forte (Orion)<br>PDT (Orion)<br>DTwP (KTL) | Older and young adults | 92-99% |
| 2003 | 3,4,5, 20-24 m<br>6y | DTwP + IPV + Hib<br>Tdap3 | <br>First ap booster vaccine in NIP | DTwP (KTL)<br>Boostrix (GSK) | Adolescents<br>Adults | 98% |
| 2005 | 3,5,12 m<br>4y<br>14-15y | DTaP2-IPV-Hib<br>DTaP2-IPV<br>Tdap3 | Introduction aP priming, 2 Bp components<br><br>Also catch-up for 11-13y | Pentavac (Sanofi)<br>Tetravac (Sanofi)<br>Boostrix (GSK) | Adolescents and children<br><br>Young adults | 97-99% |
| 2009 | 3,5,12 m<br>4y<br>14-15y | DTaP3-IPV-Hib<br>DTaP2-IPV<br>Tdap3 | 3 Bp components | Infanrix (GSK)<br>Tetravac (Sanofi)<br>Boostrix | Children<br>Adolescents<br>Young adults | 99% |
| 2012 | Military | Tdap3 | Addition of pertussis vaccination in the military, 18-25y (estimation) | Boostrix (GSK) | Young adults | 89-99% |
| 2018 | 25y | Tdap3 | Introduction of adult booster | Boostrix (GSK) | Young adults | 91% |

| Year | Scheme | Vaccine composition | Change | Pertussis vaccine name (Company) | Study cohort | Vaccination coverage [1] |
|---|---|---|---|---|---|---|
| **Netherlands** | | | | | | |
| 1954 | | DTwP | Combination vaccine became available | DTwP (RIVM) | Older adults | |
| 1957 | 3,4,5,11 m | DTwP | Start of NIP | DTwP (RIVM) | Older and young adults | 96-97% |
| 1999 | 2,3,4,11 m | DTwP-IPV + Hib | Accelerated vaccination | DTwP (RIVM) | | 97.0-97.2% |
| 2001 | 2,3,4,11 m<br>4y | DTwP-IPV + Hib<br>DT-IPV + aP3 | Introduction aP booster, 3 Bp components | DTwP (RIVM)<br>monovalent aP (GSK) | Adolescents | 97.2-97.8% |
| 2005 | 2,3,4,11 m<br>4y | DTaP3-IPV-Hib<br>DT-IPV + aP3 | Introduction aP priming, 3 Bp components | Infanrix (GSK)<br>monovalent aP (GSK) | Adolescents | 96.0% |
| 2006 | 2,3,4,11 m<br>4y | DTaP5-IPV-Hib<br>DT-IPV + aP3 | 5 Bp components | Pediacel (SP MSD)<br>monovalent aP (GSK) | Adolescents | 96.3-96.9% |
| 2011 | 2,3,4,11 m<br>4y | DTaP3-IPV-Hib-HepB + Pneu<br>DTaP3-IPV | 3 Bp components | Infanrix (GSK)<br>Infanrix (GSK) | | 94.8-96.7% |
| 2017 | 2,3,4,11 m<br>4y | DTaP3-IPV-Hib-HepB + Pneu<br>Tdap3-IPV | Reduced dose of 3 Bp components | Infanrix (GSK)<br>Boostrix (GSK) | | 93.9% |
| 2018 | 2,3,4,11 m<br>4y | DTaP5-IPV-Hib-HepB + Pneu<br>Tdap3-IPV | 5 Bp components | Vaxelis (SP MSD)<br>Boostrix (GSK) | | 93.5% |

# Chapter 5

# Memory B cell activation induced by pertussis booster vaccination in four age groups of three countries

P. Versteegen*, A. M. Barkoff*, M. Valente Pinto*, J. van de Kasteele, A. Knuutila, S. Bibi, L. de Rond, J. Teräsjärvi, K. Sanders, M. L. de Zeeuw-Brouwer, R. Luoto, H. Ten Hulscher, E. A. Clutterbuck, E. A. M. Sanders, J. Mertsola, G. A. M. Berbers, Q. He, D. F. Kelly, and A. M. Buisman. *equal contribution

Front Immunol 13 (May 23 2022): 864674.

## ABSTRACT

**Background:** Immunogenicity of acellular pertussis (aP) vaccines is conventionally assessed by measuring antibody responses but antibody concentrations wane quickly after vaccination. Memory B cells, however, are critical in sustaining long-term protection and therefore may be an important factor when assessing pertussis immunity after vaccination.

**Aim:** We studied pertussis specific memory B cell (re)activation induced by an aP booster vaccination in four different age groups within three countries.

**Materials and methods:** From a phase IV longitudinal interventional study, 268 participants across Finland, the Netherlands and the United Kingdom were included and received a 3-component pertussis booster vaccine: children (7-10y, n=53), adolescents (11-15y, n=66), young adults (20-34y, n=74), and older adults (60-70y, n=75). Memory B cells at baseline, day 28, and 1 year post-vaccination were measured by a pertussis toxin (Ptx), filamentous haemagglutinin (FHA), and pertactin (Prn) specific ELISpot assay. Antibody results measured previously were available for comparison. Furthermore, study participants were distributed into groups based on their baseline memory B cell frequencies, vaccine responses were monitored between these groups.

**Results:** Geometric mean (GM) memory B cell frequencies for pertussis antigens at baseline were low. At 28 days post-vaccination, these frequencies increased within each age group and were still elevated one year post-booster compared to baseline. Highest frequencies at day 28 were found within adolescents (GM: 5, 21, and 13, for Ptx, FHA and Prn, respectively) and lowest within older adults (GM: 2, 9, and 3, respectively). Moderate to strong correlations between memory B cell frequencies at day 28 and antibody concentrations at day 28 and 1 year were observed for Prn. Memory B cell frequencies > 1 per 100,000 PBMCs at baseline were associated with significantly higher memory responses after 28 days and 1 year.

**Conclusions:** An aP booster vaccine (re)activated memory B cells in all age groups. Still elevated memory B cell frequencies after one year indicates enhanced immunological memory. However, antigen specific memory B cell activation seems weaker in older adults, which might reflect immunosenescence. Furthermore, the presence of circulating memory B cells at baseline positively affects memory B cell responses. This study was registered at www.clinicaltrialsregister.eu: No. 2016-003678-42.

## INTRODUCTION

Pertussis can be severe and even lethal for infants, but also patients with (pulmonary) comorbidities and older adults are more prone to hospitalisation and complications [1, 2]. The incidence of pertussis has decreased enormously since the introduction of whole cell pertussis (wP) vaccines in the 1940- 1950s. However, due to its reactogenicity, wP vaccines have been replaced by acellular pertussis (aP) vaccines in many countries to achieve high vaccination coverage. Despite this high vaccination coverage, a resurgence of pertussis has been noticed in several countries where aP vaccines have been used for routine infant immunisation since the 1990s and where the disease appears to have become endemic again [3-7]. The increased circulation may be due to waning vaccine induced immunity, and adaptation of the bacterium. The switch from wP vaccines to aP vaccines for infant priming in high income countries might have influenced this resurgence. In addition, increased awareness and improved diagnostics of pertussis may also have contributed to the increase in the number of reported cases [8, 9].

Recommendations for pertussis booster vaccinations with aP vaccine vary between countries, and may be given at different ages and to different target populations such as pregnant women, military conscripts, healthcare workers and older adults. However, antibody concentrations wane rather quickly after vaccination regardless whether an infant was primed using aP or wP vaccines [10, 11]. Despite this, memory B cells may play an important role in long-term humoral immunity, through their mediation of the rapid secondary antibody response following the next encounter with pertussis. Generally, memory B cells reside in the secondary and tertiary lymphoid tissues and are reactivated by antigen encounter with or without T cell help [12]. Upon reactivation, memory B cells recirculate throughout the body and may produce either short-lived plasma cells that secrete antibodies, or renewed memory B cells, or long-lived plasma cells. Higher frequencies of antigen specific memory B cells with increasing age have been described in children, which is probably related to vaccination [13, 14]. Furthermore, the receipt of even a single dose of a wP vaccine in infants subsequently primed with an aP vaccine, seems to result in a lower risk of infection compared to individuals exclusively aP primed, even several years later [15, 16].

Recently, we have described the serological immunoglobulin (Ig) G antibody concentrations after an aP booster vaccination in four age groups and with different vaccine priming backgrounds in Finland, the Netherlands, and the United Kingdom (UK) indicating a rise in antibody concentrations upon vaccination in all age groups, with substantial inter individual differences in concentrations [10]. Differences did not seem to be consistently age-dependent. A subpopulation of this study is now further analysed.

In this study, we identified the frequencies of antigen specific memory B cells before aP booster vaccination, studied the induction of memory B cells one month post-vaccination and monitored the waning of these cells one year post-vaccination. We also assessed

potential differences in memory B cell frequencies between four age groups (children, adolescents, young adults, and older adults) within the three study countries (Finland, the Netherlands, and the UK) as well as the effect of previous priming with wP or aP vaccinations on memory B cell frequencies. Furthermore, we evaluated whether the baseline memory B cell frequencies affect vaccination responses.

## MATERIALS AND METHODS

### Study design and participants

This phase IV longitudinal interventional study conducted in Finland, the Netherlands and the UK is described in detail elsewhere [10]. In summary, healthy children (7-10 y), adolescents (11-15y), young adults (20-34 y), and older adults (60-70 y) received Boostrix-IPV (GlaxoSmithKline (GSK), Wavre, Belgium), a three component pertussis vaccine (Tdap3-IPV), between October 2017 and January 2019. Children were all aP primed, adolescents were either aP or wP primed, young adults were all wP primed, and older adults were either wP primed or unvaccinated. B cell assays were performed on a subset of the total study samples given the resources needed to undertake the assay (total n= 268 out of 379). Samples were selected according to a prespecified sample plan based on participant ID. For the memory B cell analyses the samples were collected before vaccination (baseline), and at day 28 and 1 year post-vaccination. **Figure 1** shows the number of participants per country, per age group, and per timepoint. Their characteristics are listed in **Table 1**. The trial was registered at the EU Clinical Trial database (EudraCT number 2016-003678-42).

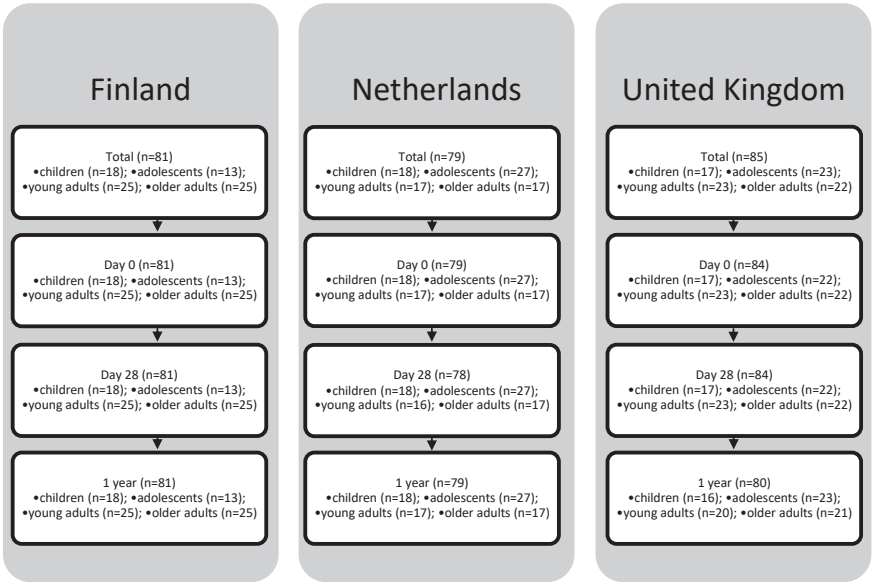



**Figure 1.** Flow diagram BERT-study.

**Table 1:** Participant characteristics.

| Characteristic | | Country | Children aged 7-10 yrs | Adolescents aged 11-15 yrs | Young adults aged 20-34 yrs | Older adults aged 60-70 yrs |
|---|---|---|---|---|---|---|
| No. of participants | Per country | FI | 18 | 13 | 25 | 25 |
| | | NL | 18 | 27 | 17 | 17 |
| | | UK | 17 | 23 | 23 | 22 |
| | Total | All | 63 | 63 | 65 | 64 |
| Age * | In years | FI | 9.0 (8.6-9.5) | 13.6 (12.9-14.4) | 30.2 (28.7-31.7) | 64.2 (63.2-65.2) |
| | | NL | 8.5 (8.3-8.7) | 13.5 (12.9-14.0) | 29.2 (27.2-31.1) | 65.6 (64.1-67.1) |
| | | UK | 9.3 (8.8-9.8) | 12.8 (12.4-13.1) | 26.1 (24.1-28.2) | 65.6 (64.3-67.0) |
| Sex# | No. female | FI | 10 [55.6] | 5 [38.5] | 21 [84.0] | 21 [84.0] |
| | | NL | 7 [38.9] | 11 [40.7] | 6 [35.3] | 10 [58.8] |
| | | UK | 8 [47.1] | 13 [56.5] | 15 [65.2] | 10 [45.5] |
| Pertussis priming background | No. aP | FI | 18 [100] | 7 [53.8] | N/A | N/A |
| | | NL | 18 [100] | 15 [55.6] | N/A | N/A |
| | | UK | 17 [100] | 23 [100] | N/A | N/A |
| | No. wP / unknown | FI | N/A | 6 [46.2] | 25 [100] | 25 [100] |
| | | NL | N/A | 12 [44.4] | 17 [100] | 17 [100] |
| | | UK | N/A | 0 | 23 [100] | 22 [100] |

* Age per country is indicated in mean with (95% CI). # Sex is indicated in number and [percentage] of female participants per country. Number of participants aP primed and wP primed in infancy is indicated in number and [percentage] per country. FI: Finland; NL: Netherlands; UK: United Kingdom. No: number. CI: confidence interval. N/A: not applicable for that age group.

### B cell analyses

Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood by density gradient centrifugation as described previously with some modifications, as lymphoprep [Axis- Shield (Progen), product no. 1114547] was used instead of vacutainer cell preparation tubes containing sodium citrate (Becton Dickinson Biosciences, CA, USA (BD) [14, 17]. PBMCs collected at baseline, day 28, and 1 year post-vaccination were frozen at -135°C after processing, and thawed for memory B cell analyses by antigen-specific ELISpot to run all timepoints per individual simultaneously. After thawing, PBMCs were stimulated for five days using AIM-V medium with AlbuMax (#31035-025, Gibco BRL), 50mM ß-mercaptoethanol (M3148, Sigma), and 10% fetal bovine serum (FBS) heat-inactivated culture medium supplied with a mixture of CpG ODN 2006 (3 µg/mL), IL2 (10 ng/mL) and IL10 (10 ng/mL). ELISpot plates were coated with pertussis antigens as described previously with minor modifications [14, 17]. Briefly, plates were coated with native pertussis toxin (Ptx, 10 µg/mL, GSK), filamentous haemagglutinin (FHA, 5 µg/mL, SP), pertactin (Prn, 5 µg/mL, GSK), and fimbriae types 2 and 3 (Fim2/3, 2.5 µg/mL, GSK) antigens. In addition, PBS was used as a negative control and either anti-human IgG or plasmablasts identification by flowcytometry pre- and post-stimulation served as positive controls. After blocking the wells with culture medium, PBMCs were put on an antigen coated plate with a concentration of 200,000 cells per well, at least in duplicate for 18-24 hours.

PBMC isolation for the memory B cell ELISpot assays were performed at the three different study sites. All study sites used the same validated protocols and same reagents; e.g. the concentration (1:5000) and batch of the secondary antibody (Goat anti-human IgG, Merck-Millipore, 401442-1mL, batch 3167145) were evaluated before the tests to ensure that there is no formation of non-specific spots. ELISpot plates were sent to the Dutch National Institute for Public Health and the Environment (RIVM) and all plates were counted using similar settings at a single ImmunoSpot S6 Ultra-V (Cellular Technology Limited, Cleveland, OH) analyser. Geometric mean (GM) frequencies were calculated for each specific antigen of memory B cells (baseline, day 28, and 1 year post-vaccination) per 100,000 PBMCs and the background frequency from the negative control was subtracted per individual. All memory B cell frequency data have been deposited in the central database of the PERISCOPE Consortium and can be accessed by a request to the PERISCOPE management team.

### Statistical analysis

For statistical analysis, a frequency of 0 memory B cells was replaced by 0.1. For the memory B cell frequencies, a linear mixed model was fitted to the log-transformed frequencies for each memory B cell frequency and antigen combination. A linear mixed model can be considered as a generalisation of a paired t-test [18]. This model describes

the GM of memory B cell frequencies while accounting for the longitudinal structure of the measurements. Timepoint of blood sampling, age group, and country were included in the model as a three-way interaction as fixed effects. Sex was included only as main effect. Participant ID was included as a random intercept in the model. This took the within-participant correlation into account. A similar model, where age group was replaced by vaccination group, was used to analyse the differences in frequencies between individuals with an aP or wP primary vaccination background.

A type III ANOVA was used to assess overall significance of the fixed effect terms. GM frequencies and their corresponding 95% confidence intervals (95% CI), as well as their mutual GM frequency ratios with corresponding 95% CI, and p-values were obtained by post hoc analysis. Satterthwaite's method was used for the mixed model denominator degrees of freedom [19, 20]. P-values were adjusted by applying the Benjamini-Hochberg procedure for multiple comparisons, controlling the false discovery rate [21].

Statistical analysis were done in R, using the lme4 package, and emmeans package [22-24].

Data regarding antigen specific IgG antibody concentrations within the study groups have been published previously [10]. In the current study, we correlated these antibody data to specific memory B cell frequencies in the total study cohort. Pearson's r was calculated for the log10 transformed data using GraphPad Prism 9.0.1 for Windows. Based on the results, heat maps were created. A correlation of exactly 1 is considered perfect, ≥ 0.70 as strong, ≥ 0.50 as moderate, and <0.30 as weak.

To identify a possible biomarker of a proper memory B cell vaccine response, cut-off values for baseline frequencies were determined by dividing the participants into ordinal groups with the following baseline GM memory B cell frequencies: ≤1, >1-2, and >2 per 100,000 PBMCs. These groups were compared to reject the null hypothesis that there is no significant difference between the groups at any timepoint (baseline, day 28, and 1 year post-vaccination). Same cut-off was also used to present the antibody GM concentrations (GMCs) of the two groups. Data analysis was performed using IBM SPSS Statistics for Windows, Version 25.0.0.0 (IBM Corp., Armonk, NY, USA).

## RESULTS

### Memory B cell frequencies in each specific age group at different timepoints

In this study, a total of 268 healthy participants across Finland, the Netherlands and the UK were included and received a Tdap3-IPV booster vaccination: children (7-10y, n=53), adolescents (11-15y, n=66), young adults (20-34y, n=74), and older adults (60-70y, n=75). Longitudinal samples were available from most participants, however in a small proportion of participants one or more timepoints were missing (**Figure 1**). The median

spot frequency in the blanc wells was 0/100,000 cells (95% CI 0-0) and the mean spot frequency was 0.21/100,000 cells (95% CI 0.18-0.23). A scan of a representative plate showing spot frequencies per antigen is available in **Supplementary Figure 1**. Generally, only minor differences were noticed in memory B cell frequencies between the three countries within each age group (**Figure 2** and **Supplementary Table 1**). When age groups were combined from all countries, baseline GM memory B cell frequencies were all > 0.1 per 100,000 PBMCs (**Table 2**). However, on an individual level the majority of participants had memory B cell frequencies of 0.1 per 100,000 PBMCs to either Ptx, and/or FHA, and/or Prn at baseline **(Table 3**). At day 28 post-vaccination we noticed significantly higher GM memory B cell frequencies within each age group for Ptx, FHA and Prn ($p \leq 0.001$ for all, **Table 2**). However, in each group we found non-responders varying from 4.6% to 37.5% of all participants (**Table 3**). The proportion of non-responders was usually highest among older adults. At 1 year post-vaccination significantly lower GM memory B cell frequencies were observed when compared to 28 days post-vaccination for all antigens among all age groups ($p \leq 0.001$ for all). GM memory B cell frequencies 1 year post-vaccination were still significantly elevated compared to baseline within all age groups and for all antigens (p values ≤ 0.001-0.05), except against Ptx in children. The GM frequencies of Ptx specific memory B cells were relatively low at each timepoint, compared to those for FHA and Prn. Memory B cell frequencies for Fim2/3 (pertussis antigen not in the vaccine) have not been included in the analyses as they were mainly low in all age groups at all timepoints (data presented in **Supplementary Figure 2**).

**Table 2:** Antigen specific geometric mean memory B cell frequencies with 95% confidence intervals and significances from all countries together were calculated using a linear mixed model on the log10 transformed data taking the longitudinal structure into account.

| Antigen | Timepoint | Children | Adolescents | Young adults | Older adults |
|---|---|---|---|---|---|
| Ptx GM Bmem frequencies / 100,000 PBMCs (CI) | day 0 | 0.5 (0.4-0.8) | 0.9 (0.6-1.3)[a,b] | 0.4 (0.3-0.6)[c] | 0.4 (0.3-0.6)[c] |
| | day 28 | 3.4 (2.2-5.1) | 4.9 (3.3-7.3)[a,b] | 2.5 (1.7-3.6)[c] | 2.0 (1.3-2.9)[c] |
| | 1 year | 0.8 (0.6-1.3) | 1.4 (1.0-2.1) | 1.0 (0.7-1.5) | 0.8 (0.5-1.2) |
| FHA GM Bmem frequencies / 100,000 PBMCs (CI) | day 0 | 2.1 (1.5-3.0)[a,b] | 2.9 (2.1-4.0)[a,b] | 0.7 (0.5-1.0)[c,d] | 0.8 (0.6-1.0)[c,d] |
| | day 28 | 18 (13-25)[b] | 21 (15-29)[b] | 19 (14-25)[b] | 8.7 (6.4-12)[a,c,d] |
| | 1 year | 4.2 (3.0-5.8)[b] | 6.7 (4.9-9.3)[a,b] | 3.7 (2.7-5.0)[c] | 2.4 (1.7-3.3)[c,d] |
| Prn GM Bmem frequencies / 100,000 PBMCs (CI) | day 0 | 0.6 (0.4-0.9)[a,b] | 0.8 (0.5-1.1)[a,b] | 0.4 (0.3-0.5)[c,d] | 0.3 (0.2-0.5)[c,d] |
| | day 28 | 8.1 (5.5-12)[b] | 13 (8.6-18)[b] | 9.2 (6.4-13)[b] | 3.0 (2.1-4.3)[a,c,d] |
| | 1 year | 1.8 (1.2-2.6)[b] | 2.7 (1.9-4.0)[b] | 1.8 (1.3-2.7)[b] | 0.8 (0.6-1.2)[a,c,d] |

Significance per antigen has been tested between age groups within a timepoint; $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$. Significantly different from: [a] young adults; [b] older adults; [c] adolescents; [d] children. Ptx: pertussis toxin; FHA: filamentous haemagglutinin; Prn: pertactin; GM: geometric mean; Bmem: memory B cell; PBMC: peripheral blood mononuclear cells; CI: 95% confidence interval.

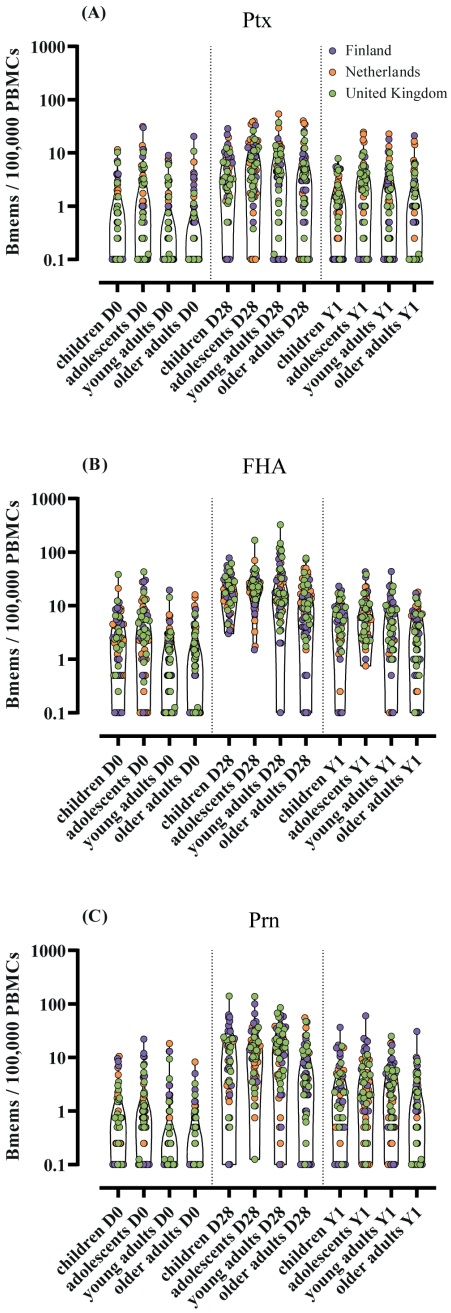



**Figure 2.** Individual memory B cell frequencies in four age groups. Bmems: memory B cell frequencies; PBMCs: peripheral blood mononuclear cells; D0: day 0 (baseline); D28: day 28 post-vaccination; Y1: 1 year post-vaccination. Memory B cell frequencies specific for **(A)**: pertussis toxin; **(B)**: filamentous haemagglutinin; and **(C)**: pertactin. Presented in truncated violin plots. Corresponding geometric mean frequencies and 95% confidence interval are presented in **Table 2**.

**Table 3.** Distribution of participants. At day 0 (baseline), the amount of participants with memory B cell frequencies ≤ 1 / 100,000 PBMCs are shown.

| Antigen | Group | Day 0 ≤ 1 spot / 100,000 PBMCs (%) | Day 28 ≤ 1 spot / 100,000 PBMCs and/or ≤ day 0 (%) | 1 year ≤ 1 spot / 100,000 PBMCs (%) |
|---|---|---|---|---|
| Ptx | Total | 169 (69.3) | 59 (24.4) | 117 (48.8) |
| | Children | 35 (66.0) | 10 (19.2) | 27 (51.9) |
| | Adolescents | 36 (58.1) | 9 (14.8) | 23 (36.5) |
| | Young adults | 49 (75.4) | 18 (27.7) | 29 (46.8) |
| | Older adults | 49 (76.6) | 24 (37.5) | 38 (60.3) |
| FHA | Total | 103 (42.2) | 15 (6.2) | 38 (15.9) |
| | Children | 15 (28.3) | 3 (5.8) | 6 (11.5) |
| | Adolescents | 16 (25.8) | 3 (4.9) | 2 (3.2) |
| | Young adults | 38 (58.5) | 3 (4.6) | 9 (14.8) |
| | Older adults | 35 (54.7) | 6 (9.4) | 21 (33.3) |
| Prn | Total | 184 (75.4) | 35 (14.5) | 90 (37.5) |
| | Children | 36 (67.9) | 7 (13.5) | 18 (34.6) |
| | Adolescents | 41 (66.1) | 3 (4.9) | 17 (27.0) |
| | Young adults | 52 (80.0) | 7 (10.8) | 21 (33.9) |
| | Older adults | 55 (85.9) | 18 (28.1) | 34 (54.0) |
| Ptx, FHA, & Prn | Total | 73 (29.9) | 4 (1.7) | 23 (9.6) |
| | Children | 11 (20.8) | - | 2 (3.8) |
| | Adolescents | 10 (16.1) | - | 1 (1.6) |
| | Young adults | 21 (32.3) | - | 5 (8.2) |
| | Older adults | 31 (48.4) | 4 (6.3) | 15 (23.8) |

At day 28 post-vaccination the amount of non-responders is shown, defined as a frequency of ≤ 1 memory B cell / 100,000 PBMCs and/or ≤ day 0. At 1 year post-vaccination again the participants with memory B cell frequencies ≤ 1 / 100,000 PBMCs are shown. Data are shown per antigen and for the combined antigens, for the total group as well as split per age group. The total group per antigen at baseline (bold) shows the distribution of participants used for **Figure 5**. Ptx: pertussis toxin; FHA: filamentous haemagglutinin; Prn: pertactin; PBMCs: peripheral blood mononuclear cells.

## Memory B cell frequency differences between age groups

GM memory B cell frequencies specific for Ptx detected at baseline were < 1 per 100,000 PBMCs for all age groups (**Table 2**). At day 28 post-vaccination, Ptx specific memory B cell frequencies were low, but significantly higher in adolescents (GM 4.9) compared to young (GM 2.5, $p < 0.05$) and older adults (GM 2.0, $p \leq 0.01$). At 1 year post-vaccination, significant differences were lost between the age groups and the GM frequencies of memory B cells were overall low. In general, adolescents had the highest GM frequency of Ptx specific memory B cells and older adults the lowest.

GM frequencies of specific memory B cells against FHA were the highest compared to the other two pertussis vaccine antigens among all age groups. At baseline, GM memory B cell frequencies were low among all age groups, although slightly higher in children and adolescents compared to young and older adults. At day 28 post-vaccination, children, adolescents and young adults (GM 18, 21, and 19, respectively) had higher frequencies of memory B cells compared to the older adults (GM 8.7, $p \leq 0.01$, $p \leq 0.001$, and $p \leq 0.001$ respectively). At 1 year post-vaccination significant differences were observed between children and older adults, between adolescents and young adults, and between adolescents and older adults. In general, adolescents had the highest GM frequencies of FHA specific memory B cells and older adults the lowest.

Prn specific GM memory B cell frequencies at baseline were < 1 per 100,000 PBMCs for all age groups. At day 28 post- vaccination frequencies were significantly higher in children, adolescents, and young adults (GM 8.1, 13 and 9.2 respectively), compared to older adults (GM 3.0, $p \leq 0.001$). One year post-vaccination, children, adolescents, and young adults still had higher frequencies of Prn specific memory B cells. In general, the Prn specific GM memory B cell frequency was the highest among adolescents and the lowest among older adults.



**Adolescents with different vaccination background**

Adolescents from Finland and the Netherlands were primed with either aP (n=22) or wP (n=18) vaccine. We observed higher GM memory B cell frequencies within the wP group compared to the aP group for all antigens at all timepoints, excluding FHA at baseline (**Figure 3**). At day 28 post-vaccination differences were most pronounced between the two groups, however not significant. At 1 year post-vaccination, GM memory B cell frequencies for wP primed individuals were still 1.5 to 2.2 fold higher when compared to those of the aP primed individuals. This comparison was not available in the UK since all adolescents were aP primed as per UK national immunisation programme.

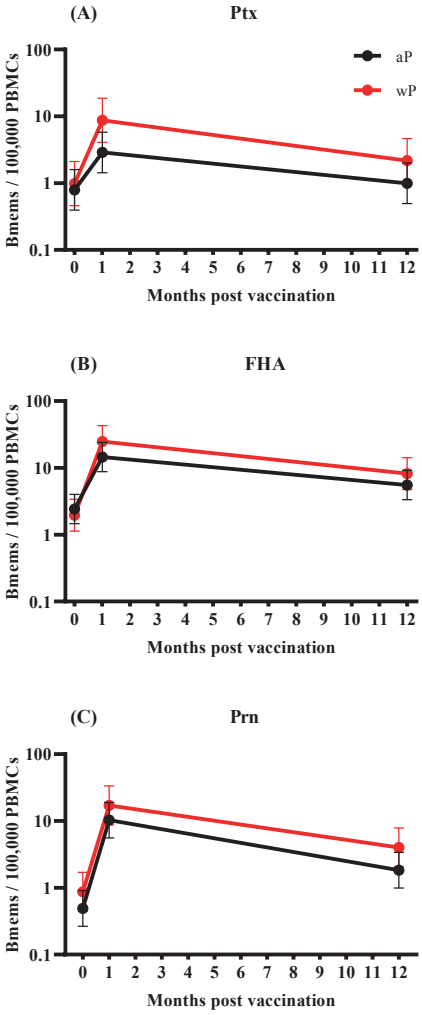



**Figure 3.** Geometric mean memory B cell frequencies per priming vaccination background with 95% confidence intervals. Significances were calculated using a linear mixed model on the log10 transformed data taking the longitudinal structure into account. Bmems: memory B cell frequencies; PBMCs: peripheral blood mononuclear cells. Memory B cell frequencies in adolescents from the Netherlands and Finland together (aP priming n=22, wP priming n=18) specific for **(A)**: pertussis toxin; **(B)**: filamentous haemagglutinin; and **(C)**: pertactin. Observed differences between the aP and wP primed cohorts did not reach significance.

## Correlation between memory B cell frequencies and IgG antibody concentrations

Correlations between memory B cells frequencies and IgG antibody concentrations against different antigens can be found in **Figure 4**. For all participants together at day 28 postvaccination, the correlation between antigen specific memory B cell frequencies and IgG antibody concentrations was moderate/strong for Prn ($r = 0.62$, $p < 0.001$),

whereas those for Ptx and FHA were weak/moderate at the same timepoint. At 1 year postvaccination, weak to moderate correlations were noticed for all antigens, still showing the highest correlation for Prn ($r = 0.46$, $p < 0.001$). Prn specific memory B cell frequencies at day 28 postvaccination correlated also moderate/strong with Prn specific antibody concentrations 1 year post-vaccination ($r = 0.62$, $p < 0.001$), whereas those correlations for Ptx and FHA were again weak/moderate. Generally, correlations for FHA were the lowest compared to Ptx and Prn. We additionally measured antigenspecific IgG producing plasma cell frequencies at day 7 postvaccination, also showing the highest correlation to memory B cell frequencies and antibody concentrations at day 28 and 1 year post-vaccination for Prn. Plasma cell methods and results are supplied in **Supplementary Figure 3**.

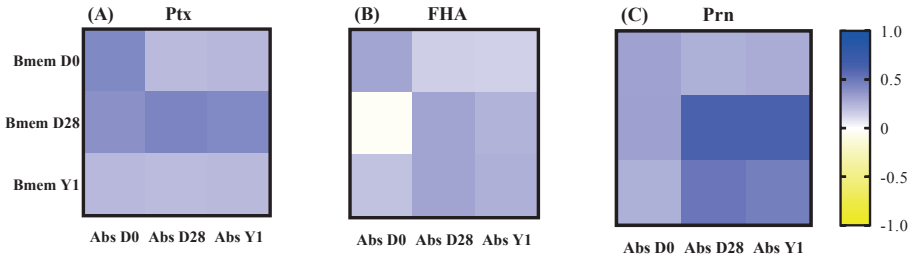



**Figure 4.** Correlations between memory B cell frequencies and antibody concentrations presented as heatmaps per antigen for all participants together based on Pearsons' correlation on the log10 transformed memory B cell frequencies and antibody concentrations. Ptx: pertussis toxin; FHA: filamentous haemagglutinin; Prn: pertactin; Bmem: memory B cell frequencies; Abs: antibody concentrations; D0: day 0 (baseline); D28: day 28 post-vaccination; Y1: 1 year post-vaccination. Correlations for **(A)**: pertussis toxin; **(B)**: filamentous haemagglutinin; and **(C)**: pertactin.

## Memory B cell frequencies at baseline as a marker of vaccine responsiveness

We studied the influence of baseline memory B cell frequencies on vaccine responses against all pertussis vaccine antigens. Study participants from all age groups of the three countries were distributed into two categories based on the previously mentioned ordinal groups. We defined a frequency cut-off of > 1 memory B cell per 100,000 PBMCs at baseline for Ptx, FHA, and Prn, which was the lowest frequency to show significant difference between the two groups. This is on average 5 times higher compared to the mean background value. These cut-offs resulted in a rather even distribution of participants within both categories (**Table 3**). Results of GM memory B cell frequencies post-vaccination in these two categories are presented in **Figures 5A–C**. Corresponding antibody GMCs at all timepoints from the two categories are displayed in **Figures 5D–F**.

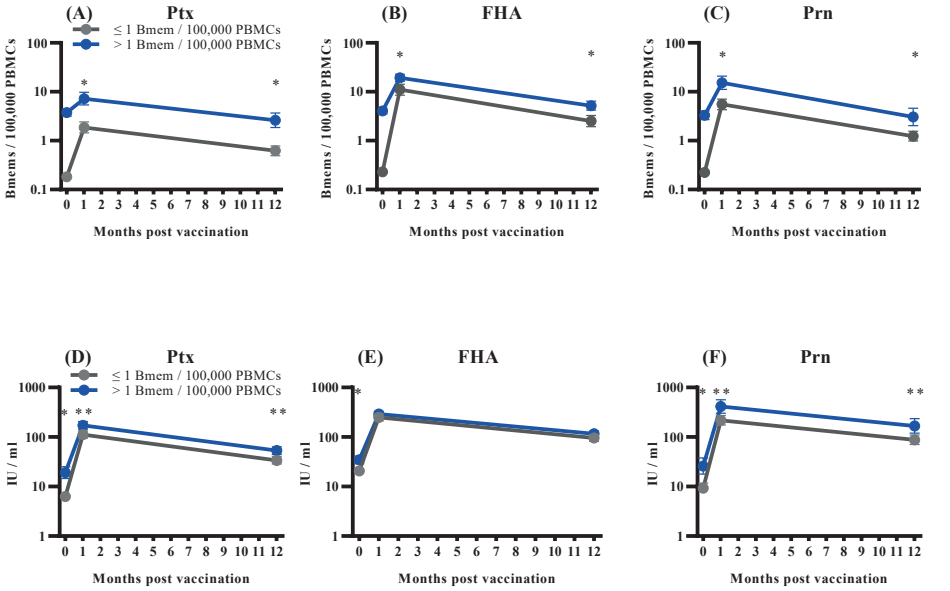



**Figure 5.** Memory B cell responses to different antigens based on a defined frequency cut-off of > 1 memory B cell / 100,000 PBMCs. Bmems: memory B cell frequencies; PBMCs: peripheral blood mononuclear cells. Geometric mean memory B cell frequencies with 95% confidence intervals for the combined age groups distributed into two groups based on baseline memory B cell frequencies for **(A)**: pertussis toxin; **(B)**: filamentous haemagglutinin; and **(C)**: pertactin. Corresponding antibody geometric mean concentrations for the two groups for **(D)**: pertussis toxin, **(E)**: filamentous haemagglutinin; and **(F)**: pertactin. Number and proportion of participants in the group with frequencies ≤ 1 memory B cell / 100,000 PBMCs can be viewed in **Table 3**. * $p < 0.001$; ** $p = 0.003$.

Significantly higher GM memory B cell frequencies were observed at day 28 and 1 year post-vaccination in individuals showing a frequency of > 1 memory B cell per 100,000 PBMCs compared to individuals showing a frequency of ≤ 1 memory B cell per 100.000 PBMCs at baseline for all three vaccine antigens ($p < 0.001$ for all). For antibodies, significantly higher GMCs were observed at baseline for all three antigens ($p < 0.001$) and these remained significant for Ptx and Prn ($p = 0.003$ at day 28 and 1 year) in individuals having memory B cells > 1 per 100,000 PBMCs at baseline. For FHA, the two groups were not significantly different at day 28 and 1 year post-vaccination as could be expected from the weak correlation between memory B cell frequencies and antibody concentrations.

## DISCUSSION

In this study, we measured antigen (Ptx, FHA, and Prn) specific memory B cell frequencies within four age groups in three countries after an aP booster vaccination. At baseline GM memory B cell frequencies were found to be low among all age groups, whereas a significant increase in memory B cell frequencies was found one month post-vaccination, to the three pertussis vaccine antigens in all age groups. The percentage of non-responders varied from 4.6 to 37.5%, with highest proportion of non-responders in older adults. Furthermore, memory B cell frequencies were still significantly elevated one year post-vaccination compared to baseline, indicating a persistent response following aP booster vaccination at all ages from 7 to 70 years of age. Generally, all age groups within the three countries showed similar patterns in memory B cell frequencies with minor country to country variations. However, variation was observed between the age groups. The lowest post-vaccination frequencies were constantly observed within the older adults (60-70 years), whereas the highest frequencies were among the adolescents (11-15 years). Furthermore, the older adults had lower memory B cell frequencies compared to the young adults (20-34 years). In addition, the presence of > 1 memory B cell per 100,000 PBMCs at baseline affects the memory B cell responses positively.



As stated, the highest post-vaccination memory B cell frequencies were found in adolescents. The infection pressure experienced by the different age groups might partly explain this. In 2017/2018, ECDC reported higher age-specific notification rates per 100,000 in adolescents compared to school-aged children and adults for all three study-countries [25]. In a Dutch population-based sero-surveillance study performed in 2016-2017, infection prevalence based on serum antibodies was highest among adolescents (11.5%), followed by school aged children and lower among adults [6]. Interestingly, this trend is similar to that of the frequencies of memory B cells found in this study. Another sero-survey performed among European young adults in 2011-2012 confirms these data and showed a low proportion of infected young adults (1.6%) in Finland [26]. Although, the study period of these studies differed, they both show the general trend of naturally circulating *B. pertussis* in these populations. Memory B cell frequencies among adolescents were slightly higher (but not significant) compared to that in children. This is in line with a previously published study among wP primed 3-9-year-olds by Hendrix et al., 2011 [13]. They showed increasing memory B cell frequencies with increasing age, however the difference between 6- and 9-year-olds was not significant which is consistent with the findings in the current study where adolescents and children had similar frequencies of memory B cells in the circulation. In this study, the older adults had significantly lower post-vaccination memory B cell frequencies compared to the other age groups. This contrasts to the previously measured IgG antibody concentrations one month and one year post-vaccination, that were not uniformly less in older adults [10]. However, lower immune responses in older adults have been previously described, and the explanation

for this is probably multifactorial [27, 28]. The first aspect to be considered in this age group is immunosenescence [28]. This phenomenon leads to weaker immune responses after an encounter with a specific stimulus in comparison with other age groups, involving both B and T cells. It is also known to compromise vaccine responses [29]. The B cell recall response is lower in older adults whereas aged memory B cells are less capable of differentiating into plasma cells upon boosting [28, 29]. This might also be applicable for in vitro stimulation of memory B cells to plasmablasts since aged memory B cells might respond less to CpG stimulation [30]. Also, the majority of the older adults comprised of individuals who have not been primarily immunised with a pertussis vaccine. However, these individuals have probably gained their primary protection against pertussis from natural infection and are probably boosted by infection multiple times in their lives [29]. Previously reported antibody concentrations measured in the participants of the current study, reported higher IgA antibody concentrations in the older adults compared to the other age groups [10]. This suggests that part of the protection in older individuals is guided towards IgA instead of IgG [29]. A further consideration for the older adults group is that repeated exposure through carriage or infection to *B. pertussis* involves specific forms of antigen which may differ in their epitope content from those antigens in aP vaccines (or those purified antigens used in assays) and result in a less effective memory response to the aP booster vaccination [31-33].

This study included participants with different vaccination backgrounds as published before by Versteegen et al., 2021 [10]. It is well known that wP vaccines contain a broad repertoire of low dose bacterial components whereas aP vaccines contain a relatively high dose of only a subset of (chemically detoxified) antigens. Immunisation using wP vaccines seems to induce immunity against the pathogen, whereas aP vaccines induce immunity against the vaccine antigens. Therefore, wP primed individuals will probably produce more antibodies when boosted by natural infection where aP primed individuals will probably produce more antibodies when boosted by aP booster vaccines [31]. However, we noticed a trend to higher memory B cell frequencies against the three measured vaccine antigens in wP compared to aP primed adolescents. One possible explanation could be the different intervals since the latest booster vaccine, as the wP primed individuals received their previous booster dose approximately two (the Netherlands) or a half (Finland) years earlier compared to their aP primed peers. In contrast to our findings, higher memory B cell frequencies have been shown in boosted 4 year old aP primed compared to wP primed individuals [34]. Interestingly, a Dutch study performed among children 9 years of age, showed that after a second aP booster at 9 years of age, wP primed individuals had higher frequencies of memory B cells than aP primed children [11]. Our results provide more support that a second aP booster results in opposite findings compared to a first booster as we observed higher frequencies of circulating memory B cells in wP primed adolescents aged 11-15 years who received their second booster. Some previous studies

have shown that long-term protection against infection after wP priming, even when mixed with aP boosters, is better compared to just aP priming [15, 16, 31, 35]. For our analysis, the small number of participants for this comparison limited the power to detect a significant difference. However, significant differences between aP and wP primed individuals have been reported in a subset of the study participants using high dimensional flow-cytometric analyses showing significantly stronger IgG1+ plasma cell responses in wP primed individuals, which was the most prominent subclass at day 7 [36]. Also stronger Th2 responses were reported in aP primed versus wP primed individuals [37]. Stronger Th2 responses in aP primed individuals have been described often, in young children as well as in adolescents [38]. Therefore, age difference does not seem to explain why aP primed young children have better B cell responses following an aP booster while in adolescence wP primed individuals have higher frequencies. However, it is possible that the amount of aP boosters might be of more influence on this issue. A group of wP primed young adults (20-34 years) was also included in this study. Results from this group indicated a lower frequency of memory B cell frequencies in young adults compared to adolescents, but similar or even higher frequencies compared to the children's group. Most young adults have never received additional pertussis booster vaccinations, which may indicate that wP priming is more efficient than aP priming in long-term immunity [39]. However, it is also possible that these individuals have had their immunity boosted by mild infections from time to time [6, 40]. To strengthen this aspect, a recently published seroprevalence study among adults in childbearing age (20-39 years) showed clear circulation of pertussis in this age group in several European countries [26].



We demonstrated best correlation between memory B cell frequencies and long-term antibody concentrations for Prn. Jahnmatz et al., 2014 compared B cell responses to humoral immunity after a fifth dose of a single component pertussis vaccine (TdAP1, 20 µg PT) versus a five component pertussis vaccine (TdaP5, 2.5 µg PT, 5 µg FHA, 3 µg Prn, and 5 µg Fim2/3). They showed a good correlation between antibody concentrations and memory B cell frequencies to Ptx with the high dose AP1 vaccine. However, with the low dose aP5 vaccine, there was a poor correlation for Ptx, FHA and Prn [41]. The latter finding is quite similar to our findings (excluding Prn), whereas a relatively low dose of a three component pertussis vaccine (TdaP3-IPV, 8 µg PT, 8 µg FHA, and 2.5 µg Prn) was used in this study. This may either suggest that B cell memory is enhanced less efficiently after a multi-component aP booster, or by the lower dose of the antigens included. It has been shown that FHA and Prn are both immunogenic [42, 43]. Studies by Jahnmatz et al., 2014 and Schure et al., 2013 show that antibody concentrations post aP booster are higher for Prn than for FHA in children, whereas in our study groups (all except older adults), these values were close to each other [10, 41, 44]. FHA is known to be cross-reactive with species other than *Bordetella*, such as *C. pneumoniae* and *M. pneumoniae* and is therefore likely to be boosted more often [45]. Prn is only expressed by the *Bordetella* genus and might

have cross-reactive properties between different species of *Bordetella* [46]. However, due to the immune pressure, circulating strains have changed during the years and a significant increase of Prn-negative *Bordetella* isolates was noticed around 2012 in Europe, leading to boost of immunity to other antigens than Prn [47, 48]. This might also explain the best correlation found between vaccination and memory B cell frequencies for Prn since Prn is least affected by infection and therefore most directly affected by vaccination [49]. Ptx specific memory B cell frequencies seem to be more elevated after infection than vaccination and remain more elevated even nine months post-infection [49]. This indicates high stimulation of memory B cells to Ptx in vivo during an infection in contrast to vaccination, which might be explained by the altered immunogenicity caused by the chemically modified PT in aP vaccines [31]. Vaccine responses against pertussis toxin might benefit from the inclusion of genetically detoxified Ptx instead of chemically detoxified Ptx in future vaccines [33]. Memory B cell responses against native Ptx (as we measure it) are most interesting to study someone's current immune status against *Bordetella pertussis*, since Ptx is the most pathogenic product of this bacterium. However, Prn is probably most informative to study magnitude and duration of vaccine responses, since this is least affected by infection at present.

When we compared the participants divided into two categories based on the baseline memory B cell frequencies, we noticed significantly higher memory B cell frequencies specific to Ptx, FHA, and Prn in participants with > 1 memory B cell per 100,000 PBMCs at baseline, which can be interpreted as the presence of circulating memory B cells. As expected, antibody concentrations for Ptx and Prn were also significantly higher at all timepoints in individuals with circulating memory B cells at baseline. For FHA differences between the two categories were not significant at day 28 and 1 year which was expected considering the previously described correlation coefficient between memory B cells and antibody concentrations for this antigen. This interesting finding might be considered as a biomarker to assess the persistence of B cell memory following primary or booster pertussis vaccination, capable of producing a robust memory response among school-aged children, adolescents, and young and older adults. Another study, with a slightly different approach, found higher vaccine responses in participants with higher baseline antibody concentrations [50]. Furthermore, similar results with baseline IgG-Ptx antibody concentrations have been observed for Ptx neutralising antibody titres [51].

The strengths of this study are in concurrently assessing B cell (re)activation in three countries in four age groups with identical clinical and laboratory methodologies. All protocols used for measuring memory B cell results were harmonised between the countries. Furthermore, the participants in this study were monitored for up to one year providing longitudinal data on memory B cell responses after an aP booster vaccination. In addition, all plates were measured centrally at the RIVM, which excludes the possibility

of any result-based bias due to multi-site ELISpot measuring approaches. Limitations of this multicentre trial include the small number of adolescents included in the wP versus aP analysis, which may be reflected in the results between these two groups as we observed elevated, but not significantly higher, memory B cell frequencies for the wP primed participants.

To conclude, we showed that memory B cell frequencies are highly elevated after aP boosting and that frequencies were still elevated after one year. Furthermore, country-to-country variations were small indicating that responses to aP are similar regardless of the pertussis epidemiology in different populations. Our results also show that memory B cell frequencies are reduced in the older adults probably due to immunosenescence. Circulating memory B cells seem most pronounced during adolescence, which is probably a consequence of both vaccinations and natural boosting in this age group. Baseline presence of circulating memory B cells was found to predict the outcome of memory B cell responses after a booster dose. From this it can be deduced that, in order to induce a good booster response, it is important to have circulating memory B cells when a subsequent booster is administered. Therefore, circulating memory B cells might be a marker to determine intervals for boosting pertussis immunity.



## DATA AVAILABILITY STATEMENT

Individual participant data that underlie the results reported in this article, have been de-identified and deposited in the central database of the PERISCOPE Consortium and can be accessed by a request to the PERISCOPE management team.

## ETHISCS STATEMENT

This human clinical study was designed and conducted in accordance with the provisions of the Declaration of Helsinki (1996) and the International Conference on Harmonisation Guidelines for Good Clinical Practice. The trial was registered at the EU Clinical Trial database (EudraCT number 2016- 003678-42) and was approved by the Medical Research Ethics Committees United (MEC-U, NL60807.100.17-R17.039) in the Netherlands, the South Central - Hampshire B Research Ethics Committee (REC, 19/SC/0368) in the UK, and the MREC UTU (ETMK Dnro: 129/1800/2017) in Finland. Written informed consent was obtained from all adult participants, and parents or legal guardian of minors, at the start of the study.

## AUTHOR CONTRIBUTIONS

GB, DK and JM were responsible for the conception and design of the study with contribution of QH, A-MBu, ES, PV, A-MBa, MP. PV, A-MBa, MV, AK, SB, LR, JT, KS, HH, RL,

EC, and MlZ-B contributed substantially to the data acquisition. JK was responsible for the data analysis with contribution from A-MBa, PV, AK, and MV. PV, A-MBa, MV, A-MBu, DK, QH contributed substantially to the interpretation of the data. PV, A-MBa, MV wrote the first draft of the manuscript and A-MBu, DK, QH, GB, JM, JK, AK, SB, LR, JT, KS, HH, RL, EC, ES, and M-lZ-B revised it critically for important intellectual content. All authors provided approval for publication of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

## FUNDING

PERISCOPE has received funding from the Innovative Medicines Initiative 2 Joint Undertaking under grant agreement No 115910. This Joint Undertaking receives support from the European Union's Horizon 2020 research and innovation programme and EFPIA and BMGF.

## ACKNOWLEDGEMENTS

We are grateful to Natasha Kaagman from the RIVM, for Amy Beveridge from the University of Oxford, and for Elina Tenhu, Elisa Knuutila, and Tuula Rantasalo from the University of Turku for their support in the assay performance. We also want to thank our collaborators from the Radboud Institute for Molecular Life Sciences for their help in sample processing. Dominic F Kelly receives salary support from the Oxford NIHR Biomedical Research Centre.

## AUTHOR DISCLAIMER

Results reflect the authors' view, the Innovative Medicines Initiative 2 Joint Undertaking is not responsible for any use that may be made of the information it contains.

## CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## PUBLISHER'S NOTE

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

## REFERENCES


1. Cherry, J.D., Pertussis in Young Infants Throughout the World. Clin Infect Dis, 2016. 63(suppl 4): p. S119-S122. doi: 10.1093/cid/ciw550.
2. Kandeil, W., et al., The burden of pertussis in older adults: what is the role of vaccination? A systematic literature review. Expert Rev Vaccines, 2019. 18(5): p. 439-455. doi: 10.1080/14760584.2019.1588727.
3. Safarchi, A., et al., Genomic dissection of Australian Bordetella pertussis isolates from the 2008-2012 epidemic. J Infect, 2016. 72(4): p. 468-477. doi: 10.1016/j.jinf.2016.01.005.
4. Bowden, K.E., et al., Molecular epidemiology of the pertussis epidemic in Washington State in 2012. J Clin Microbiol, 2014. 52(10): p. 3549-3557. doi: 10.1128/jcm.01189-14.
5. Choi, Y.H., et al., Investigating the pertussis resurgence in England and Wales, and options for future control. BMC Med, 2016. 14(1): p. 121. doi: 10.1186/s12916-016-0665-8.
6. Versteegen, P., et al., More than 10 years after introduction of an acellular pertussis vaccine in infancy: a cross-sectional serosurvey of pertussis in the Netherlands. The Lancet Regional Health – Europe, 2021. 10: p. 100196.
7. Tan, T., E. Trindade, and D. Skowronski, Epidemiology of pertussis. Pediatr Infect Dis J, 2005. 24(5 Suppl): p. S10-S18. doi: 10.1097/01.inf.0000160708.43944.99.
8. Nieves, D.J. and U. Heininger, Bordetella pertussis. Microbiol Spectr, 2016. 4(3): p. EI10-0008-2015. doi: 10.1128/microbiolspec.EI10-0008-2015.
9. Cherry, J.D., Pertussis: challenges today and for the future. PLoS Pathog, 2013. 9(7): p. e1003418. doi: 10.1371/journal.ppat.1003418.
10. Versteegen, P., et al., Responses to an acellular pertussis booster vaccination in children, adolescents, and young and older adults: A collaborative study in Finland, the Netherlands, and the United Kingdom. EBioMedicine, 2021. 65: p. 103247.
11. van der Lee, S., et al., Whole-Cell or Acellular Pertussis Primary Immunizations in Infancy Determines Adolescent Cellular Immune Profiles. Front Immunol, 2018. 9: p. 51. doi: 10.3389/fimmu.2018.00051.
12. Kurosaki, T., K. Kometani, and W. Ise, Memory B cells. Nature Reviews Immunology, 2015. 15(3): p. 149-159. doi: 10.1038/nri3802.
13. Hendrikx, L.H., et al., Identifying long-term memory B-cells in vaccinated children despite waning antibody levels specific for Bordetella pertussis proteins. Vaccine, 2011. 29(7): p. 1431-1437. doi: 10.1016/j.vaccine.2010.12.033.
14. Buisman, A.M., et al., Long-term presence of memory B-cells specific for different vaccine components. Vaccine, 2009. 28(1): p. 179-186. doi: 10.1016/j.vaccine.2009.09.102.
15. Sheridan, S.L., et al., Waning vaccine immunity in teenagers primed with whole cell and acellular pertussis vaccine: recent epidemiology. Expert Rev Vaccines, 2014. 13(9): p. 1081-1106. doi: 10.1586/14760584.2014.944167.
16. Sheridan, S.L., et al., Number and order of whole cell pertussis vaccines in infancy and disease protection. Jama, 2012. 308(5): p. 454-456. doi: 10.1001/jama.2012.6364.
17. Jahnmatz, M., et al., Optimization of a human IgG B-cell ELISpot assay for the analysis of vaccine-induced B-cell responses. J Immunol Methods, 2013. 391(1-2): p. 50-59. doi: 10.1016/j.jim.2013.02.009.

**18.** Guo, B. and Y. Yuan, A comparative review of methods for comparing means using partially paired data. Stat Methods Med Res, 2017. 26(3): p. 1323-1340. doi: 10.1177/0962280215577111.

**19.** Giesbrecht, F.G. and J.C. Burns, Two-Stage Analysis Based on a Mixed Model: Large-Sample Asymptotic Theory and Small-Sample Simulation Results. Biometrics, 1985. 41(2): p. 477-486. doi: 10.2307/2530872.

**20.** Hrong-Tai Fai, A. and P.L. Cornelius, Approximate F-tests of multiple degree of freedom hypotheses in generalized least squares analyses of unbalanced split-plot experiments. Journal of Statistical Computation and Simulation, 1996. 54(4): p. 363-378. doi: 10.1080/00949659608811740.

**21.** Benjamini, Y. and Y. Hochberg, Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. Journal of the Royal Statistical Society. Series B (Methodological), 1995. 57(1): p. 289-300.

**22.** R Core Team, R: A language and environment for statistical computing. R Foundation for Statistical Computing. 2021: Vienna, Austria.

**23.** Bates, D., et al., Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software; Vol 1, Issue 1 (2015), 2015: p. 1-48. doi: 10.18637/jss.v067.i01.

**24.** Lenth, R.V., Emmeans: estimated marginal means, aka least-squares means. R package version 1.6.2-1. 2021.

**25.** ECDC. Disease data from ECDC Surveillance Atlas for pertussis. 2019 [cited 2019 20 September]; Available from: http://ecdc.europa.eu/en/pertussis/surveillance-and-disease-data/atlas].

**26.** Wehlin, L., et al., Pertussis seroprevalence among adults of reproductive age (20-39 years) in fourteen European countries. Apmis, 2021. 129(9): p. 556-565. doi: 10.1111/apm.13165.

**27.** Pool, V., et al., Humoral immunity 10years after booster immunization with an adolescent and adult formulation combined tetanus, diphtheria, and 5-component acellular pertussis vaccine in the USA. Vaccine, 2018. 36(17): p. 2282-2287. doi: 10.1016/j.vaccine.2018.03.029.

**28.** Gustafson, C.E., et al., Influence of immune aging on vaccine responses. J Allergy Clin Immunol, 2020. 145(5): p. 1309-1321. doi: 10.1016/j.jaci.2020.03.017.

**29.** Crooke, S.N., et al., Immunosenescence and human vaccine immune responses. Immun Ageing, 2019. 16: p. 25. doi: 10.1186/s12979-019-0164-9.

**30.** Frasca, D., et al., Unique biomarkers for B-cell function predict the serum response to pandemic H1N1 influenza vaccine. Int Immunol, 2012. 24(3): p. 175-182. doi: 10.1093/intimm/dxr123.

**31.** Eberhardt, C.S. and C.A. Siegrist, What Is Wrong with Pertussis Vaccine Immunity? Inducing and Recalling Vaccine-Specific Immunity. Cold Spring Harb Perspect Biol, 2017. 9(12): p. a029629. doi: 10.1101/cshperspect.a029629.

**32.** Knuutila, A., et al., Differences in epitope-specific antibodies to pertussis toxin after infection and acellular vaccinations. Clin Transl Immunology, 2020. 9(8): p. e1161. doi: 10.1002/cti2.1161.

**33.** Sutherland, J.N., et al., Antibodies recognizing protective pertussis toxin epitopes are preferentially elicited by natural infection versus acellular immunization. Clin Vaccine Immunol, 2011. 18(6): p. 954-962. doi: 10.1128/cvi.00561-10.

**34.** Hendrikx, L.H., et al., Impact of infant and preschool pertussis vaccinations on memory B-cell responses in children at 4 years of age. Vaccine, 2011. 29(34): p. 5725-5730. doi: 10.1016/j.vaccine.2011.05.094.

**35.** Sheridan, S.L., et al., Unexpectedly limited durability of immunity following acellular pertussis vaccination in preadolescents in a North American outbreak. Clin Infect Dis, 2012. 55(10): p. 1434-1435; author reply 1435-6. doi: 10.1093/cid/cis672.

**36.** Diks, A.M., et al., Age and Primary Vaccination Background Influence the Plasma Cell Response to Pertussis Booster Vaccination. Vaccines, 2022. 10(2): p. 136.

**37.** Lambert, E.E., et al., Uncovering Distinct Primary Vaccination-Dependent Profiles in Human Bordetella Pertussis Specific CD4+ T-Cell Responses Using a Novel Whole Blood Assay. Vaccines, 2020. 8(2): p. 225.

**38.** Saso, A., B. Kampmann, and S. Roetynck, Vaccine-Induced Cellular Immunity against Bordetella pertussis: Harnessing Lessons from Animal and Human Studies to Improve Design and Testing of Novel Pertussis Vaccines. Vaccines (Basel), 2021. 9(8): p. 877. doi: 10.3390/vaccines9080877.

**39.** Klein, N.P., et al., Comparative effectiveness of acellular versus whole-cell pertussis vaccines in teenagers. Pediatrics, 2013. 131(6): p. e1716-e1722. doi: 10.1542/peds.2012-3836.

**40.** van der Lee, S., et al., Enhanced Bordetella pertussis acquisition rate in adolescents during the 2012 epidemic in the Netherlands and evidence for prolonged antibody persistence after infection. Euro Surveillance, 2017. 22(47): p. 17-00011. doi: 10.2807/1560-7917.ES.2017.22.47.17-00011.

**41.** Jahnmatz, M., et al., Pertussis-specific memory B-cell and humoral IgG responses in adolescents after a fifth consecutive dose of acellular pertussis vaccine. Clin Vaccine Immunol, 2014. 21(9): p. 1301-1308. doi: 10.1128/cvi.00280-14.

**42.** Hellwig, S.M., et al., Crucial role of antibodies to pertactin in Bordetella pertussis immunity. J Infect Dis, 2003. 188(5): p. 738-742. doi: 10.1086/377283.

**43.** Knight, J.B., et al., Immunogenicity and protective efficacy of a recombinant filamentous haemagglutinin from Bordetella pertussis. Clin Exp Immunol, 2006. 144(3): p. 543-551. doi: 10.1111/j.1365-2249.2006.03097.x.

**44.** Schure, R.M., et al., Differential T- and B-cell responses to pertussis in acellular vaccine-primed versus whole-cell vaccine-primed children 2 years after preschool acellular booster vaccination. Clin Vaccine Immunol, 2013. 20(9): p. 1388-1395. doi: 10.1128/cvi.00270-13.

**45.** Vincent, J.M., et al., Prolonged afebrile nonproductive cough illnesses in American soldiers in Korea: a serological search for causation. Clin Infect Dis, 2000. 30(3): p. 534-539. doi: 10.1086/313707.

**46.** He, Q., et al., Protective role of immunoglobulin G antibodies to filamentous hemagglutinin and pertactin of Bordetella pertussis in Bordetella parapertussis infection. Eur J Clin Microbiol Infect Dis, 1996. 15(10): p. 793-798. doi: 10.1007/bf01701521.

**47.** Barkoff, A.M., et al., Pertactin-deficient Bordetella pertussis isolates: evidence of increased circulation in Europe, 1998 to 2015. Euro Surveill, 2019. 24(7): p. 1700832. doi: 10.2807/1560-7917.Es.2019.24.7.1700832.

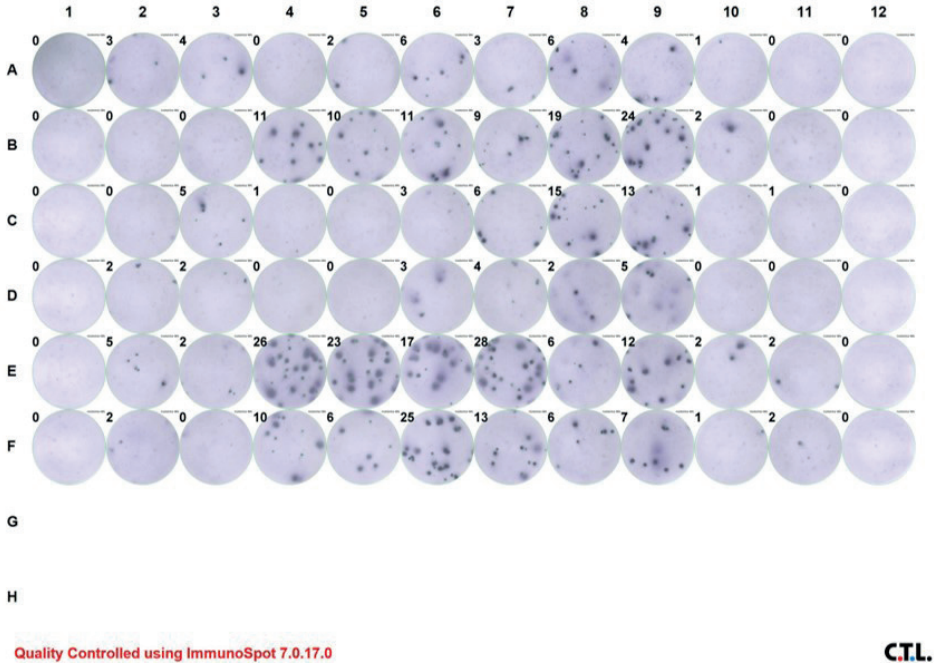
**48.** Hegerle, N., et al., Evolution of French Bordetella pertussis and Bordetella parapertussis isolates: increase of Bordetellae not expressing pertactin. Clin Microbiol Infect, 2012. 18(9): p. E340-E346. doi: 10.1111/j.1469-0691.2012.03925.x.

**49.** van Twillert, I., et al., Age related differences in dynamics of specific memory B cell populations after clinical pertussis infection. PLoS One, 2014. 9(1): p. e85227. doi: 10.1371/journal.pone.0085227.

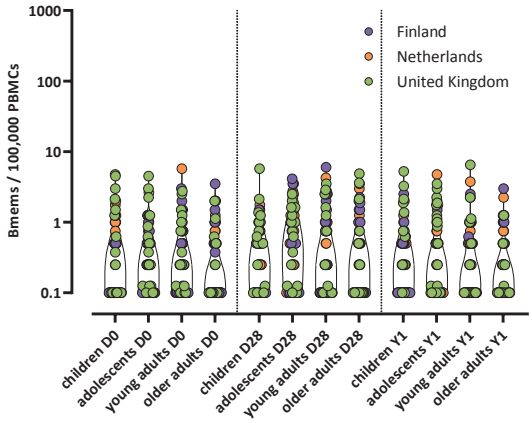
**50.** Tran Minh, N.N., et al., Acellular vaccines containing reduced quantities of pertussis antigens as a booster in adolescents. Pediatrics, 1999. 104(6): p. e70. doi: 10.1542/peds.104.6.e70.

**51.** Knuutila, A., et al., Pertussis toxin neutralizing antibody response after an acellular booster vaccination in Dutch and Finnish participants of different age groups. Emerging Microbes & Infections, 2022. 11: p. 956-963.

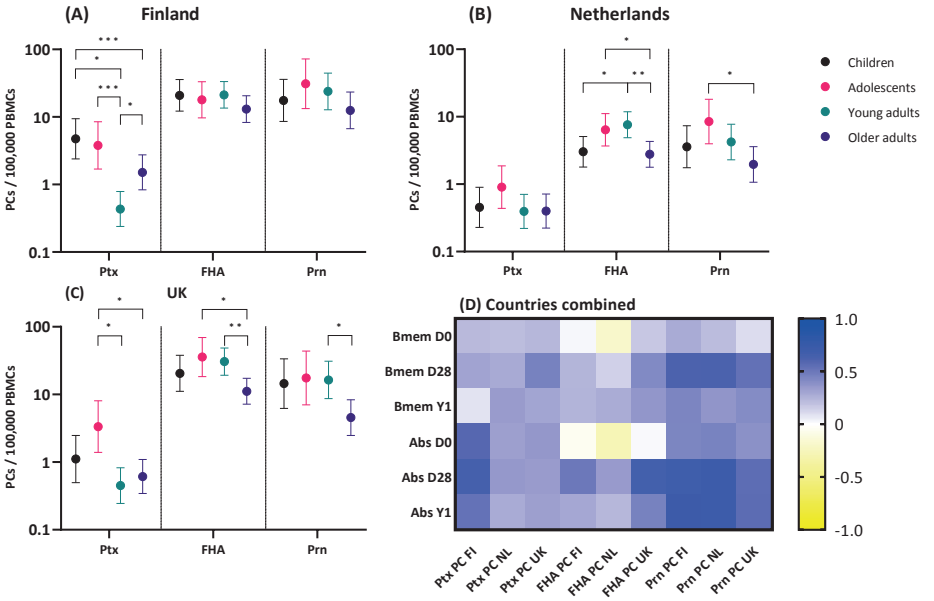
## SUPPLEMENTARY MATERIAL




**Supplementary Figure 1.** Scan of a representative ELISpot plate. Column 1: PBS (Blanc), Column 2&3:Pertussis toxin; column 4&5 pertactin; column 6&7: filamentous haemagglutinin; column 8&9: tetanus toxin; column 10&11: fimbriae types 2 and 3; column 12: PBS (blanc). Row A: participant X at day 0 (baseline); Row B: participant X at day 28 post-vaccination; Row C: participant X at 1 year post-vaccination; Row D: participant XX at day 0 (baseline); Row E: participant XX at day 28 post-vaccination; Row F: participant XX at 1 year post-vaccination.




**Supplementary Figure 2.** Individual spot frequencies for fimbriae types 2 and 3. Memory B cell spot frequencies specific for fimbriae types 2 and 3 in four age groups. Fimbriae types 2 and 3 are pertussis antigens but are not present in the current study vaccine. Spot frequencies have not been included in the linear mixed model because the frequencies were low in all age groups and at all timepoints.




**Supplementary Figure 3.** Plasma cell data. PBMCs collected at day 7 (± 1day) were processed and used for an ex-vivo ELISpot assay within 24 hours. Geometric means with 95% confidence intervals were calculated using a linear model fitted to the log-transformed spot frequencies for each spot number and antigen combination. Age group and country were included in the model as a two-way interaction. Number of participants per country varies from 73 to 84. Frequencies between countries differed and are therefore presented separately. Ptx: pertussis toxin; FHA: filamentous haemagglutinin; Prn: pertactin; * $p \leq 0{\cdot}05$; ** $p \leq 0{\cdot}01$; *** $p \leq 0{\cdot}001$. We present plasma cell frequencies for participants from A. Finland; B. the Netherlands; and C. the United Kingdom. Between the age groups similar pattern of frequencies could be detected in all three countries for the Prn-specific plasma cell numbers. Differences in the magnitude of plasma cell frequencies between the countries, might be due to variety in time of both sampling and storage of the whole blood. PBMC isolation was usually performed within 4-6 hours after blood draw in Finland and the UK, but due to logistics this took up to 24 hours in the Netherlands. D. Correlation heatmap based on Pearsons' correlation on the log10 transformed values of plasma cell frequencies versus their corresponding memory B cell frequencies and corresponding antibody concentrations per country. FI: Finland; NL: Netherlands; UK: United Kingdom; PC: plasma cell frequencies; Bmem: memory B cell frequencies; Abs: antibody concentrations; D0: day 0 (baseline); D28: day 28 post-vaccination; Y1: 1 year post-vaccination; and D7: day 7 post-vaccination. Prn specific plasma cell frequencies per country at day 7 correlated best to antibody concentrations and memory B cell frequencies.

**Supplemental Table 1.** Antigen specific geometric mean spot frequencies per age group and country.

| Antigen | Timepoint | Country | Children | Adolescents | Young adults | Older adults |
|---|---|---|---|---|---|---|
| Ptx GM spot frequencies (CI) in spots/100,000 PBMCs | Day 0 | FI | 0.3 (0.2-0.6) | 0.8 (0.4-1.9) | 0.2 (0.1-0.4) | 0.3 (0.2-0.5) |
| | | NL | 0.7 (0.4-1.5) | 0.8 (0.5-1.5) | 0.7 (0.3-1.4) | 0.7 (0.4-1.5) |
| | | UK | 0.7 (0.3-1.4) | 0.9 (0.5-1.7) | 0.4 (0.2-0.7) | 0.3 (0.1-0.5) |
| | Day 28 | FI | 2.8 (1.4-5.7) | 5.4 (2.4-12) | 1.0 (0.6-1.9) | 1.2 (0.7-2.3) |
| | | NL | 4.7 (2.3-9.5) | 4.2 (2.4-7.4) | 4.6 (2.2-9.3) | 2.8 (1.3-5.7) |
| | | UK | 2.9 (1.4-6.0) | 5.1 (2.7-9.6) | 3.1 (1.7-5.8) | 2.2 (1.2-4.1) |
| | 1 year | FI | 0.4 (0.2-0.9) | 0.8 (0.3-1.8) | 0.7 (0.4-1.2) | 0.3 (0.2-0.6) |
| | | NL | 1.0 (0.5-1.9) | 1.8 (1.0-3.1) | 1.7 (0.8-3.6) | 1.4 (0.7-2.8) |
| | | UK | 1.4 (0.7-2.9) | 2.1 (1.1-3.8) | 1.0 (0.5-1.9) | 1.1 (0.6-2.0) |
| FHA GM spot frequencies (CI) in spots/100,000 PBMCs | Day 0 | FI | 1.5 (0.9-2.7) | 3.9 (2.0-7.7) | 0.5 (0.3-0.8) | 0.3 (0.2-0.5) |
| | | NL | 2.7 (1.5-4.8) | 1.6 (1.0-2.6) | 1.0 (0.6-1.9) | 1.7 (0.9-3.1) |
| | | UK | 2.4 (1.3-4.3) | 3.8 (2.3-6.4) | 0.8 (0.5-1.4) | 0.8 (0.5-1.3) |
| | Day 28 | FI | 18 (9.9-31) | 18 (9.2-36) | 12 (7.2-19) | 3.5 (2.1-5.7) |
| | | NL | 15 (8.5-28) | 18 (11-29) | 22 (12-39) | 16 (8.6-28) |
| | | UK | 21 (12-38) | 28 (16-46) | 26 (15-43) | 12 (7.3-21) |
| | 1 year | FI | 3.3 (1.8-5.8) | 9.9 (5.0-20) | 3.8 (2.3-6.2) | 1.7 (1.0-2.7) |
| | | NL | 3.7 (2.1-6.6) | 5.2 (3.3-8.4) | 3.1 (1.7-5.8) | 4.2 (2.3-7.5) |
| | | UK | 5.9 (3.2-11) | 5.9 (3.6-9.8) | 4.2 (2.4-7.1) | 2.0 (1.2-3.3) |
| Prn GM spot frequencies (CI) in spots/100,000 PBMCs | Day 0 | FI | 0.4 (0.2-0.7) | 0.9 (0.4-2.0) | 0.3 (0.2-0.5) | 0.2 (0.1-0.4) |
| | | NL | 0.8 (0.4-1.6) | 0.5 (0.3-0.9) | 0.5 (0.2-1.0) | 0.4 (0.2-0.8) |
| | | UK | 0.8 (0.4-1.6) | 0.9 (0.5-1.7) | 0.3 (0.2-0.6) | 0.4 (0.2-0.8) |
| | Day 28 | FI | 8.6 (4.4-17) | 23 (11-51) | 12 (7-21) | 1.7 (1.0-3.0) |
| | | NL | 8.0 (4.1-16) | 9.5 (5.5-16) | 4.5 (2.3-9.0) | 4.1 (2.1-8.2) |
| | | UK | 7.7 (3.9-15) | 8.9 (4.9-16) | 15 (8.1-27) | 3.9 (2.1-7.1) |
| | 1 year | FI | 2.0 (1.0-4.0) | 4.8 (2.2-11) | 2.0 (1.1-3.5) | 0.6 (0.3-1.1) |
| | | NL | 1.5 (0.8-2.9) | 1.9 (1.1-3.3) | 1.2 (0.6-2.4) | 0.9 (0.5-1.8) |
| | | UK | 1.8 (0.9-3.6) | 2.3 (1.2-4.1) | 2.6 (1.4-4.9) | 1.0 (0.5-1.8) |

Geometric mean memory B cell spot frequencies with 95% confidence intervals from four age groups per country were calculated using a linear mixed model on the log10 transformed data taking the longitudinal structure into account. Ptx: pertussis toxin; FHA: filamentous haemagglutinin; Prn: pertactin; CI: 95% confidence interval.

# Chapter 6

# Age and primary vaccination background influence the plasma cell response to pertussis booster vaccination

A.M. Diks, P. Versteegen, C. Teodosio, R. J. Groenland, B. de Mooij, A. M. Buisman, A. Torres-Valle, M. Pérez-Andrés, A. Orfao, G. A. M. Berbers, J. J. M. van Dongen, M. A. Berkowska, and on behalf of the IMI-2 PERISCOPE Consortium.

Vaccines 10, no. 2 (Jan 18 2022): 136.

## ABSTRACT

Pertussis is a vaccine-preventable disease caused by the bacterium Bordetella pertussis. Over the past years, the incidence and mortality of pertussis increased significantly. A possible cause is the switch from whole-cell to acellular pertussis vaccines, although other factors may also contribute. Here, we applied high-dimensional flow cytometry to investigate changes in B cells in individuals of different ages and distinct priming backgrounds upon administration of an acellular pertussis booster vaccine. Participants were divided over four age cohorts. We compared longitudinal kinetics within each cohort and between the different cohorts. Changes in the B-cell compartment were correlated to numbers of vaccine-specific B- and plasma cells and serum Ig levels. Expansion and maturation of plasma cells 7 days postvaccination was the most prominent cellular change in all age groups and was most pronounced for more mature IgG1+ plasma cells. Plasma cell responses were stronger in individuals primed with whole-cell vaccine than in individuals primed with acellular vaccine. Moreover, IgG1+ and IgA1+ plasma cell expansion correlated with FHA-, Prn-, or PT- specific serum IgG or IgA levels. Our study indicates plasma cells as a potential early cellular marker of an immune response and contributes to understanding differences in immune responses between age groups and primary vaccination backgrounds.

## INTRODUCTION

Pertussis is a vaccine-preventable respiratory disease caused by the bacterium *Bordetella pertussis* (Bp). Since the introduction of the first pertussis vaccines in the 1940s and 1950s containing whole inactivated bacteria (whole-cell pertussis; wP), the incidence and mortality of pertussis have dramatically decreased [1]. However, the wP vaccine itself has a relatively high reactogenicity profile [2, 3]. Therefore, from the early 1980s onwards, many (developed) countries started to replace the wP vaccine by an acellular pertussis (aP) vaccine, which had a more favorable reactogenicity profile [3, 4]. In The Netherlands, this change took place on 1 January 2005. The aP vaccines contain purified Bp components, such as pertussis toxoid (PT), filamentous hemagglutinin (FHA), pertactin (Prn), and fimbriae 2 and 3 (Fim2/3). In The Netherlands, the combined DTaP-IPV-Hib-HepB vaccine (providing protection against diphtheria, tetanus, pertussis, polio, Haemophilus influenzae type b (Hib), and hepatitis B) is used for primary vaccination. The booster vaccinations given at a later age are often Tdap vaccines [5].

Immune surveillance data have shown that despite high vaccination coverage in many countries, there has been an increase in pertussis cases in the past decennia [6, 7]. This increase is not only seen in aP-using countries but was also reported in countries that primarily used wP vaccines at the time of investigation [6, 8]. Several explanations for this increase have been proposed. First of all, improved awareness, surveillance, and diagnostics may increase the detection rate [9]. Furthermore, several new Bp strains have been described. These strains lack antigens present in the aP vaccine (such as FHA- or Prn-deficient strains), or PtxP3 strains that have adapted to suppress host immunity by producing higher levels of PT [10-13]. Lastly, there may be increased carriership within the population as well as faster waning of protective immunity in aP-primed individuals. Initial studies comparing the efficacy of aP vs. wP vaccines showed a similar short-term protection [14, 15]. However, later long-term studies showed that protection lasted shorter when using aP vaccines [16-19]. Further, baboon models have shown that aP-induced immunity does not prevent transmission, immunity induced by wP vaccines leads to a faster clearance of bacteria, and immunity generated by infection prevents colonization [20]. These combined data point at the need for mucosal immunity to prevent or reduce colonization and carriership.

An improved vaccine, immunization program, and/or route of administration seem necessary to combat pertussis. This implies a need to first understand the mechanism underlying protection induced by aP and wP vaccines (their differences and similarities). So far, no true correlate of protection (neither serological nor cellular) has been established for pertussis, and this would greatly aid evaluation of newly developed vaccines. This is one of the pillars of the Innovative Medicines Initiative (IMI)-2 PERISCOPE Consortium (PERtussIS COrrelates of Protection Europe), which aims to increase the scientific

understanding of pertussis-related immunity in humans, identify new biomarkers of protection, and generate technology and infrastructure for the future development of improved pertussis vaccines [21].

Several (recent) studies within and outside the IMI-2 PERISCOPE Consortium have shown that initial priming against pertussis (aP or wP vaccine) influences protection against disease as well as the immune response to (future) booster vaccinations [16, 18, 22-25]. For example, Hendrikx et al. found that in aP-primed children, antigen (Ag)-specific IgG4 serum levels were higher compared with those in wP-primed children [23]. Furthermore, Da Silva et al. showed that, even after receiving aP booster vaccinations, initial priming (wP or aP vaccine) determined the Ag-specific CD4 T-cell response [24]. Similarly, Lambert et al. showed that CD4 T cells isolated from recently aP-boosted individuals could be separated in a principal component analysis (PCA) view based on priming background. Here, an aP priming background resulted in a more Th2-related response compared to a wP priming background [22].

Neither vaccine-induced nor infection-induced immunity leads to lifelong protection against pertussis. Thus, the use of booster vaccinations later in life is a topic relevant for public health, as people with waned immunity can become carriers and, thus, a source of transmission. Moreover, older adults can be more vulnerable to severe disease outcomes [26]. Several studies have shown that aP boosters are effective and well-tolerated in (older) adults [27, 28]. Recently, Versteegen et al. investigated the specific serological response to an aP booster vaccination in four cohorts of different ages and primary vaccination backgrounds in The Netherlands, Finland, and the UK (IMI-2 PERISCOPE study acronym: BERT, Booster pertussis vaccination study) [29]. Here, they found that all age cohorts showed a good response upon booster vaccination, with only limited differences between the different age cohorts for the Bp-specific IgG levels. However, Ag-specific serum IgA (both pre- and postvaccination) increased with age, likely caused by (mild) exposures to Bp over time.

Previous studies on influenza have shown that up to 80% of the circulating IgG plasma cells 7 days after vaccination can be vaccine-specific [30, 31]. This, combined with the low numbers of circulating plasma cells at baseline (median counts <5 cells / µl [32], implies that the plasma cell system is a relatively 'clean' system to monitor. Thus, flow cytometry may serve as a faster and less laborious approach to study vaccination-elicited plasma cells than typical Ag-specific approaches such as Enzyme-Linked Immunospot (ELISpot). Recently, our team used high-dimensional flow cytometry to investigate overtime cellular kinetics in 10 healthy (wP-primed) adults upon aP vaccination. We were able to demonstrate a clear expansion and maturation of plasma cells (especially IgG1+), and a strong correlation between IgG1+ memory B-cell expansion and the magnitude of the Ag-specific IgG serum response [33]. Here, we extended our exploratory study by the analysis of participants of

different ages and different priming backgrounds after receiving an aP booster (Boostrix-IPV, GlaxoSmithKline (GSK), Wavre, Belgium). We included 48 individuals enrolled in the Dutch cohort used in the IMI-2 PERISCOPE–BERT study (periscope-project.eu/patients/study-2-bert/ accessed on 11 January 2022) at predefined time points, with the primary objective of describing the kinetics of circulating B-cell populations in four cohorts of different ages and with different priming backgrounds.

## MATERIALS AND METHODS

### Study design and sample collection

This study comprises one of the exploratory objectives of the Dutch 'BERT study', which was initiated by the IMI-2 PERISCOPE Consortium. It was approved by Medical Research Ethics Committees United (MEC-U, NL60807.100.17-R17.039) and registered at the EU Clinical trial registry (EudraCT number 2016-003678-42). To be eligible for this study, participants had to (1) be generally healthy; (2) have no recent evidence of serious disease— i.e., requiring the use of immunosuppressive or immunomodulating medication—within the 3 months prior to inclusion; (3) received all regular vaccines according to Dutch National Immunization Program (www.rivm.nl/en/national-immunisation-programme, accessed on 18 November 2020) as appropriate for their age. An extensive description of the cohort and a complete overview of all inclusion and exclusion criteria was published recently by Versteegen and colleagues [29]. For convenience, an overview of inclusion and exclusion criteria specific for this study is provided in Supplementary Materials, **Supplementary Table 1**. A fraction of the participants of this aP vaccination study was subjected to additional exploratory analysis, such as mass cytometry, evaluation of mucosal antibodies, NGS, or in-depth flow cytometry. The exploratory substudy monitoring the fluctuations in circulating B-cell subsets at baseline and days 7, 14, and 28 after vaccination is discussed in this manuscript. Here, 48 individuals were selected from four cohorts of different ages and distinct priming backgrounds at infancy: children, 7–10 y/o, aP-primed; adolescents, 11–15 y/o, aP- or wP-primed (aiming for equal distribution of priming background); young adults, 20–34 y/o, wP-primed; older adults 60–70 y/o, in whom vaccination history was unknown (presumably, wP-primed or not vaccinated). Participants were selected from the study cohort of the overarching 'BERT study'. For each cohort, the first 12 participants to be included for the BERT study were also included in this flow cytometric study. Dropouts were replaced by individuals that were included but had not yet started the BERT study. As we aimed for an equal distribution of priming background in the adolescent cohort, inclusion of participants for this cohort was guided by priming background and order of inclusion in the BERT cohort.

The study was conducted by the Spaarne Academy (Spaarne Hospital, Hoofddorp, The Netherlands). Written informed consent was obtained at the start of the study. Participants

were vaccinated intramuscularly with the Boostrix-IPV vaccine after their first blood donation (baseline). Boostrix-IPV is a reduced-antigen Tdap–IPV booster vaccine, which contains diphtheria toxoid (Diph) (≥2.5 Limit of flocculation (Lf)); tetanus toxoid (Tet) (≥5 Lf); three Bordetella pertussis proteins, PT (8 μg), FHA (8 μg), Prn (2.5 μg); and inactivated poliovirus (Mahoney strain, 40 D-Antigen units (DU); MEF-1 strain, 8 DU; Saukett strain, 32 DU) and aluminum hydroxide as adjuvant [34]. Peripheral blood samples were collected in blood collection tubes using heparin as anticoagulant and in serum collection tubes at baseline, day 7, and day 28 after vaccination. An additional peripheral blood sample was taken at day 14 in participants aged 20–34 and aged 60–70. Individuals were excluded and replaced by a new participant if a blood sample at day 0 or 28 could not be obtained.

### Evaluation of antigen-specific immunoglobulin levels in serum

Serological analysis was performed in all collected samples. Levels of IgG directed against Tet, PT, FHA, Prn, and Fim2/3, and levels of IgA directed against PT, FHA, Prn, and Fim2/3 were determined by multiplex immunoassay (MIA) at the Dutch National Institute for Public Health and the Environment (RIVM, The Netherlands) [35]. The serum antibody responses raised against Bp-antigens during the PERISCOPE–BERT study have been extensively discussed by Versteegen and colleagues [29].

### Detection of vaccine-specific antibody-producing plasma cells and memory B cells

Analysis of numbers of IgG and IgA producing plasma cells and memory B cells was performed in the majority of the samples included in this study. B cells producing IgG directed against PT, FHA, Prn, and Tet, and B cells producing IgA direct against PT, FHA, and Prn were measured using ELISpot assay at the RIVM. This procedure has been described previously [36]. In short, peripheral blood mononuclear cells (PBMCs) were isolated using a density gradient. For antibody-producing plasma cells, PBMCs at day 7 postvaccination were directly transferred to Ag-coated ELISpot filter plates (duplicates). For the detection of vaccine-specific memory B cells, PBMCs collected at day 0 and day 28 were collected and stored at −135 °C. Thawed PBMCs were stimulated for 5 days using a culture medium containing CpG, IL-2, and IL-10. Next, cells were transferred to Ag-coated plates (duplicates). Numbers of Ag-specific antibody-producing cells—appearing as spots—were measured using an ImmunoSpot S6 Ultra-V analyzer (Cellular Technology Limited, Cleveland, OH). Uncoated wells filled with PBS served as negative control and were used to subtract background signal. Wells with a signal below the limit of quantification were set at 0.1 cell/$10^5$ PBMCs. Cumulative IgG and IgA spot counts for all antigens measured were used for analyses.

**Longitudinal flow cytometric analysis of circulating B-cell subsets**

All peripheral blood samples were subjected to high-throughput flow cytometric immunophenotyping of the B-cell compartment. Here, we used a recently developed BIGHtube: the B-cell and plasma cell tube (BIGH) allows identification of >100 populations of B and plasma cells distinguished based on their maturation stage and expressed Ig subclasses [32, 37] (Antibody panel and phenotypic description of the identified B-cell subsets: **Supplementary Tables 2 and 3**).

Samples were processed according to the bulk lysis protocol using $10 \times 10^6$ cells followed by intracellular staining, as described before [33] (protocols available on www. EuroFlow.org (accessed on 9 October 2017)), with the addition of membrane staining with CD45-AlexaFluor700.

In short, based on the white blood cell count (as determined by an automated hematological analyzer (Sysmex XP-300, Sysmex Europe GmbH, Norderstedt, Germany)), one or multiple tubes were filled with up to 2 mL of blood, after which ammonium chloride was added up to a total volume of 50 mL. After a 15 min incubation at room temperature on a roller bank to lyse non-nucleated red blood cells, cells were washed, counted on a Sysmex XP-300, and pooled to a total of $10 \times 10^6$ cells. Next, cells were stained with an antibody cocktail directed against surface markers for 30 min in the dark with the BIGH panel (**Supplementary Table 2**). This was followed by a cytoplasmic staining for intracellular Igs using the Fix & Perm reagent kit (Nordic MUbio, Susteren, The Netherlands) according to the manufacturer's protocol. Finally, samples were washed and resuspended in PBS for immediate acquisition (or stored for max ~3 h at 4 °C).

For precise enumeration of cell numbers, we used Perfect-Count Microspheres™ (Cytognos) according to the EuroFlow SOP (protocol available on www.EuroFlow.org, accessed on 9 October 2017). In short, exactly 50 µL of well-mixed Perfect-Count Microspheres™ were added to exactly 50 µL of peripheral blood. Then, antibodies directed against CD19, CD3, and CD45 were added and the sample was incubated for 30 min in the dark. Next, 500 µL of NH4Cl was added and after 10 min incubation, samples were ready for immediate acquisition. Using this tube, we could identify and quantify total leukocytes and lymphocytes, B, T, and NK cells in each sample. All samples were acquired at the Flow cytometry Core Facility of LUMC, using a BD FACS LSR Fortessa 4L (BD Biosciences, San Jose, CA, USA) or a BD FACS LSR Fortessa X-20 flow cytometer (BD Biosciences), which were calibrated daily according to EuroFlow guidelines, as previously described [38, 39].

**Data analysis and statistics**

To ensure objective data analysis and minimize operator-induced variability, all data were analyzed using the automated gating and identification (AGI) module of the Infinicyt

software (Infinicyt™ Software v2.0, Cytognos). This AGI module makes use of clustering algorithms and comparison with fully annotated reference flow cytometry (FCS) data files of healthy individuals to assign clusters of events to a population [40]. Importantly, when there was no perfect fit for a cluster of events, this was marked as a 'check' population and the software indicated to which populations this cluster may correspond. These check events were assigned manually according to the proposed gating strategies for the BIGH panel (**Supplementary Table 3**) [32, 37].

For visualization and statistical analysis, the GraphPad Prism 8.1.1 software (GraphPad, San Diego, CA, USA) was used. First, normality of distribution of major cell populations at baseline was evaluated using D'Agostino–Pearson Normality test. As not all major cell populations were normally distributed, a nonparametric approach was applied. To test longitudinal changes within each cohort, the Wilcoxon signed-rank test for paired samples was used. This was corrected for multiple testing by Bonferroni correction (in case of three sampling timepoints, $p < 0.0167$; in case of four sampling timepoints, $p < 0.0083$ was considered significant). To compare differences between the four cohorts at days 0, 7, and 28, the Kruskal–Wallis approach was used, followed by Dunn's test. At day 14, only samples from the two adult cohorts were collected; therefore, the Mann–Whitney test was used instead of Kruskal–Wallis at day 14. This was corrected for multiple testing by Bonferroni correction ($p < 0.0125$ was considered significant). Correlations were determined using Spearman's Ranking Correlation. Correlation coefficients with a $p < 0.05$ were considered significant. Within these significant correlations, correlation coefficients −0.6—were considered weak correlations, whereas correlation coefficients >0.6 or < 0.01 was considered significant). Lastly, in the comparison between aP- and wP-primed individuals (comparison of ratio over baseline at days 7 and 28), we performed Mann–Whitney, followed by Bonferroni correction ($p < 0.025$ was considered significant).

## RESULTS

### Study cohorts

All participants enrolled in the study between October 2017 and March 2018. In total, 12 children (age: 7–10, aP-primed, m/f ratio: 6/6), 12 adolescents (age: 11–15, 7 individuals wP-primed, m/f ratio: 2/5; 5 individuals aP-primed, m/f ratio: 4/1), 12 young adults (age 20–34, wP-primed, m/f ratio: 7/5), and 12 older adults (age 60–70, presumably wP-primed or not vaccinated, m/f ratio: 4/7) completed this study (as part of the PERISCOPE– BERT study). Three children who were initially enrolled dropped out and were replaced by three new participants. From all acquired samples, two baseline B-cell samples were lost due to technical problems (one child and one young adult). Finally, one older adult was excluded due to (potentially) clonal expansion of B cells and replaced by a new participant.

For most participants, the leukocyte, lymphocyte, T-cell, B-cell, and NK-cell counts at baseline were within the normal age-matched range (**Table 1**, **Supplementary Table 4**), or, in case of minor deviations, fell into the normal range at later time points [41, 42]. Leukocytes, lymphocytes, and T cells remained mostly stable over the time of analysis. Although NK-cell numbers showed a minor decrease at day 28, this was most likely not related to the vaccination response (day 0 vs. 28, $p \leq 0.01$; day 7 vs. 28, $p \leq 0.05$, **Supplementary Figure 1**). There were no statistically significant differences in absolute leukocyte, lymphocyte, T-cell, and NK-cell counts at baseline between age cohorts. Thus, regarding the numbers of leukocytes, lymphocytes, B cells, T cells, and NK cells, our participants were healthy representatives of the general population.

### Higher counts of naive B cells and plasma cells in children

B-cell numbers are known to decrease over time from an average of 1400 cells/µL in children < 2 years to 200 cells / µl in adults [32,41]. This trend was also visible in our dataset, where children had more B cells than the adult groups (**Table 1**). This difference was mainly due to high numbers of pre-germinal center (naive) B cells in children. Although memory B-cell numbers were also higher in children than in adults (160 cells/µL in children vs. 85.7 cells/µL in young adults, n.s., and 160 cells/µL in children vs. 63.8 cells/µL in older adults, $p \leq 0.01$), these differences were less prominent, and mainly restricted to IgG1+ and IgG3+ memory B cells. Finally, several plasma cell subsets were significantly more abundant in children than in adults (IgG1+, IgG3+, and IgD+ plasma cells), but due to their overall low frequencies, this did not have a major impact on total B-cell numbers. Limited differences in B-cell subset numbers were observed between adolescents and adults (**Table 1**). These differences were predominantly found in pre-germinal center (naive) B cells and individual plasma cell subsets. Thus, baseline cell numbers of B-cell subsets differed between the cohorts, which was in line with previously published data of age-matched individuals [32].

### Expansion of plasma cells as the most prominent cellular B-cell change after vaccination

We have recently shown that aP booster vaccination in (wP-primed) adults triggers several cellular changes, of which the expansion of (predominantly) IgG1+ plasma cells at day 7 is most prominent [33]. Now, we set out to determine whether the same types of changes occur in vaccinated individuals, irrespective of age and primary vaccination background.

**Table 1. Baseline** distribution of normal B-cell and plasma cell subsets (cells/µL) in each age cohort. Ages of the cohorts of which reference values were used: children 5–9 y/o, adolescents 10–17 y/o, young adults 18–39 y/o, older adults 60–79 y/o. The reference values as indicated in this table were selected from the study performed by Blanco et al. [32]. In the before-mentioned study, reference values for B-cell subsets are provided for all age ranges (from cord blood until older adults >80 y/o). When referring to the reference values indicated here, please refer to the original source data.

| **Cohort** | **Children (ch)** | | | **Adolescents (ad)** | | | **Young Adults (yo)** | | | **Older Adults (ol)** | | | **Statistical Differences Between Cohorts** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Age | 7–10 y/o | | | 11–15 y/o | | | 20–34 y/o | | | 60–70 y/o | | | | | | | |
| Priming background | aP | | | Mixed aP and wP | | | wP | | | No vaccination history available (presumably wP-primed or not vaccinated) | | | No significant differences found within the other combination (yo vs. ol) | | | | |
| N= | 11 † | | | 12 | | | 11 † | | | 12 | | | | | | | |
| | median | min. | max. | median | min. | max. | median | min. | max. | median | min. | max. | ch vs. yo | ch vs. ol | ch vs. ad | ad vs. yo | ad vs. ol |
| total B-cell<br>reference cell counts<br>cell counts | 451<br>496.0 | 157<br>346.0 | 725<br>966.0 | 360<br>430.0 | 174<br>133.0 | 630<br>667.0 | 220<br>279.0 | 41<br>141.0 | 470<br>481.0 | 173<br>244.0 | 36<br>53.5 | 384<br>396.0 | ** | *** | ns | ns | * |
| pre-GC B-cell<br>reference cell counts<br>cell counts | 391.0 | NI<br>173.0 | 731.0 | 326.5 | NI<br>96.3 | 529.0 | 194.0 | NI<br>53.2 | 342.0 | 195.0 | NI<br>31.5 | 273.0 | ** | ** | ns | * | ** |
| immature B-cell<br>reference cell counts<br>cell counts | 41<br>26.6 | 12<br>9.5 | 84<br>70.4 | 37<br>20.8 | 11<br>1.6 | 111<br>43.7 | 5.6<br>5.9 | 0.25<br>1.1 | 24<br>19.8 | 6.1<br>4.7 | 0.69<br>0.6 | 36<br>9.2 | ** | **** | ns | * | ** |
| CD5+ naive B-cell<br>reference cell counts<br>cell counts | 89.7 | NI<br>25.0 | 248.0 | 49.8 | NI<br>5.2 | 166.0 | 30.9 | NI<br>7.8 | 152.0 | 15.3 | NI<br>2.4 | 47.0 | * | *** | ns | ns | * |
| naive B-cell<br>reference cell counts<br>cell counts | 265<br>260.0 | 68<br>124.0 | 505<br>483.0 | 189<br>238.5 | 75<br>89.4 | 401<br>335.0 | 111<br>160.0 | 13<br>44.0 | 288<br>186.0 | 109<br>150.0 | 20<br>24.9 | 280<br>250.0 | ** | * | ns | ns | * |
| memory B-cell<br>reference cell counts<br>cell counts | 123<br>160.0 | 64<br>76.1 | 282<br>252.0 | 68<br>96.2 | 31<br>33.6 | 1<br>60<br>193.0 | 91<br>85.7 | 23<br>31.3 | 221<br>282.0 | 56<br>63.8 | 13<br>21.1 | 128<br>171.0 | ns | ** | ns | ns | ns |
| IgMD+ memory cells<br>reference cell counts<br>cell counts | 54<br>65.7 | 23<br>30.7 | 147<br>129.0 | 29<br>48.8 | 17<br>16.0 | 78<br>141.0 | 38<br>37.4 | 7.9<br>10.0 | 122<br>121.0 | 27<br>20.8 | 7.4<br>8.6 | 72<br>93.2 | * | **** | ns | ns | ns |

| Cohort | Children (ch) | | | Adolescents (ad) | | | Young Adults (yo) | | | Older Adults (ol) | | | Statistical Differences Between Cohorts | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| IgG1+ memory cells | | | | | | | | | | | | | | | | | |
| reference cell counts | 30 | 12 | 86 | 18 | 7 | 42 | 18 | 3.2 | 40 | 9.1 | 1.3 | 22 | * | **** | ns | ns | ** |
| cell counts | 47.8 | 15.2 | 81.7 | 17.3 | 6.8 | 44.7 | 13.2 | 5.4 | 72.5 | 16.5 | 2.8 | 27.8 | | | | | |
| IgG2+ memory cells | | | | | | | | | | | | | | | | | |
| reference cell counts | 4.4 | 0.7 | 15 | 3.0 | 0.7 | 10 | 5.9 | 1.6 | 30 | 3.6 | 1.0 | 11 | * | *** | ns | ns | ns |
| cell counts | 3.8 | 1.6 | 10.3 | 2.8 | 1.4 | 4.4 | 3.1 | 1.1 | 12.3 | 2.8 | 0.6 | 13.4 | | | | | |
| IgG3+ memory cells | | | | | | | | | | | | | | | | | |
| reference cell counts | 7.4 | 2.4 | 16 | 3.0 | 1.1 | 8.3 | 3.0 | 0.5 | 8.4 | 2.3 | 0.4 | 8.1 | * | ** | ns | ns | ns |
| cell counts | 7.9 | 3.2 | 13.0 | 3.1 | 0.6 | 7.1 | 1.8 | 0.8 | 6.4 | 2.1 | 0.5 | 7.4 | | | | | |
| IgG4+ memory cells | | | | | | | | | | | | | | | | | |
| reference cell counts | 0.4 | <0.01 | 2.0 | 0.2 | <0.01 | 2.9 | 0.4 | <0.01 | 2.4 | 0.4 | <0.01 | 2.1 | ns | *** | ns | ns | ns |
| cell counts | 0.2 | <0.05 | 2.2 | 0.3 | <0.05 | 1.0 | 0.5 | 0.1 | 6.1 | 0.3 | 0.1 | 1.2 | | | | | |
| IgA1+ memory cells | | | | | | | | | | | | | | | | | |
| reference cell counts | 12 | 4.5 | 24 | 9.0 | 2.9 | 21 | 11 | 2.1 | 43 | 6.2 | 2.2 | 22 | ns | ns | ns | ns | ns |
| cell counts | 16.3 | 6.4 | 34.7 | 11.4 | 4.4 | 14.7 | 8.0 | 3.8 | 42.4 | 11.2 | 4.2 | 30.4 | | | | | |
| IgA2+ memory cells | | | | | | | | | | | | | | | | | |
| reference cell counts | 3.2 | 1.0 | 13 | 2.7 | 0.8 | 5.9 | 4.1 | 1.2 | 18 | 3.4 | 0.7 | 9.0 | ns | ** | ns | ns | ns |
| cell counts | 4.8 | 1.4 | 10.3 | 2.7 | 0.5 | 6.2 | 3.1 | 0.7 | 17.5 | 2.7 | 0.8 | 9.6 | | | | | |
| IgD+ memory cells | | | | | | | | | | | | | | | | | |
| reference cell counts | 1.1 | <0.01 | 2.9 | 0.3 | <0.01 | 1.7 | 0.2 | <0.01 | 2.4 | 0.01 | <0.01 | 1.2 | * | ** | ns | ns | ns |
| cell counts | 1.0 | 0.3 | 2.1 | 0.4 | <0.05 | 1.4 | 0.2 | <0.05 | 2.7 | 0.2 | <0.05 | 0.8 | | | | | |
| IgH- memory cells | | | | | | | | | | | | | | | | | |
| reference cell counts | | NI | | | NI | | | NI | | | NI | | ns | ns | ns | ns | ns |
| cell counts | 2.2 | 0.6 | 4.4 | 1.5 | 0.4 | 2.7 | 1.2 | 0.5 | 1.8 | 0.9 | 0.3 | 3.0 | | | | | |
| total plasma cells | | | | | | | | | | | | | | | | | |
| reference cell counts | 13 | 3.5 | 45 | 8.5 | 1.3 | 27 | 4.4 | 1.1 | 25 | 1.2 | 0.3 | 7.1 | ns | * | ns | ns | ns |
| cell counts | 8.5 | 3.8 | 12.9 | 3.4 | 1.6 | 13.1 | 2.6 | 1.2 | 8.5 | 1.6 | 0.2 | 6.1 | | | | | |
| IgM+ plasma cells | | | | | | | | | | | | | | | | | |
| reference cell counts | 1.4 | 0.6 | 14 | 0.8 | 0.2 | 5.7 | 0.4 | 0.05 | 4.7 | 0.1 | 0.01 | 0.8 | ns | ns | ns | ns | ns |
| cell counts | 1.3 | 0.3 | 2.9 | 0.6 | 0.4 | 2.0 | 0.4 | 0.1 | 2.8 | 0.1 | <0.05 | 0.8 | | | | | |
| IgG1+ plasma cells | | | | | | | | | | | | | | | | | |
| reference cell counts | 1.9 | 0.1 | 7.7 | 1.1 | 0.1 | 4.8 | 0.4 | 0.05 | 4.4 | 0.1 | 0.01 | 0.6 | ** | ** | ns | ns | ns |
| cell counts | 1.5 | 0.2 | 2.7 | 0.4 | 0.1 | 5.6 | 0.4 | 0.1 | 1.3 | 0.2 | 0.1 | 0.6 | | | | | |

| Cohort | Children (ch) | | | Adolescents (ad) | | | Young Adults (yo) | | | Older Adults (ol) | | | Statistical Differences Between Cohorts | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| IgG2+ plasma cells | | | | | | | | | | | | | | | | | |
| reference cell counts | 0.7 | 0.07 | 2.3 | 0.5 | 0.08 | 0.8 | 0.2 | <0.01 | 2.6 | 0.09 | <0.01 | 1.6 | ns | ns | ns | ns | ns |
| cell counts | 0.8 | 0.2 | 1.1 | 0.3 | 0.1 | 1.5 | 0.2 | <0.05 | 0.6 | 0.1 | <0.05 | 1.2 | | | | | |
| IgG3+ plasma cells | | | | | | | | | | | | | | | | | |
| reference cell counts | 0.2 | <0.01 | 1.3 | 0.08 | <0.01 | 0.4 | 0.03 | <0.01 | 0.3 | <0.01 | <0.01 | 0.2 | * | ** | ns | ns | ns |
| cell counts | 0.2 | <0.05 | 0.5 | <0.05 | <0.05 | 0.9 | <0.05 | <0.05 | 0.1 | <0.05 | <0.05 | 0.1 | | | | | |
| IgG4+ plasma cells | | | | | | | | | | | | | | | | | |
| reference cell counts | 0.02 | <0.01 | 0.2 | <0.01 | <0.01 | 0.2 | <0.01 | <0.01 | 0.4 | <0.01 | <0.01 | 0.1 | ns | ns | ns | ns | ns |
| cell counts | <0.05 | <0.05 | 0.2 | <0.05 | <0.05 | 0.2 | <0.05 | <0.05 | 0.7 | <0.05 | <0.05 | 0.1 | | | | | |
| IgA1+ plasma cells | | | | | | | | | | | | | | | | | |
| reference cell counts | 4.4 | 0.6 | 16 | 3.1 | 0.5 | 14 | 1.7 | 0.3 | 6.9 | 0.4 | 0.04 | 3.3 | ns | ns | ns | ns | ns |
| cell counts | 3.4 | 0.6 | 5.6 | 1.3 | 0.4 | 4.1 | 1.1 | 0.4 | 3.3 | 0.7 | 0.1 | 3.0 | | | | | |
| IgA2+ plasma cells | | | | | | | | | | | | | | | | | |
| reference cell counts | 1.5 | 0.3 | 3.5 | 1.0 | 0.3 | 3.6 | 0.7 | 0.2 | 4.2 | 0.3 | 0.06 | 1.2 | ns | ns | ns | ns | ns |
| cell counts | 1.0 | 0.3 | 2.7 | 2.7 | 0.5 | 6.2 | 0.3 | 0.1 | 1.1 | 0.2 | 0.1 | 1.2 | | | | | |
| IgD+ plasma cells | | | | | | | | | | | | | | | | | |
| reference cell counts | 0.04 | <0.01 | 0.8 | <0.01 | <0.01 | 2.0 | <0.01 | <0.01 | 1.1 | <0.01 | <0.01 | 0.2 | ** | ** | * | ns | ns |
| cell counts | 0.1 | <0.05 | 0.6 | <0.05 | <0.05 | 0.1 | <0.05 | <0.05 | 0.1 | <0.05 | <0.05 | <0.05 | | | | | |

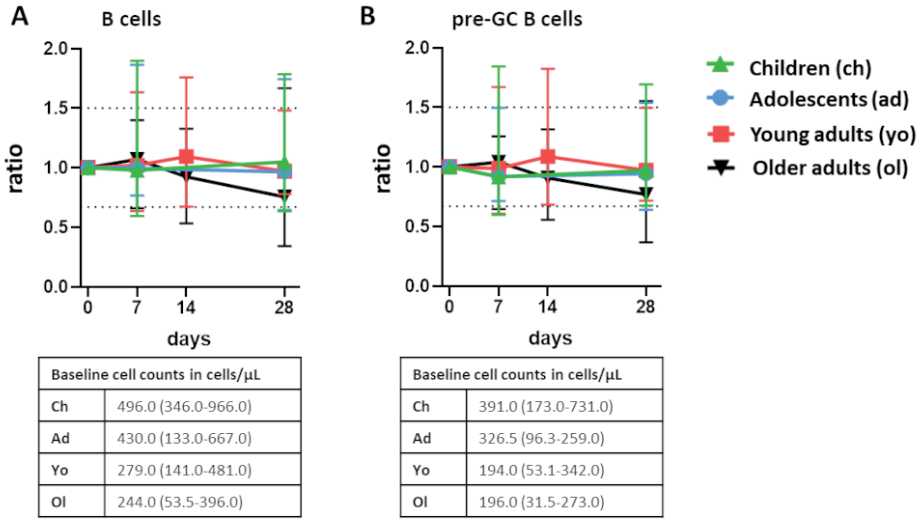
† in children and young adults, for one included donor no baseline B-cell data was available. NI = not indicated. * $p < 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; **** $p \leq 0.0001$.

Total B cells, pre-germinal center B cells, and memory B cells remained relatively stable and did not show any consistent fluctuations over time following vaccination. However, in all participants, plasma cells underwent significant expansion between baseline and day 7 ($p \leq 0.01$ for children, and $p \leq 0.001$ for adolescents and the adult cohorts, **Figure 1**). The magnitude of this expansion was highly similar in children, adolescents, and young adults (ratio to baseline: 2.6–3.4) and was significantly higher in older adults compared with children (ratio: 2.6 in children vs. 5.7 in older adults, $p \leq 0.01$). Total plasma cell numbers returned to baseline at day 14 or, if day 14 was not measured, day 28 ($p \leq 0.001$ for adolescents, young and older adults; in the children cohort, plasma cell counts were still slightly elevated at day 28). Thus, the expansion of plasma cells 7 days postvaccination was the most prominent change in all age groups.

### Despite individual differences, skewing towards IgG1+ plasma cell responses in all cohorts

Cell expansion at day 7 was not equally pronounced in all plasma cell subsets. It was most prominent in IgG1+ plasma cells (ratio to baseline: 5.7–17.6, depending on cohort, $p \leq 0.001$ in all cohorts, with a higher increase in older adults compared with children, $p < 0.05$). This was followed by IgG3+ plasma cells (ratio: 3.6–6.5, $p \leq 0.01$ in children and adolescents, and $p \leq 0.001$ in young and older adults, **Figure 2A**). IgG4+ plasma cells were significantly increased in children and adolescents (ratio: 5.1 and 7.2, respectively, $p \leq 0.01$). IgG2+ plasma cells were significantly increased in adolescents only (ratio: 1.8, $p \leq 0.001$). IgA1+ plasma cells were significantly increased in both adult cohorts (ratio: 2.1 and 3.9 in younger and older adults, $p \leq 0.01$ and $p \leq 0.001$, respectively). Although IgM+ plasma cells seemed to expand at day 7 in older adults, there was a large variation between individuals and the difference was not statistically significant.

Despite individual changes in response patterns between the cohorts, IgG1+ plasma cells were the most expanded subset. They constituted between 43% (young adults) and 61% (adolescents) of all plasma cells at the peak of expansion, while only at most 18% at baseline (**Figure 2B**). Although the ratio (to baseline) of plasma cells was often higher in adults than in children, children had higher baseline IgG1+ plasma cell numbers, and also higher IgG1+ plasma cell numbers at day 7 (median cell count at day 7: 7.64 cells/µL in children vs. 3.81 cells/µL in older adults, ns). Thus, the expansion of IgG1+ plasma cells was the most prominent in all cohorts. Both an increase over baseline (ratio) and in absolute cell numbers were observed.




| Baseline cell counts in cells/µL | |
|---|---|
| Ch | 496.0 (346.0-966.0) |
| Ad | 430.0 (133.0-667.0) |
| Yo | 279.0 (141.0-481.0) |
| Ol | 244.0 (53.5-396.0) |

| Baseline cell counts in cells/µL | |
|---|---|
| Ch | 391.0 (173.0-731.0) |
| Ad | 326.5 (96.3-259.0) |
| Yo | 194.0 (53.1-342.0) |
| Ol | 196.0 (31.5-273.0) |

| Baseline cell counts in cells/µL | |
|---|---|
| Ch | 160.0 (76.1-252.0) |
| Ad | 96.2 (33.6-193.0) |
| Yo | 85.7 (31.3-282.0) |
| Ol | 63.8 (21.1-171.0) |

| Baseline cell counts in cells/µL | |
|---|---|
| Ch | 8.5 (3.8-12.9) |
| Ad | 3.4 (1.6-13.1) |
| Yo | 2.6 (1.2-8.5) |
| Ol | 1.6 (0.2-6.1) |

**Figure 1.** Postvaccination fluctuations of major B-cell subsets. Fluctuations of (**A**) total B cells (**B**) pre-germinal center (pre-GC) B cells, (**C**) memory B cells, and (**D**) plasma cells postvaccination presented as ratio over baseline (median, min-max). For total B cells, pre-GC B cells, and memory B cells, the dashed lines indicate a ratio over baseline of 0.67 and 1.5. For plasma cells, the dashed lines indicate a ratio over baseline of 0.5 and 2.0. Underneath each graph, the baseline cell counts per cohort are presented in cells/µL (median, min-max). To assess longitudinal changes within each cohort, Wilcoxon matched pair signed-rank test followed by Bonferroni correction was used. To test differences between cohorts at one timepoint, Kruskal–Wallis followed by Dunn's test was used, with exception of the comparison at day 14. At day 14, only blood samples from the adult cohorts were collected; here, the Mann–Whitney test followed by Bonferroni correction was used. For longitudinal changes, only significant differences compared with baseline are shown. Significant longitudinal differences within a cohort are indicated as **, $p \leq 0.01$; ***, $p \leq 0.001$. Significant differences between cohorts at the same time point are indicated as ##, $p \leq 0.01$.

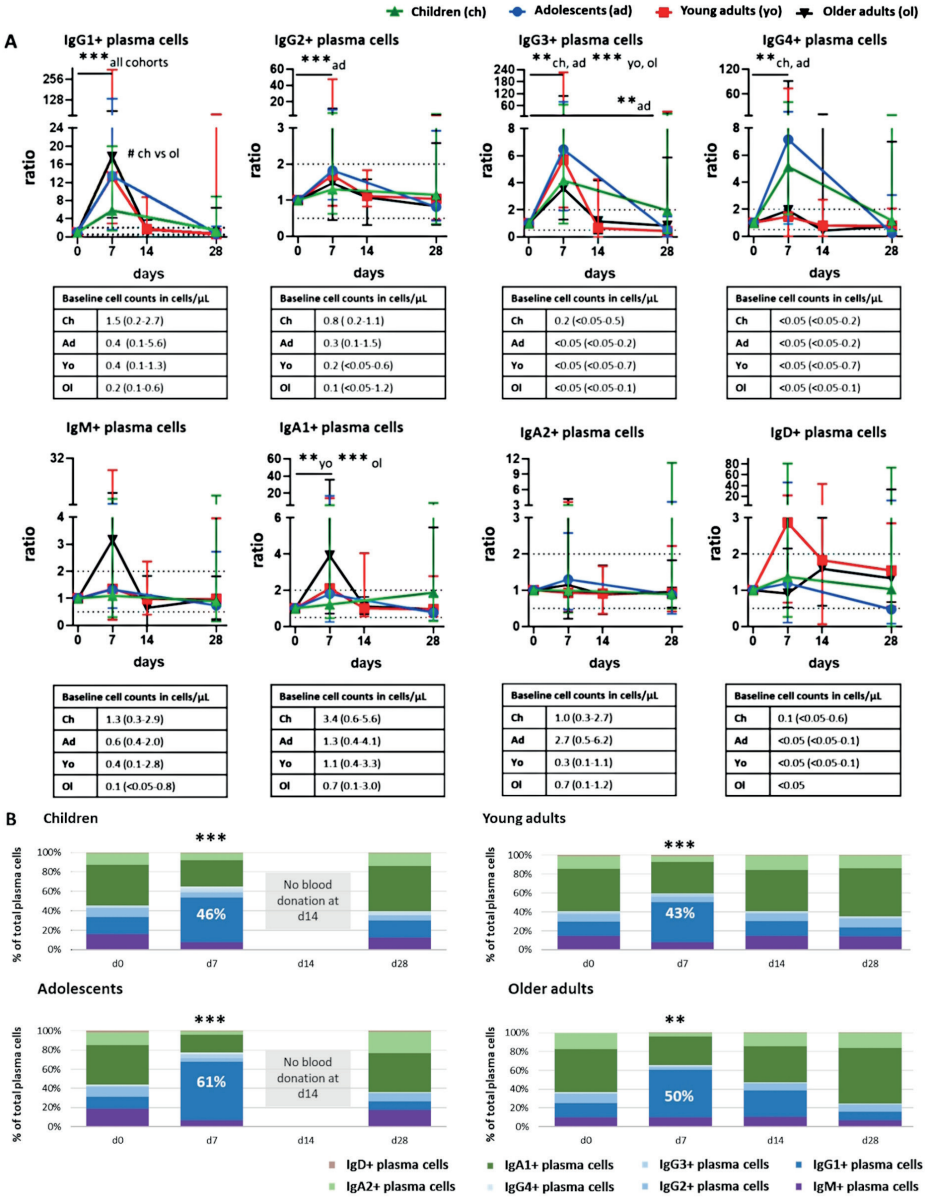



**Figure 2.** Individuals of all age groups underwent expansion of IgG1+ plasma cells at day 7 postvaccination. (**A**) Quantitative changes in plasma cells expressing different Ig subclasses, presented as ratio over baseline (median, min-max). Each symbol represents a median value with range. Dashed lines indicate a ratio over baseline of 0.5 and 2. Underneath each graph, the baseline cell counts per cohort are presented in cells/μL (median, min-max). Wilcoxon matched pair signed-rank test followed by Bonferroni correction was used to assess longitudinal differences within each cohort. Differences in ratio at day 7 between cohorts were assessed using Kruskal–Wallis followed by Dunn's test. (**B**) Over-time distribution of plasma cells expressing different Ig subclasses. Median values for each population were used to construct the plots. Wilcoxon matched pair signed-rank test followed by Bonferroni correction was used to assess longitudinal differences

in the percentage of IgG1+ cells in the total plasma cell compartment within each cohort. Differences in the percentage of IgG1+ cells in total plasma cell compartment between cohorts were assessed using Kruskal–Wallis followed by Dunn's test but did not yield significant differences. For pediatric cohorts, no blood samples were collected at day 14; the Mann–Whitney test followed by Bonferroni correction was used. For longitudinal changes, only significant differences compared with baseline are shown. Significant longitudinal differences within a cohort are indicated as **, $p \leq 0.01$; ***, $p \leq 0.001$. Significant differences between cohorts at the same time point are indicated as #, $p < 0.05$. d = days after vaccination.

### Maturation of plasma cells over time following vaccination

Newly generated plasma cells migrate from germinal centers via the blood stream to become long-lived antibody-secreting plasma cells in the bone marrow and other peripheral tissues. Over time, they gradually lose expression of CD20 and gain expression of CD138 [43] (**Figure 3A**). We used this information to divide plasma cells into consecutive maturation stages and to trace their maturation over time after booster vaccination.

Plasma cells representing all maturation stages were expanded at day 7 (**Supplementary Figure 2** for total plasma cells, **Figure 3** for IgG1+ plasma cells). This expansion was limited in the least mature CD20+CD138- plasma cells (ratio to baseline: up to 3.1 in IgG1+ plasma cells in children, **Figure 3B**), clearer in intermediate CD20-CD138- plasma cells (ratio to baseline: up to 22.7 in IgG1+ plasma cells in young adults, in all cohorts $p \leq 0.01$ or $p \leq 0.001$), and most prominent in the most mature CD20-CD138+ plasma cells (ratio to baseline: up to of 115.6 in young adults, in all cohorts $p \leq 0.01$ or $p \leq 0.001$). Similar to what was observed for total plasma cells, expansion of IgG1+ plasma cells belonging to different maturation stages was least prominent in children. In all cohorts, a significant increase in the percentage of the most mature plasma cells was observed, except for the adolescent cohort, showing just a trend. At day 7 postvaccination, most mature plasma cells constituted between 42% and 53% of IgG1+ plasma cells (**Figure 3C**). Thus, the expansion of plasma cells at day 7 postvaccination was accompanied by a shift towards a more mature plasma cell phenotype in all cohorts.

As an expansion of IgA1+ plasma cells was observed in the adult cohorts, we evaluated maturation of IgA1+ plasma cells at the peak of expansion as well. In children, no increase in more mature (CD20-CD138- and CD20-CD138+) IgA1+ plasma cells was observed at the peak of expansion. In adolescents, a small increase of the most mature (CD20- CD138+) IgA1+ plasma cells was observed, and in both adult cohorts, an increase in both intermediate and the most mature (CD20-CD138- and CD20-CD138+) IgA1+ plasma cells was observed at the peak of plasma cell expansion (adolescent cohort: $p < 0.05$, adult cohorts: $p \leq 0.01$). When comparing all cohorts at 7 days postvaccination, a higher expansion of the most mature IgA1+ plasma cells was observed in older adults compared with children ($p \leq 0.01$). Thus, the expansion and maturation of IgA1+ plasma cells seemed to increase with the age of the cohort. However, within the oldest cohort, no correlation was found between total IgA plasma cells, IgA1+ plasma cells, or vaccine-specific IgA cells, and age.

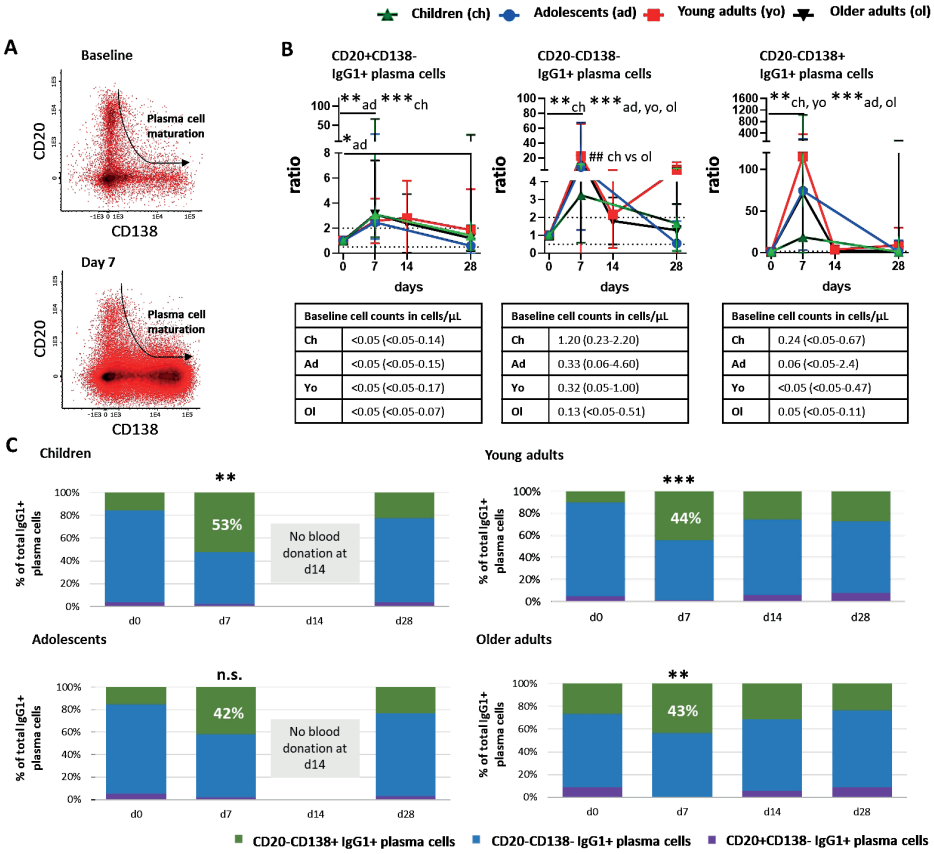



**Figure 3.** Over-time maturation of IgG1+ plasma cells. (**A**) Representative dot plots showing the phenotypical changes during plasma cell maturation. The top plot shows a baseline situation and the bottom plot shows a situation at day 7 postvaccination. Each dot represents an individual cell. The arrow indicates the direction of changes during maturation. (**B**) Over-time quantitative changes in IgG1+ plasma cells belonging to different maturation stages, presented as ratio over baseline (median, min-max). Dashed lines indicate a ratio over baseline of 0.5 and 2. Underneath each graph, a table shows the baseline cell counts of that population in cells/µL (median, min-max). (**C**) Over-time distribution of IgG1+ plasma cells representing different maturation stages with total IgG1+ plasma cells based on expression of CD20 and CD138. Median values for each population were used to construct the plots. Wilcoxon matched pair signed-rank test followed by Bonferroni correction was used to assess longitudinal differences in percentage of CD20-CD138+ cells in total IgG1+ plasma cells within each cohort. Differences in the percentage of CD20-CD138+ cells in total IgG1+ plasma cells between cohorts were assessed using Kruskal–Wallis followed by Dunn's test but did not yield significant differences. For pediatric cohorts, no blood samples were collected at day 14; the Mann–Whitney test followed by Bonferroni correction was used. For longitudinal changes, only significant differences compared with baseline are shown. Significant longitudinal differences within a cohort are indicated as *, $p < 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$. Significant differences between cohorts at the same time point are indicated as ##, $p \leq 0.01$. d = days after vaccination.

## No clear changes in the memory B-cell compartment over time following vaccination

While mature antibody-secreting plasma cells predominantly reside in bone marrow and only transiently appear in blood, memory B cells form the circulating component of immunological memory. In the steady state, memory B cells (directed against various antigens) are known to be abundant in blood of both children and adults, and undergo limited quantitative changes following antigen exposure [44, 45]. Still, memory B cells with specific reactivities can be detected in the blood stream [45-47]. In our previous study, we showed that even minor expansions in circulating memory B cells can strongly correlate with a postvaccination increase in Bp-specific serum Ig levels [33]. Therefore, we set out to determine whether any quantitative changes can be observed at selected time points and whether the same pattern is shared by different cohorts.

Neither total memory B cells nor any of the major memory B-cell subsets underwent consistent quantitative changes over time following vaccination (**Figure 4A**). One exception was the IgG4+ memory B-cell subset in adolescents, which showed a minor but significant increase at day 28 compared with baseline levels. Moreover, at 14 days postvaccination, there was a minor but significant difference between the number of IgG3+ memory B cells between young and older adults, although there were no significant longitudinal changes within any of the groups. Within each cohort, the distribution of memory B-cell subsets was stable over the time of analysis (**Figure 4B**). However, upon further subdivision of memory B cells based on the expression of CD20, CD21, CD24, and CD27, two memory B-cell subsets underwent significant fluctuations over time (**Supplementary Figure 3**). In adolescents, there was a significant increase in IgG1+ CD20++CD21-CD24+ memory B cells at day 28 after vaccination. Moreover, in adolescents and older adults, there was a significant increase in IgG1+ CD20++CD21-CD24-CD27+ memory B cells at days 14 or 28 after vaccination compared with baseline. Therefore, it is possible that memory B cells specific to Boostrix-IPV vaccine reside within these CD20++CD21- memory B cells. Here, the use of an Ag-specific approach should lead to additional insights. No significant differences were observed between the cohorts. Although most of subsets defined within IgG1+ memory B cells were significantly more numerous in children than in both adult cohorts (data not shown), this was mainly due to higher numbers of IgG1+ memory B cells in children at baseline. Thus, except for a few minor fluctuations, no differences in the number of memory B cells were observed after Tdap booster vaccination.

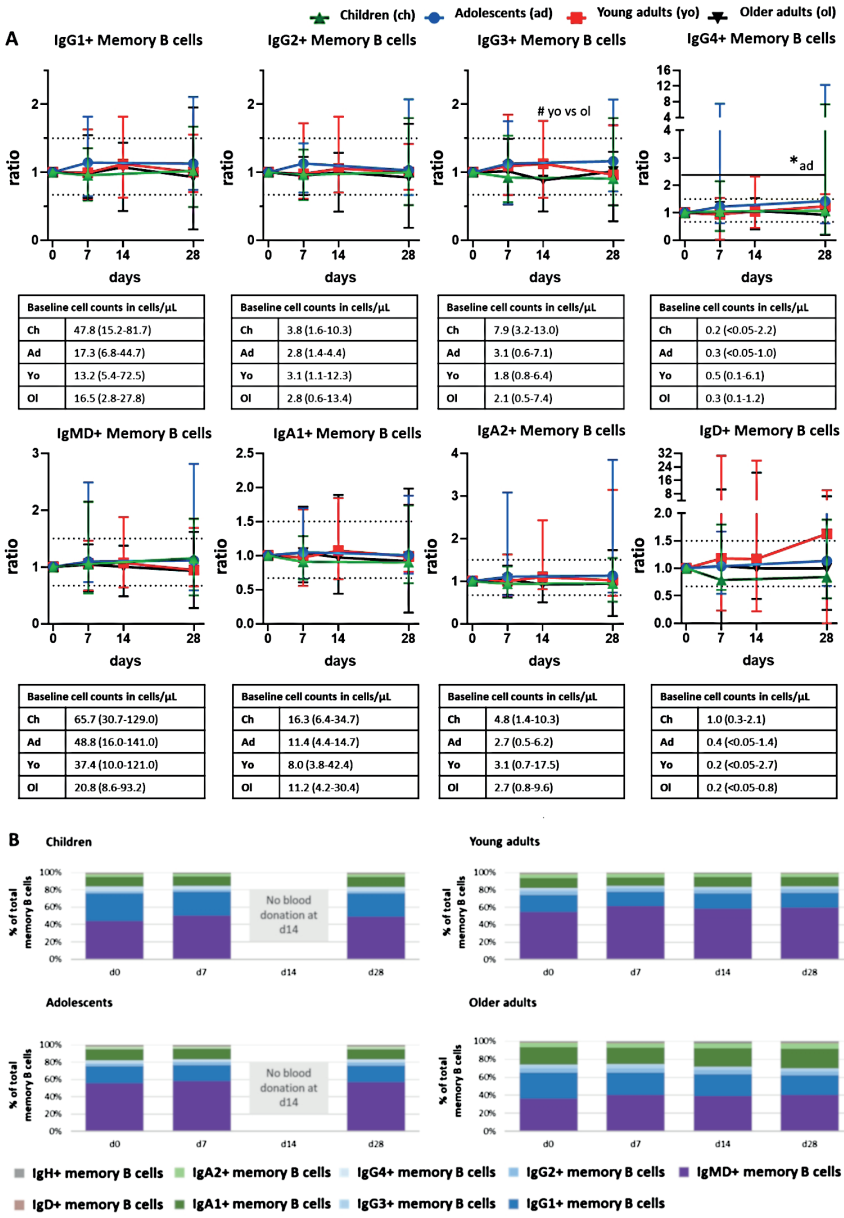



**Figure 4.** Stable distribution of memory B-cell subsets over time after vaccination. (**A**) Quantitative changes in memory B cells expressing different Ig subclasses, presented as ratio over baseline (median, min-max). Dashed lines indicate a ratio over baseline of 0.67 and 1.5. Underneath each graph, a table shows the baseline cell counts of that population in cells/µL (median, min-max). (**B**) Over-time distribution of memory B cells expressing different Ig subclasses within the total memory B-cell compartment. Median values for each population were used to construct the plots. Wilcoxon matched pair signed-rank test followed by Bonferroni correction was used to assess longitudinal differences within each cohort. Differences between cohorts were assessed using Kruskal–Wallis followed by Dunn's test but did not yield significant differences. For pediatric cohorts, no blood samples were collected at day 14; the Mann–Whitney test followed by

Bonferroni correction was used. For longitudinal changes, only significant differences compared with baseline are shown as *, $p < 0.05$. Significant differences between cohorts at the same time point are indicated as #, $p < 0.05$. d = days after vaccination.

## Good correlation between the increase in plasma cell numbers with the vaccine-specific antibody-producing cells

Plasma cells are the main producers of antibodies, and, in a recall response, are mainly generated from Ag-specific memory B cells originating from a previous encounter. To support our flow cytometry-based monitoring of memory B- and plasma cell fluctuations, we determined the increase in numbers of vaccine-specific plasma and memory B cells upon vaccination via ELISpot.

Previous studies on influenza have found that Ag-specific plasma cells generated after vaccination represent up to 80% of the total plasma cell pool [30, 31]. Therefore, we correlated numbers of total plasma cells (non-Ag-specific) as determined by flow cytometry to those specific for the vaccine components as determined by ELISpot analysis. In our Tdap-IPV vaccination study, the absolute increase in IgG and IgA plasma cell numbers from baseline to day 7 and the number of vaccine-specific IgG- and IgA-producing plasma cells at day 7 showed a positive correlation, indicating that the increase in total IgG and IgA plasma cells is explained by the increase in Ag-specific IgG and IgA plasma cells (IgG: $r = 0.59$, $p < 0.0001$; IgA: $r = 0.60$, $p < 0.0001$) (**Figure 5**).

Next, we evaluated whether the expansion of circulating IgG+ and IgA+ plasma cells at day 7 could predict the increase in serum IgG and IgA at day 28 and year 1 postvaccination. We correlated the increase in IgG+ and IgA+ plasma cells (measured by flow cytometry) with the levels of vaccine-specific serum IgG and IgA at day 28 and year 1. Although plasma cells had no predictive value for serum IgG levels, a positive correlation was found for serum IgA levels ($r = 0.3944$, $p = 0.0067$). Although weaker, this correlation was still present at year 1 postvaccination ($r = 0.3403$, $p = 0.0207$).

For memory B cells, no correlation was observed between the ELISpot readout and flow cytometry readout (**Supplementary Figure 4**). Neither flow cytometry IgG nor IgA memory readout correlated with vaccine-specific serum Igs at day 28 or year 1 (data not shown). These findings indicate that for analysis of memory B cells by flow cytometry, an Ag-specific approach is required. As differences in cellular changes could be found between the different age groups, we also tested for correlations between cell expansion (ratio over baseline) and vaccine-specific serum Ig (IU/mL) within each age group (**Supplementary Figure 5**). No correlations between cell expansion and serum Ig levels were found.

Thus, the expansion of total plasma cells measured by flow cytometry correlated with the expansion of Ag-specific plasma cells measured by ELISpot. In this regard, flow cytometry and ELISpot can provide complementary data. This is not observed for memory B cells.

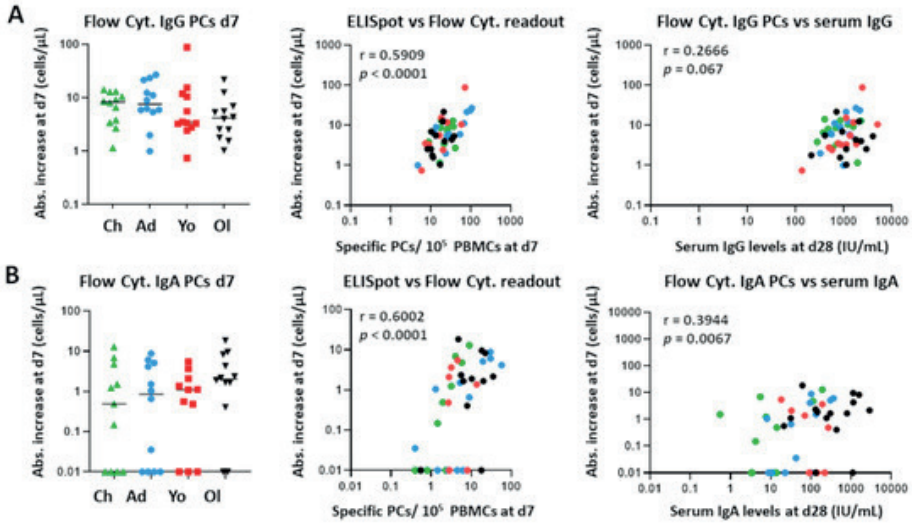



**Figure 5.** Correlation between cellular changes as measured by flow cytometry and ELISpot, and the correlation between plasma cell expansion and vaccine-specific serum Igs 28 days postvaccination. (**A**) Left panel: expansion of IgG+ plasma cells (day 7) per individual, expressed as absolute increase in cells/µL. Middle panel: correlation between the ELISpot and flow cytometry readout for IgG+ plasma cells. Right panel: correlation between the increase in plasma cells (as measured by flow cytometry) and the vaccine-specific serum IgG levels (day 28). (**B**) Left panel: expansion of IgA1+ plasma cells (day 7) per individual, expressed as absolute increase in cells/µL. Middle panel: correlation between the ELISpot and flow cytometry readout for IgA+ plasma cells. Right panel: correlation between the increase in plasma cells (as measured by flow cytometry) and the vaccine-specific serum IgA levels (day 28). Each dot represents a single donor. Of note, for visualization purposes, all absolute increases lower than 0.01 were set to 0.01. The original values were used to calculate the Spearman correlations. Flow Cyt. = flow cytometry; PCs = plasma cells; d = days after vaccination; Abs. = absolute.



## Weak positive correlation between plasma cell expansion and vaccine-component-specific ig levels

As the response to individual vaccine components may differ, we next correlated the IgG1+ and IgA1+ plasma cell expansions at day 7 postvaccination with the levels of serum IgG and IgA directed against individual pertussis vaccine components. Additionally, we tested whether the degree of maturation correlated with vaccine-component-specific IgG or IgA levels. Within the IgG1+ plasma cells, the strongest correlations (with a correlation coefficient between 0.3–0.5) were found between PT- or Prn-specific IgG levels and total IgG1+ plasma cells or CD20-CD138- IgG1+ plasma cells (**Supplementary Table 5** (IgG)). No correlations were observed between IgG1+ memory B-cell expansion and vaccine component-specific serum IgG. Correlations between IgA1+ plasma cells and serum IgAs against individual pertussis components were higher and more frequent (ranging between 0.3–0.6, **Supplementary Table 6**). Most correlations were found between FHA-specific serum IgAs and the increase in total IgA1+ plasma cells, CD20-CD138- IgA1+ plasma cells, and CD20-CD138+ IgA1+ plasma cells. Considerably fewer correlations were found between

PT- and Prn-specific serum IgAs and the increase in IgA1+ plasma cells. Interestingly, a positive correlation was found between the maximum expansion of IgA1+ memory B cells and the FHA-specific serum IgAs at year 1 postvaccination (ratio over baseline).

### More prominent cellular responses in participants primed with wP vaccine

Due to the change in the National Immunization Program on 1 January 2005, all children and 5 out of 12 adolescents were primed with an aP vaccine, while 7 adolescents and all young adults received the Dutch wP vaccine in childhood (presumably, older adults were vaccinated with wP or had received no pertussis vaccination during childhood). It has been previously shown that the primary vaccine can impact both T-cell and antibody responses [16, 18, 22-24]. As antibodies are the product of plasma cells, we studied whether B-cell and plasma cell responses are also influenced by the primary vaccine. To avoid the effect of age, we first compared both subgroups of adolescents.

Over-time changes in numbers of total, naive, and memory B cells were minor and comparable between wP- and aP-primed adolescents (**Figure 6A**). In contrast, changes in plasma cell numbers were much more prominent in adolescents who were primed with the Dutch wP vaccine vs. aP-primed adolescents (ratio to baseline: 4.8 vs. 1.5 at day 7 in total plasma cell numbers). From all plasma cells, the differences were the clearest for IgG1+ (ratio: 23.9 vs. 4.5 at day 7), IgG3+ (ratio: 19.7 vs. 3.7 at day 7), and IgG4+ (ratio: 9.2 vs. 3.2 at day 7) plasma cells. Only the difference in IgG1+ plasma cell expansion reached statistical significance ($p \leq 0.01$), possibly due to the low number of participants in both groups. Moreover, plasma cell maturation at the peak of expansion was more prominent in wP-primed adolescents in whom the most mature CD20-CD138+ plasma cells constituted 46% of IgG1+ plasma cells in contrast to 39% in aP-primed adolescents (**Figure 6A**,**B**). Thus, the type of primary vaccination background seems to influence the plasma cell response to later booster vaccinations. In our study, the plasma cell response was stronger and more diverse in wP-primed adolescents.

To exclude that differences observed in the adolescent cohort were caused by the different sex distribution between aP- and wP-primed individuals, we assessed the impact of sex on the cell expansion in the young adult cohort and extrapolated this to the adolescent cohort. The young adult cohort was well sex-balanced and had a homogenous priming background. We evaluated the expansion of total B cells, plasma cells, IgG1–3+ plasma cells, and IgA1+ plasma cells in males and females. No significant differences were observed between male and female responses (**Supplementary Figure 6**). Therefore, we concluded that the sex imbalance in the adolescent cohort did not influence the plasma cell expansion as found in this study.

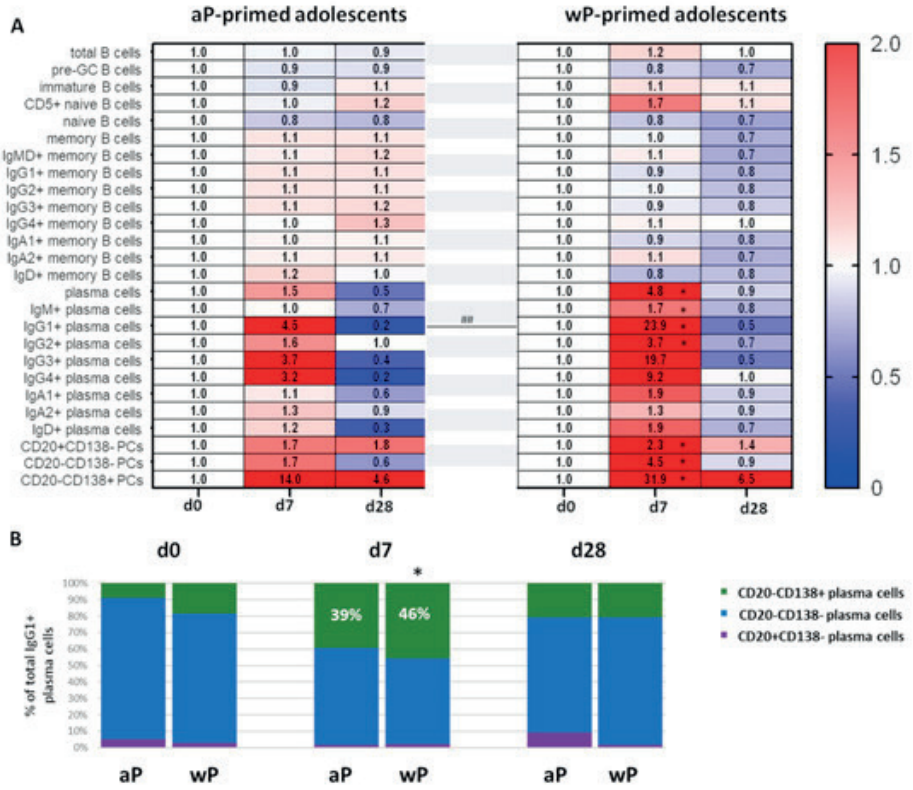



**Figure 6.** IgG1+ plasma cell expansion and maturation are more prominent in age-matched participants after wP priming. (**A**) Heatmap showing over-time changes in memory B-cell and plasma cell subsets in aP-primed ($n$ = 5) or wP-primed ($n$ = 7) adolescents (median values). (**B**) Over-time distribution of IgG1+ plasma cells representing different maturation stages within total IgG1+ plasma cells. Median values for each population were used to construct the plots. Wilcoxon matched pair signed-rank test followed by Bonferroni correction was used to assess longitudinal differences in percentage of CD20-CD138+ cells in total IgG1+ plasma cells within each cohort. Differences in the percentage CD20-CD138+ cells in total IgG1+ plasma cells between cohorts were assessed using Kruskal–Wallis followed by Dunn's test but did not yield significant differences. For longitudinal changes, only significant differences compared to baseline are shown. Significant longitudinal differences within a cohort are indicated as *, $p < 0.05$. Significant differences between cohorts at the same time point are indicated as ##, $p \leq 0.01$. d = days after vaccination; aP = acellular pertussis vaccine; wP = whole-cell pertussis vaccine; pre-GC = pre-Germinal Center.

The adolescent cohort only consisted of 12 individuals, which is rather small for statistical analysis. Therefore, although we found some age-dependent differences in cellular responses, we grouped all aP-primed individuals (children and adolescents) and all wP-primed individuals (adolescents and young adults; not the older adults, because of their uncertain vaccination background status) to increase the size of the aP- vs. wP-primed study cohorts (**Supplementary Figure 7A**). In this comparison, expansions of total and

IgG1+ plasma cells were again more pronounced in wP-primed participants. Differences in other plasma cell subsets did not reach statistical significance. Finally, based on ratio to baseline, the (total) plasma cell maturation was more prominent in wP-primed individuals compared with aP-primed individuals, and differed significantly for intermediate mature plasma cells. The percentage of the most mature cells in the IgG1+ plasma cell population did not significantly differ between the two groups (**Supplementary Figure 7**).

In summary, we showed that, irrespective of the age of vaccinated individuals, the most prominent cellular changes occurred in the numbers of circulating plasma cells. Despite some age-related differences, the expansion and maturation of IgG1+ plasma cells at day 7 postvaccination are a shared phenomenon. This expansion of plasma cells measured by flow cytometry was complementary to the increase of vaccine-specific plasma cell numbers determined by ELISpot. Positive correlations between plasma cell expansion and postvaccination Ag-specific serum Ig levels were observed, mainly when correlating with the individual Bp components (Bp: FHA, Prn, and PT). Finally, plasma cell responses were stronger in individuals who were wP-primed.

## DISCUSSION

In this study, we applied high-dimensional flow cytometry to investigate changes in B cells in individuals of different ages and primary vaccination backgrounds upon administration of an aP booster vaccine and correlated these findings with vaccine-specific Ig levels in serum. In all age groups, expansion and maturation of plasma cells 7 days postvaccination was the most prominent cellular change. Although in children the expansion of plasma cells was less prominent than in adults (ratio to baseline), they had more plasma cells at peak levels due to their initially high plasma cell numbers. Furthermore, total and IgG1+ plasma cell responses were stronger in individuals primed with the Dutch wP vaccine than in individuals who were primed with aP vaccines. No consistent over-time memory B-cell fluctuations were observed. No strong correlation between plasma cell expansion or memory B-cell expansion with vaccine-specific serum Ig levels was observed, yet the absolute increase in IgA plasma cells at day 7 correlated weakly with the IgA serum levels at day 28 (IU/mL). Furthermore, weak positive correlations were observed between the expansion of IgG1+ and IgA1+ plasma cells and FHA-, Prn-, and PT-specific serum IgG or IgA levels postvaccination. Although serology provides insight into Ag-specific Ig levels and function, analysis of circulating immune cells may result in a deeper understanding of the processes induced by the vaccine and the cellular changes preceding Ig production. Our study points at plasma cells as a potential cellular marker of an immune response and contributes to a better understanding of the immune responses (to booster vaccinations) between different age groups and different primary vaccination backgrounds.

To ensure objective data analysis, we used the automated gating and identification (AGI) tool in the Infinicyt software. This tool was shown to reduce intra- and interoperator variability and increase reproducibility of the analysis [40, 48-50]. This is especially important for studies with big data from multiple samples, which cannot be analyzed by a single operator within a reasonable timeframe. Irrespective of the new analysis strategy, these data corroborated major findings from our previous study, where data were subjected to manual analysis [33]. This automated analysis approach, in combination with the standardized EuroFlow sample processing and acquisition procedures, allows for identification of fluctuations in small populations of cells such as different plasma cell maturation stages.

Levels of Ag-specific serum Igs are routinely used as readout for vaccine efficacy. In many cases, a rise in Ag-specific IgG levels is associated with response to vaccination, and for several vaccines—e.g., against rotavirus—an increase in IgA levels has been indicated as a correlate of protection [23, 45, 51-53]. As Igs are the product of terminally differentiated B cells (plasma cells), the B-cell compartment may harbor new correlates or biomarkers of ongoing immune responses. Indeed, we found that expansion and maturation of circulating plasma cells 7 days after booster vaccination was the most prominent cellular change. The generation of mainly IgG1+ plasma cells is in line with previous serology-based studies, where within Bp-specific serum IgGs mostly IgG1 antibodies were found, with minor contribution of IgG2, -3, and -4 [51, 54]. The positive correlation between the numbers of total plasma cells with the vaccine-specific plasma cell numbers supports the assumption that most of the plasma cells at the peak of expansion are vaccine-specific.



The IgA response, observed mostly in the adult cohorts, is likely a result of immunological memory generated by previous (subclinical) infection of the respiratory tract, where a mucosal response against Bp was launched. As Bp circulates within the population, causing outbreaks every 2–5 years, the adult cohorts have likely encountered Bp multiple times during life, which explains the more prominent IgA1+ plasma cell response in these groups [55-58]. In contrast, the expansion of IgG4+ plasma cells was mostly seen in the pediatric cohorts, which may be explained by the predominant aP priming in these cohorts; this has been shown to induce a more Th2-related response as well as increased vaccine-specific serum IgG4 [23, 24].

In addition to the expansion of IgG1+ and IgA1+ plasma cells in adults, and the IgG1+ and IgG4+ response in the pediatric cohorts, which are in line with previous (cellular and serology-based) studies, we also observed a prominent increase in IgG3+ plasma cells in all cohorts [23, 33, 51, 54]. A potential explanation for this phenomenon might be that, in addition to the memory B cells, there are naive B cells that recognize the antigens and undergo first-step IgG3 class switching and affinity maturation [59].

The difference in plasma cell—and thus, antibody—production can have consequences for the type and efficacy of the launched immune response. IgG1 and IgG3 antibodies have stronger opsonizing capacities compared with IgG4 antibodies [60]. The mixed IgG1-IgG3-IgG4 response observed in the mostly aP-primed pediatric cohorts may lead to competition for Bp antigens in future encounters, possibly leading to less efficient bacterial clearance compared with the IgG1-IgG3 (and IgA1) response observed in the adult cohorts [59, 61]. Lastly, the prominent contribution of IgA1+ plasma cells to responses observed in the adult cohorts, which is likely an indicator of previous pertussis encounters, may imply existence of effective mucosal defense mechanisms, and more efficient protection against bacterial translocation in IgA-producing individuals. Comparison of repertoires and reactivities of IgA in mucosa and in circulation could provide better insights into this phenomenon and value of IgA as a biomarker of protection.

Maturation of plasma cells (total and IgG1+) was observed irrespective of age and priming background. The clear expansion and maturation of total and IgG1+ plasma cells are in line with our previous findings and may be explained by the prolonged retention of newly generated plasma cells in the periphery as well as the competition for bone marrow niches with pre-existing long-lived plasma cells [33, 62].

In this study, several differences between the aP- and wP-primed cohorts were observed. Although the sizes of the age-matched adolescent cohorts were too limited to reach statistically significant conclusions, major observations were confirmed by analysis of all individuals with known primary vaccination background. Remarkably, this difference based on primary vaccination background was not observed in the overarching part of the BERT study, where the Bp-specific Ig responses of 85 Dutch and Finnish adolescents pre- and postvaccination were evaluated [29].

The formulation of aP and wP vaccines differs with regards to number of antigens and the total antigenic load, with wP vaccines containing the broad variety of pertussis antigens and aP vaccines containing high concentrations of a restricted number of antigens. In consequence, wP priming is likely to trigger a more diverse antibody response. Since consecutive boosters lead to a more specific, but also more restricted response, this initial broad priming can be beneficial in case of encountering future (mutated) bacterial strains [10, 12, 13]. Interestingly, in this study, we showed that compared to aP-primed individuals, individuals primed with the Dutch wP vaccine have a stronger response upon aP booster vaccination. It would be of interest to visualize potential differences in breadth of an immune response against Bp antigens. Moreover, since the initial type of priming vaccine seems to imprint future responses to given antigens, it should be carefully considered in the design of future vaccines and vaccination strategies. This may also hold true for diseases other than pertussis, such as COVID-19.

To identify unique and shared patterns between groups, we primarily focused on normalized data (represented as the ratio to baseline). However, we also showed that, in line with published studies, children had overall higher leukocyte and B-cell counts compared with (older) adults [32, 41]. Specifically, the cell count of naive B and T cells—and thus, the available naive repertoire—is substantially higher in youth [32, 48]. Therefore, despite a lower increase in cells expressed as ratio to baseline, children and adolescents may still produce a stronger and more diverse immune responses than adults.

In this study, memory B-cell fluctuations were limited. As the frequencies of Ag- specific memory B cells are low, as demonstrated by previous studies using ELISpot assays an increase in only these Ag-specific memory B cells may not have an impact on the total memory B-cell population [36, 63]. Indeed, in this study, no correlation was found between the memory B-cell fluctuations measured by flow cytometry and the vaccine-specific memory B cells by ELISpot. However, we observed an increase in CD20++CD21- IgG1+ memory B cells in older adults and adolescents at days 14 and 28 after vaccination, respectively. Interestingly, several studies reported an increased percentage of Ag-specific CD27+CD21-/dim B cells 14 days after influenza vaccination [64, 65]. There is no consensus about the exact function of these CD21-/dim B cells, but it has been suggested that CD21- / dim B cells are exhausted cells or, as described in autoimmunity and chronic infection, are anergic [66, 67]. In this context, it is not unlikely that cells that have responded to an antigen multiple times would acquire this phenotype. However, Lau and colleagues suggest that CD21-/dim B cells are primed for plasma cell differentiation [64]. Ag-specific flow cytometry studies should give insight into the exact function of this B-cell subset.



No correlation was found between expansion of memory B cells and Ag-specific serum IgG levels at day 28. Previously, we observed a clear correlation between the expansion of IgG1+ memory B cells and the vaccine-specific IgG levels at day 21 in a cohort of 10 healthy adults [33]. Moreover, we found that although in the majority of participants memory B cells showed maximum expansion at 14 days after vaccination, the expansion of memory B cells was not as synchronized in time as the plasma cell expansion, implying that, in some participants, we may not have sampled at the most optimal timepoint [33], especially in the children and adolescent cohorts, where the sampling times were limited to days 0, 7, and 28. This difference in timing of memory B-cell responses might be related to the immune status of each individual at baseline and makes the use of memory B cells as correlates of protection more difficult.

Neither aP nor wP vaccination yield a response that fully mimics natural infection; especially, the IgA response seems to be limited upon vaccination and mostly relies upon previous encounters with Bp. To overcome this limitation, multiple novel pertussis vaccines and alternative delivery routes are being developed, such as nasal delivery of a vaccine or the use of life-attenuated Bp strains (BPZE1) [68-70]. It would be of interest to

evaluate how (cellular) immune responses induced by these vaccine candidates compare with cellular kinetics induced by intramuscular aP and wP vaccines, as well as (controlled) human infection. Such comparison between aP booster vaccination and (controlled) human infection is currently ongoing within the IMI-2 PERISCOPE program [71, 72]. These studies will create a solid basis for evaluation of novel vaccination approaches.

## CONCLUSIONS

Analysis of circulating immune cells results in a deeper understanding of the processes induced by vaccination and the cellular changes preceding Ig production. Irrespective of the age of vaccinated individuals, the most prominent cellular changes occurred in the numbers of circulating plasma cells. The expansion and maturation of IgG1+ plasma cells at day 7 postvaccination were a shared phenomenon. Plasma cell expansion, as determined by flow cytometry, was complementary to the increase of vaccine-specific plasma cell numbers as determined by ELISpot. Positive correlations between plasma cell expansion and postvaccination Ag-specific serum Ig levels were observed, mainly when correlating with the individual Bp components. Finally, plasma cell responses were stronger in individuals who were wP-primed. Thus, our study contributes to a better understanding of the immune responses (to booster vaccinations) between different age groups and different primary vaccination backgrounds.

## AUTHOR CONTRIBUTION

Conceptualization, C.T., A.-M.B., M.P.-A., A.O., G.A.M.B., J.J.M.v.D. and M.A.B.; methodology, A.T.-V., M.P.-A. and A.O.; validation, R.J.G. and B.d.M.; formal analysis, A.M.D., P.V., C.T., R.J.G., B.d.M. and M.A.B.; investigation, A.M.D., P.V., C.T., R.J.G., B.d.M. and M.A.B.; data curation, A.M.D., P.V. and G.A.M.B.; writing—original draft preparation, A.M.D. and M.A.B.; writing—review & editing, A.M.D., P.V., C.T., R.J.G., B.d.M., A.-M.B., A.T.-V., M.P.-A., A.O., G.A.M.B., J.J.M.v.D. and M.A.B.; visualization, A.M.D.; supervision, A.-M.B., M.P.-A., A.O., G.A.M.B., J.J.M.v.D. and M.A.B.; project administration, P.V., A.-M.B., G.A.M.B., J.J.M.v.D. and M.A.B.; funding acquisition, A.O., G.A.M.B. and J.J.M.v.D. All authors have read and agreed to the published version of the manuscript.

## FUNDING

This PERISCOPE project has received funding from the Innovative Medicines Initiative 2 Joint Undertaking under grant agreement No 115910. This Joint Undertaking receives support from the European Union's Horizon 2020 research and innovation program and EFPIA and BMGF. The Joint Undertaking is not responsible for any use that may be made of the information this manuscript contains.

## INSTITUTIONAL REVIEW BOARD STATEMENT

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Medical Research Ethics Committees United (MEC-U, NL60807.100.17-R17.039) on 2017-09-01 and registered at the EU Clinical trial registry (EudraCT number 2016-003678-42).

## INFORMED CONSENT STATEMENT

Written informed consent was obtained from all subjects (or their parent/guardian) involved in the study.

## DATA AVAILABILITY STATEMENT

The data presented in this study are available on request from the corresponding author.

## ACKNOWLEDGMENTS

The authors would like to thank all research nurses and clinical trial staff, especially J. Zonneveld and G. van Asselts (Spaarne Gasthuis), for their support in sample collection, performing home visits, and management of the clinical data. We also acknowledge M. van Houten (Spaarne Gasthuis) for her help with the trial coordination, clinical trial management, and clinical sample collection. We thank P. van Gageldonk, N. Kaagman and L. de Rond from National Institute of Public Health and the Environment for measuring the Ag-specific serum Ig levels and antibody producing cells. We express gratitude to all participants and their families for their participation in this study. Moreover, the authors gratefully acknowledge the Flow cytometry Core Facility at LUMC (coordinated by K. Schepers, M. Hameetman, run by operators S. van de Pas, D. Lowie, J. Jansen, I.J. Reyneveld, and former operators E. de Haas and G. de Roo) for their support.

## CONFLICTS OF INTEREST

A.M.D., C.T., M.P.-A., A.O., J.J.M.v.D. and M.A.B. report inventorship of the patent “Means and methods for multiparameter cytometry-based leukocyte subsetting” (NL2844751, filing date 5 November 2019) [37], owned by the EuroFlow Consortium. In addition, J.J.M.v.D. and A.O. report to be chairman and co-chairman of the EuroFlow scientific foundation, which receives royalties from licensed patents, which are collectively owned by the participants of the EuroFlow Foundation. These royalties are exclusively used for continuation of the EuroFlow collaboration and sustainability of the EuroFlow consortium. Lastly, J.J.M.v.D. and A.O. report an Educational Services Agreement from BD Biosciences (San José, CA) and a Scientific Advisor Agreement with Cytognos; all related fees and honoraria go to LUMC and USAL, respectively.

## REFERENCES


1. Atkinson, W., Epidemiology and Prevention of Vaccine-Preventable Diseases, Centers for Disease Control and Prevention. 2006, Washington, DC, USA: Department of Health & Human Services,.
2. Cherry, J.D., Historical review of pertussis and the classical vaccine. J Infect Dis, 1996. 174 Suppl 3: p. S259-63.
3. Lambert, L.C., Pertussis vaccine trials in the 1990s. J Infect Dis, 2014. 209 Suppl 1(Suppl 1): p. S4-9.
4. Edwards, K.M., et al., Comparison of 13 acellular pertussis vaccines: overview and serologic response. Pediatrics, 1995. 96(3 Pt 2): p. 548-57.
5. Rijksvaccinatieprogramma [EN: National Immunization Programme]. 18 November 2020]; Available from: https://rijksvaccinatieprogramma.nl/english.
6. Tan, T., et al., Pertussis Across the Globe: Recent Epidemiologic Trends From 2000 to 2013. Pediatr Infect Dis J, 2015. 34(9): p. e222-32.
7. Versteegen, P., et al., More than 10 years after introduction of an acellular pertussis vaccine in infancy: a cross-sectional serosurvey of pertussis in the Netherlands. The Lancet Regional Health – Europe, 2021. 10: p. 100196.
8. Heininger, U., et al., Comparative Epidemiologic Characteristics of Pertussis in 10 Central and Eastern European Countries, 2000-2013. PLoS One, 2016. 11(6): p. e0155949.
9. Berbers, G.A.M., S.C. de Greeff, and F.R. Mooi, Improving pertussis vaccination. Hum Vaccin, 2009. 5(7): p. 497-503.
10. Bart, M.J., et al., Complete Genome Sequences of 11 Bordetella pertussis Strains Representing the Pandemic ptxP3 Lineage. Genome Announc, 2015. 3(6).
11. Mooi, F.R., et al., Bordetella pertussis Strains with Increased Toxin Production Associated with Pertussis Resurgence. Emerging Infectious Disease journal - CDC, 2009. 15(8).
12. Martin, S.W., et al., Pertactin-negative Bordetella pertussis strains: evidence for a possible selective advantage. Clin Infect Dis, 2015. 60(2): p. 223-7.
13. Lam, C., et al., Rapid increase in pertactin-deficient Bordetella pertussis isolates, Australia. Emerg Infect Dis, 2014. 20(4): p. 626-33.
14. Sato, Y., M. Kimura, and H. Fukumi, Development of a pertussis component vaccine in Japan. Lancet, 1984. 1(8369): p. 122-6.
15. Gustafsson, L., et al., A controlled trial of a two-component acellular, a five-component acellular, and a whole-cell pertussis vaccine. N Engl J Med, 1996. 334(6): p. 349-55.
16. Liko, J., S.G. Robison, and P.R. Cieslak, Priming with whole-cell versus acellular pertussis vaccine. N Engl J Med, 2013. 368(6): p. 581-2.
17. Klein, N.P., et al., Waning Tdap Effectiveness in Adolescents. Pediatrics, 2016. 137(3): p. e20153326.
18. Sheridan, S.L., et al., Number and order of whole cell pertussis vaccines in infancy and disease protection. Jama, 2012. 308(5): p. 454-456. doi: 10.1001/jama.2012.6364.
19. Klein, N.P., et al., Comparative effectiveness of acellular versus whole-cell pertussis vaccines in teenagers. Pediatrics, 2013. 131(6): p. e1716-e1722. doi: 10.1542/peds.2012-3836.
20. Warfel, J.M., L.I. Zimmerman, and T.J. Merkel, Acellular pertussis vaccines protect against disease but fail to prevent infection and transmission in a nonhuman primate model. Proc Natl Acad Sci U S A, 2014. 111(2): p. 787-792, DOI: 10.1073/pnas.1314688110.

**21.** PERISCOPE Consortium, PERISCOPE: road towards effective control of pertussis. Lancet Infect Dis, 2019. 19(5): p. e179-e186, DOI: 10.1016/s1473-3099(18)30646-7.

**22.** Lambert, E.E., et al., Uncovering Distinct Primary Vaccination-Dependent Profiles in Human Bordetella Pertussis Specific CD4+ T-Cell Responses Using a Novel Whole Blood Assay. Vaccines, 2020. 8(2): p. 225.

**23.** Hendrikx, L.H., et al., Different IgG-subclass distributions after whole-cell and acellular pertussis infant primary vaccinations in healthy and pertussis infected children. Vaccine, 2011. 29(40): p. 6874-80.

**24.** da Silva Antunes, R., et al., Th1/Th17 polarization persists following whole-cell pertussis vaccination despite repeated acellular boosters. J Clin Invest, 2018. 128(9): p. 3853-3865.

**25.** van der Lee, S., et al., Whole-Cell or Acellular Pertussis Primary Immunizations in Infancy Determines Adolescent Cellular Immune Profiles. Front Immunol, 2018. 9: p. 51. doi: 10.3389/fimmu.2018.00051.

**26.** Kandeil, W., et al., The burden of pertussis in older adults: what is the role of vaccination? A systematic literature review. Expert Rev Vaccines, 2019. 18(5): p. 439-455. doi: 10.1080/14760584.2019.1588727.

**27.** Van Damme, P., et al., Immunogenicity of the reduced-antigen-content dTpa vaccine (Boostrix(®)) in adults 55 years of age and over: a sub-analysis of four trials. Vaccine, 2011. 29(35): p. 5932-9.

**28.** Theeten, H., et al., Primary vaccination of adults with reduced antigen-content diphtheria-tetanus-acellular pertussis or dTpa-inactivated poliovirus vaccines compared to diphtheria-tetanus-toxoid vaccines. Curr Med Res Opin, 2007. 23(11): p. 2729-39.

**29.** Versteegen, P., et al., Responses to an acellular pertussis booster vaccination in children, adolescents, and young and older adults: A collaborative study in Finland, the Netherlands, and the United Kingdom. EBioMedicine, 2021. 65: p. 103247.

**30.** Ellebedy, A.H., et al., Defining antigen-specific plasmablast and memory B cell subsets in human blood after viral infection or vaccination. Nat Immunol, 2016. 17(10): p. 1226-34.

**31.** Wrammert, J., et al., Rapid cloning of high-affinity human monoclonal antibodies against influenza virus. Nature, 2008. 453(7195): p. 667-71.

**32.** Blanco, E., et al., Age-associated distribution of normal B-cell and plasma cell subsets in peripheral blood. J Allergy Clin Immunol, 2018. 141(6): p. 2208-2219.e16.

**33.** Diks, A.M., et al., Highly Sensitive Flow Cytometry Allows Monitoring of Changes in Circulating Immune Cells in Blood After Tdap Booster Vaccination. Front Immunol, 2021. 12: p. 666953.

**34.** GlaxoSmithKline. Boostrix Polio; Product Summary. 7 July 2020]; Available from: https://www.geneesmiddeleninformatiebank.nl/Bijsluiters/h35123.pdf.

**35.** van Gageldonk, P.G.M., et al., Development and validation of a multiplex immunoassay for the simultaneous determination of serum antibodies to Bordetella pertussis, diphtheria and tetanus. J Immunol Methods, 2008. 335(1-2): p. 79-89, DOI: 10.1016/j.jim.2008.02.018.

**36.** Buisman, A.M., et al., Long-term presence of memory B-cells specific for different vaccine components. Vaccine, 2009. 28(1): p. 179-186. doi: 10.1016/j.vaccine.2009.09.102.

**37.** van Dongen, J.J.M.O.d.M.C.E.V., J.A.; Goncalves Grunho Teodosio, C.I.; Perez Y Andres, M.; Almeida Parra, J.M.; Van den Bossche, W.B.L.; Botafogo Goncalves, V.D.; Berkowska, M.A.; Van der Pan, K.; Blanco Alvarez, E.; Diks, A.M.; Pinto; Pinto Damasceno, D.; Hernández Delgado, A., Means and Methods for Multiparameter Cytometry-Based Leukocyte Subsetting.

**38.** Kalina, T., et al., Quality assessment program for EuroFlow protocols: summary results of four-year (2010-2013) quality assurance rounds. Cytometry A, 2015. 87(2): p. 145-56.

**39.** Kalina, T., et al., EuroFlow standardization of flow cytometer instrument settings and immunophenotyping protocols. Leukemia, 2012. 26(9): p. 1986-2010.

**40.** Pedreira, C.E., et al., From big flow cytometry datasets to smart diagnostic strategies: The EuroFlow approach. J Immunol Methods, 2019. 475: p. 112631.

**41.** Comans-Bitter, W.M., et al., Immunophenotyping of blood lymphocytes in childhood. Reference values for lymphocyte subpopulations. J Pediatr, 1997. 130(3): p. 388-93.

**42.** Kumar, P.C., M.L., Clinical Medicine. 8th ed. 2012, UK: Saunders Elsevier: Edinburgh.

**43.** Perez-Andres, M., et al., Human peripheral blood B-cell compartments: a crossroad in B-cell traffic. Cytometry B Clin Cytom, 2010. 78 Suppl 1: p. S47-60.

**44.** Tangye, S.G. and D.M. Tarlinton, Memory B cells: effectors of long-lived immune responses. Eur J Immunol, 2009. 39(8): p. 2065-75.

**45.** Hendrikx, L.H., et al., Enhanced memory B-cell immune responses after a second acellular pertussis booster vaccination in children 9 years of age. Vaccine, 2011. 30(1): p. 51-8.

**46.** Crotty, S., et al., Cutting edge: long-term B cell memory in humans after smallpox vaccination. J Immunol, 2003. 171(10): p. 4969-73.

**47.** Hendrikx, L.H., et al., Identifying long-term memory B-cells in vaccinated children despite waning antibody levels specific for Bordetella pertussis proteins. Vaccine, 2011. 29(7): p. 1431-1437. doi: 10.1016/j.vaccine.2010.12.033.

**48.** Botafogo, V., et al., Age Distribution of Multiple Functionally Relevant Subsets of CD4+ T Cells in Human Blood Using a Standardized and Validated 14-Color EuroFlow Immune Monitoring Tube. Front Immunol, 2020. 11: p. 166.

**49.** Linskens, E., et al., Improved Standardization of Flow Cytometry Diagnostic Screening of Primary Immunodeficiency by Software-Based Automated Gating. Front Immunol, 2020. 11: p. 584646.

**50.** Flores-Montero, J., et al., EuroFlow Lymphoid Screening Tube (LST) data base for automated identification of blood lymphocyte subsets. J Immunol Methods, 2019. 475: p. 112662.

**51.** Giammanco, A., et al., Analogous IgG subclass response to pertussis toxin in vaccinated children, healthy or affected by whooping cough. Vaccine, 2003. 21(17-18): p. 1924-31.

**52.** Vesikari, T., et al., Immunogenicity and safety of the human rotavirus vaccine Rotarix co-administered with routine infant vaccines following the vaccination schedules in Europe. Vaccine, 2010. 28(32): p. 5272-9.

**53.** Plotkin, S.O., W.; Offit, P., Vaccines (Section 1, Chapter 2). 2008, Saunders Elsevier: Philadelphia: PA, USA. p. 17-36.

**54.** van der Lee, S., et al., Whole-cell or acellular pertussis vaccination in infancy determines IgG subclass profiles to DTaP booster vaccination. Vaccine, 2018. 36(2): p. 220-226.

**55.** van der Maas, N.A.T.d.M., H.E.; Heuvelman, C.J.; van Gent, M.; Mooi, F.R., Kinkhoestsurveillance in 2013 en 2014—RIVM Briefrapport 2014-0165. Rijksinstituut voor volksgezondheid en milieu (RIVM): Bilthoven: The Netherlands, 2014. p. 36.

**56.** Cherry, J.D., Epidemic pertussis in 2012--the resurgence of a vaccine-preventable disease. N Engl J Med, 2012. 367(9): p. 785-7.

**57.** Subissi, L., et al., Assessment of IgA anti-PT and IgG anti-ACT reflex testing to improve Bordetella pertussis serodiagnosis in recently vaccinated subjects. Clin Microbiol Infect, 2020. 26(5): p. 645. e1-645.e8.

**58.** Hendrikx, L.H., et al., Serum IgA responses against pertussis proteins in infected and Dutch wP or aP vaccinated children: an additional role in pertussis diagnostics. PLoS One, 2011. 6(11): p. e27681.

**59.** Diavatopoulos, D.A. and K.M. Edwards, What Is Wrong with Pertussis Vaccine Immunity? Why Immunological Memory to Pertussis Is Failing. Cold Spring Harb Perspect Biol, 2017. 9(12).

**60.** Brüggemann, M., et al., Comparison of the effector functions of human immunoglobulins using a matched set of chimeric antibodies. J Exp Med, 1987. 166(5): p. 1351-61.

**61.** Weiss, A.A., et al., Acellular pertussis vaccines and complement killing of Bordetella pertussis. Infect Immun, 2004. 72(12): p. 7346-51.

**62.** Odendahl, M., et al., Generation of migratory antigen-specific plasma blasts and mobilization of resident plasma cells in a secondary immune response. Blood, 2005. 105(4): p. 1614-21.

**63.** Nanan, R., et al., Acute and long-term effects of booster immunisation on frequencies of antigen-specific memory B-lymphocytes. Vaccine, 2001. 20(3-4): p. 498-504.

**64.** Lau, D., et al., Low CD21 expression defines a population of recent germinal center graduates primed for plasma cell differentiation. Sci Immunol, 2017. 2(7).

**65.** Koutsakos, M., et al., Circulating T(FH) cells, serological memory, and tissue compartmentalization shape human influenza-specific B cell immunity. Sci Transl Med, 2018. 10(428).

**66.** Andrews, S.F. and P.C. Wilson, The anergic B cell. Blood, 2010. 115(24): p. 4976-8.

**67.** Isnardi, I., et al., Complement receptor 2/CD21- human naive B cells contain mostly autoreactive unresponsive clones. Blood, 2010. 115(24): p. 5026-36.

**68.** Lin, A., et al., Live attenuated pertussis vaccine BPZE1 induces a broad antibody response in humans. J Clin Invest, 2020. 130(5): p. 2332-2346.

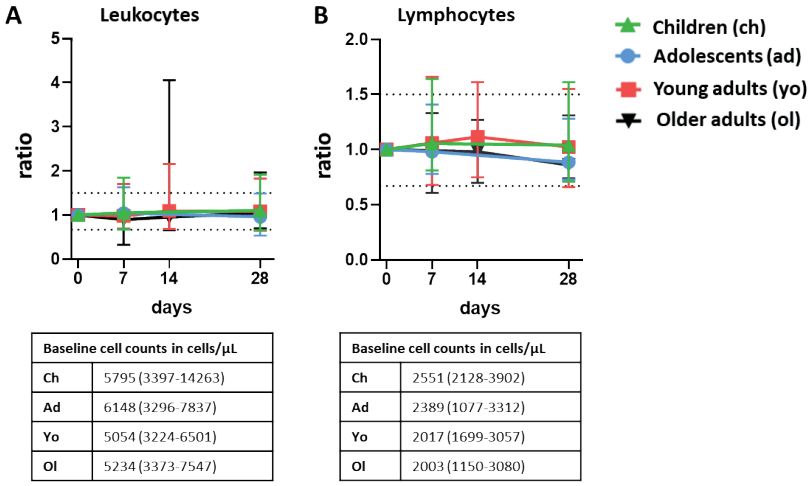
**69.** Thorstensson, R., et al., A phase I clinical study of a live attenuated Bordetella pertussis vaccine--BPZE1; a single centre, double-blind, placebo-controlled, dose-escalating study of BPZE1 given intranasally to healthy adult male volunteers. PLoS One, 2014. 9(1): p. e83449.

**70.** Boehm, D.T., et al., Intranasal acellular pertussis vaccine provides mucosal immunity and protects mice from Bordetella pertussis. NPJ Vaccines, 2019. 4: p. 40.

**71.** de Graaf, H., et al., Investigating Bordetella pertussis colonisation and immunity: protocol for an inpatient controlled human infection model. BMJ Open, 2017. 7(10): p. e018594.

**72.** de Graaf, H., et al., Controlled Human Infection With Bordetella pertussis Induces Asymptomatic, Immunizing Colonization. Clin Infect Dis, 2020. 71(2): p. 403-411.

# SUPPLEMENTARY MATERIALS



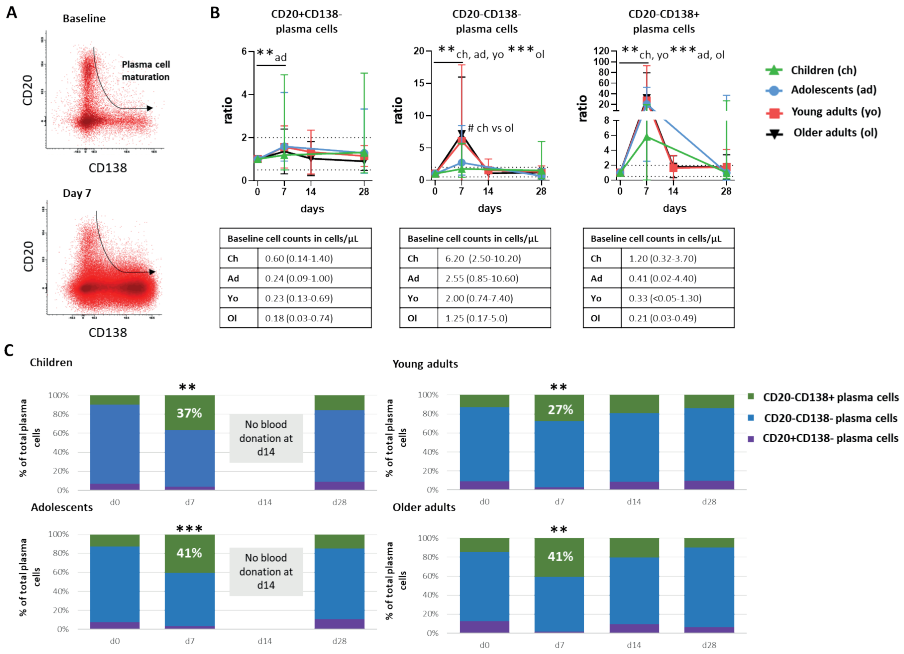

| Baseline cell counts in cells/µL | |
|---|---|
| Ch | 5795 (3397-14263) |
| Ad | 6148 (3296-7837) |
| Yo | 5054 (3224-6501) |
| Ol | 5234 (3373-7547) |

| Baseline cell counts in cells/µL | |
|---|---|
| Ch | 2551 (2128-3902) |
| Ad | 2389 (1077-3312) |
| Yo | 2017 (1699-3057) |
| Ol | 2003 (1150-3080) |

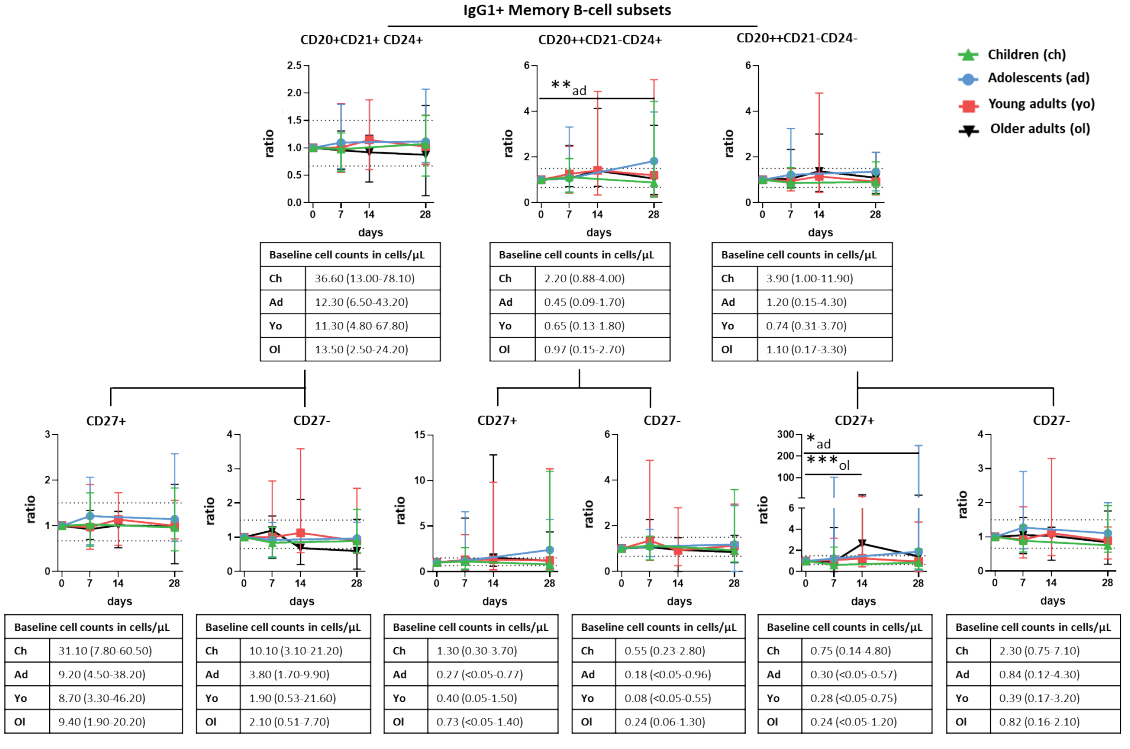
| Baseline cell counts in cells/µL | |
|---|---|
| Ch | 1806 (1132-3308) |
| Ad | 1674 (814-2466) |
| Yo | 1481 (1204-2405) |
| Ol | 1481 (794-2312) |

| Baseline cell counts in cells/µL | |
|---|---|
| Ch | 210 (123-508) |
| Ad | 268 (65-456) |
| Yo | 214 (115-713) |
| Ol | 282 (87-547) |

**Supplementary Figure 1.** No clear over-time post-vaccination changes in major populations in any of the donor groups. The post-vaccination fluctuations of (**A**) leukocytes, (**B**) lymphocytes, (**C**) T cells, and (**D**) NK cells, presented as ratio over baseline (median, min-max). Dashed linesindicate a ratio of 0.67 and 1.5 compared to baseline. Underneath each graph, the baseline cell counts per cohort are presented in cells/µL (median, min-max). To assess longitudinal changes within each cohort, Wilcoxon matched pair signed-rank test followed by Bonferroni correction was used. To test differences between cohorts at one timepoint, Kruskal-Wallis followed by Dunn's test was used, with exception of the comparison at day 14. At day 14, only blood samples from the adult cohorts were collected. Here, the Mann-Whitney test followed by Bonferroni correction was used. For longitudinal changes, only significant differences compared to baseline are shown. *, $p < 0.05$; **, $p \leq 0.01$.




**Supplementary Figure 2.** Over time maturation of total plasma cells. **(A)** Representative plots showing the phenotypical changes during plasma cell maturation. Each dot represents an individual cell. The arrow indicates the direction of changes during maturation. **(B)** Over-time quantitative changes in plasma cells belonging to different maturation stages, presented as ratio over baseline (median, min-max). Dashed lines indicate a ratio of 0.5 and 2.0 compared to baseline. Underneath each graph, the baseline cell counts per cohort are indicated in cells/µL (median, min-max). **(C)** Over-time distribution of plasma cells representing different maturation stages within total plasma cells. Median values for each population were used to construct the plots. Wilcoxon matched pair signed-ranked test followed by Bonferroni correction was used to assess longitudinal differences in percentage of CD20-CD138+ cells in total plasma cells within each cohort. Differences in the percentage CD20-CD138+ cells in total plasma cells between cohorts were assessed using Kruskal-Wallis followed by Dunn's test, but did not yield significant differences. At day 14, only blood samples from the adult cohorts were collected. Here, the Mann-Whitney test followed by Bonferroni correction was used. For longitudinal changes, only significant differences compared to baseline are shown. Significant longitudinal differences within a cohort as indicated with **, $p \leq 0.01$; ***, $p \leq 0.001$. Significant differences between cohorts at the same time point are indicated with #, $p < 0.05$.




| Baseline cell counts in cells/µL | |
|---|---|
| Ch | 36.60 (13.00-78.10) |
| Ad | 12.30 (6.50-43.20) |
| Yo | 11.30 (4.80-67.80) |
| Ol | 13.50 (2.50-24.20) |

| Baseline cell counts in cells/µL | |
|---|---|
| Ch | 2.20 (0.88-4.00) |
| Ad | 0.45 (0.09-1.70) |
| Yo | 0.65 (0.13-1.80) |
| Ol | 0.97 (0.15-2.70) |

| Baseline cell counts in cells/µL | |
|---|---|
| Ch | 3.90 (1.00-11.90) |
| Ad | 1.20 (0.15-4.30) |
| Yo | 0.74 (0.31-3.70) |
| Ol | 1.10 (0.17-3.30) |

| Baseline cell counts in cells/µL | |
|---|---|
| Ch | 31.10 (7.80-60.50) |
| Ad | 9.20 (4.50-38.20) |
| Yo | 8.70 (3.30-46.20) |
| Ol | 9.40 (1.90-20.20) |

| Baseline cell counts in cells/µL | |
|---|---|
| Ch | 10.10 (3.10-21.20) |
| Ad | 3.80 (1.70-9.90) |
| Yo | 1.90 (0.53-21.60) |
| Ol | 2.10 (0.51-7.70) |

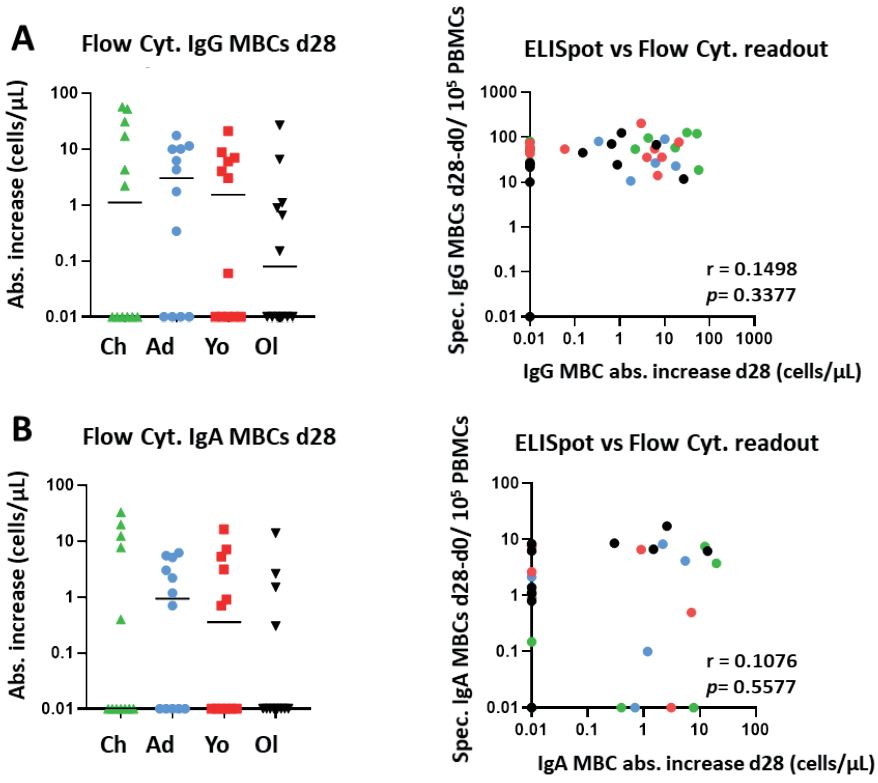
| Baseline cell counts in cells/µL | |
|---|---|
| Ch | 1.30 (0.30-3.70) |
| Ad | 0.27 (<0.05-0.77) |
| Yo | 0.40 (0.05-1.50) |
| Ol | 0.73 (<0.05-1.40) |

| Baseline cell counts in cells/µL | |
|---|---|
| Ch | 0.55 (0.23-2.80) |
| Ad | 0.18 (<0.05-0.96) |
| Yo | 0.08 (<0.05-0.55) |
| Ol | 0.24 (0.06-1.30) |

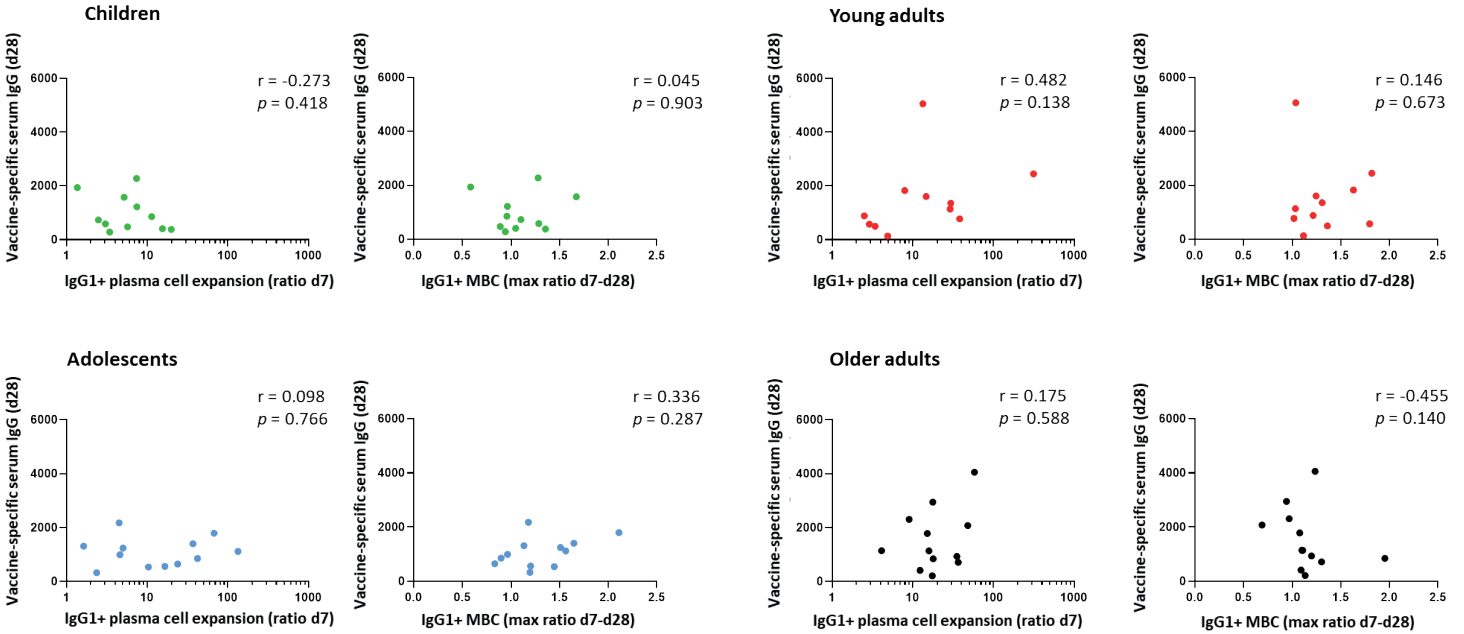
| Baseline cell counts in cells/µL | |
|---|---|
| Ch | 0.75 (0.14-4.80) |
| Ad | 0.30 (<0.05-0.57) |
| Yo | 0.28 (<0.05-0.75) |
| Ol | 0.24 (<0.05-1.20) |

| Baseline cell counts in cells/µL | |
|---|---|
| Ch | 2.30 (0.75-7.10) |
| Ad | 0.84 (0.12-4.30) |
| Yo | 0.39 (0.17-3.20) |
| Ol | 0.82 (0.16-2.10) |

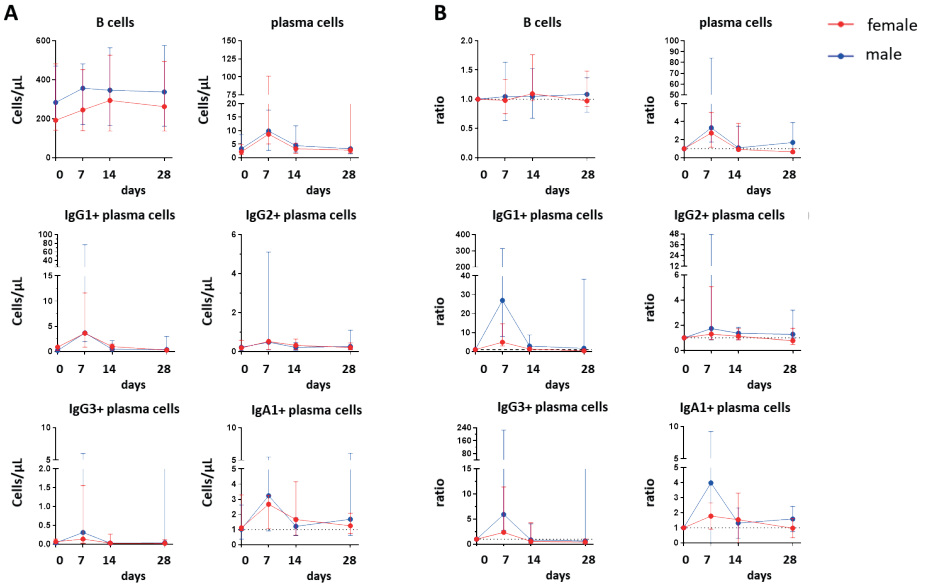
**Supplementary Figure 3.** No significant changes in IgG1+ memory B-cell subsets upon vaccination. Over- time quantitative changes in IgG1+ memory B-cell subsets, presented as ratio over baseline (median, min-max). Dashed lines indicate a ratio over baseline of 0.67 and 1.5. Underneath each graph, the baseline cell counts per cohort are presented in cells/µL (median, min-max). Wilcoxon matched pair signed-ranked test followed by Bonferroni correction was used to assess differences in ratio compared to baseline over time. For longitudinal changes, only significant differences compared to baseline are shown. *, $p < 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$.



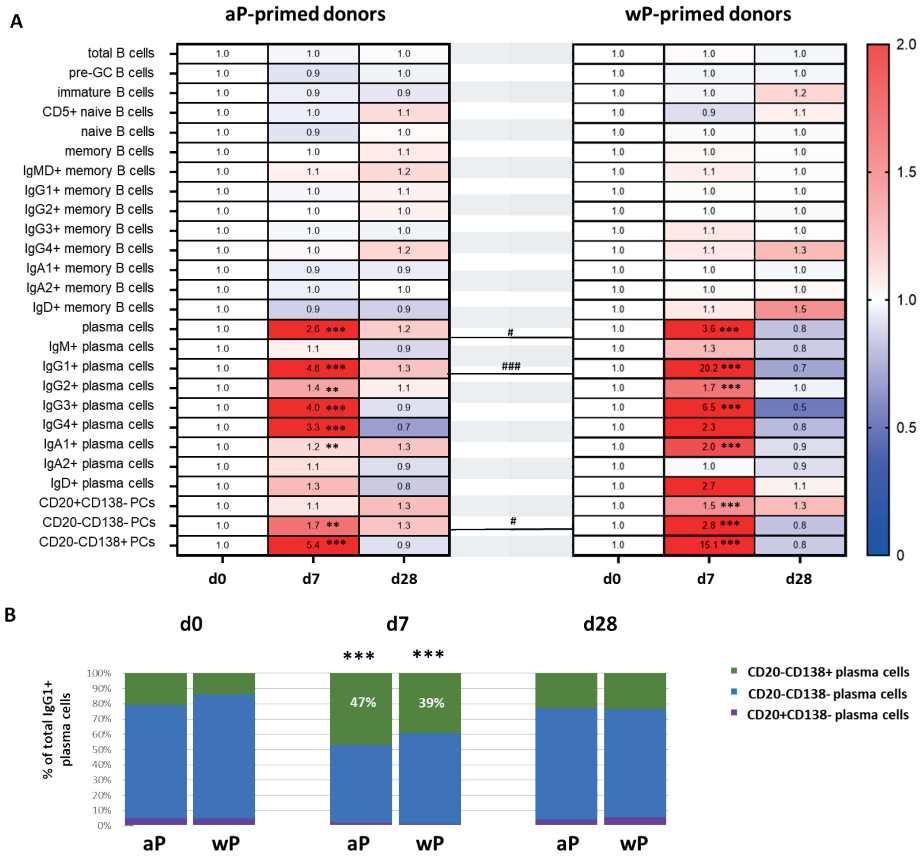

**Supplementary Figure 4.** Correlation between cellular changes as measured by flow cytometry and ELISpot. **(A)** Left panel: expansion of IgG+ Memory B cells (day 28) per individual, expressed as absolute increase in cells/µL. Right panel: correlation between the ELISpot readings and the flow cytometry readout for IgG+ Memory B cells. **(B)** Left panel: expansion of IgA+ Memory B cells (day 28) per individual, expressed as absolute increase in cells/µL. Right panel: correlation between the ELISpot readings and the flow cytometry readout for IgA+ Memory B cells. Of note; for visualization purposes, all absolute increases lower than 0.01 were set to 0.01. The original values were used to calculate the Spearman Correlations. Flow Cyt. = flow cytometry; spec.= specific (in this case, specific for the tested vaccine antigens); MBC = Memory B cells; PBMC= peripheral blood mononuclear cells; d= days after vaccination; r= Spearman's Correlation coefficient; abs.= absolute.




**Supplementary Figure 5.** Correlation between cellular changes and the vaccine-specific serum IgG level post-vaccination as determined by Spearman's Ranking Correlation per age cohort. Per cohort the left plot shows the correlation between the maximum expansion of IgG1 plasma cells (day 7) and vaccine-specific serum IgG (directed against FHA, Prn, PT and Tet) (day 28). The right plots show the correlation between the maximum expansion of IgG1 memory B cells (day 14 or day 28) and vaccine-specific serum IgG (directed against FHA, Prn, PT and Tet) (day 28). MBC = memory B cell; r= Spearman's correlation coefficient; d= days after vaccination.




**Supplementary Figure 6.** Impact of sex on cellular responses after vaccination in the young adult cohort (all wP-primed). Flow cytometry-derived cell numbers (absolute count in cells/µL (**A**) and ratio over baseline (**B**)) and their changes over time in an age-matched, wP-primed male (n= 7) and female (n= 5) cohort. Of note, for one male participant, no baseline B-cell data was available. Therefore, in the graphs showing the ratio over baseline, data of 6 males are shown, whereas absolute counts include the data of 7 males. Graphs indicate median + range. Dashed line indicates ratio of 1.0 (baseline value).




**Supplementary Figure 7.** IgG1+ and total plasma cell expansion is more prominent in non-age-matched donors after wP priming. **(A)** Heatmap showing over-time changes in memory B-cell and plasma cell subsets in aP-primed (12 children + 5 adolescents) and wP-primed (7 adolescents and 12 young adults; not the older adults, because of their uncertain vaccination status) donors. **(B)** Over-time distribution of IgG1+ plasma cells representing different maturation stages with total IgG1+ plasma cells. Median values for each population were used to construct the plots. Wilcoxon matched pair signed-ranked test followed by Bonferroni correction was used to assess longitudinal differences in percentage of CD20-CD138+ cells in total IgG1+ plasma cells within each cohort. Differences in the percentage CD20-CD138+ cells in total IgG1+ plasma cells between cohorts were assessed using Kruskal-Wallis followed by Dunn's test, but did not yield significant differences. For longitudinal changes, only significant differences compared to baseline are shown. Significant longitudinal differences within a cohort as indicated with ***, $p \leq 0.001$. Significant differences between cohorts at the same time point are indicated with #, $p < 0.05$; ###, $p \leq 0.001$. D= days after vaccination; aP = acellular pertussis vaccine; wP = whole cell pertussis vaccine.

**Supplementary Table 1.** Complete overview of the inclusion and exclusion criteria for this study. This table is adjusted from a previous manuscript describing the inclusion and exclusion criteria of the total BERT study cohort (Versteegen, P., et al., EBioMedicine, 2021)[29].

**Inclusion criteria**

In order to be eligible to participate in this study, participants must meet all of the following criteria:

| |
|---|
| • normal general health; |
| • within the right age group for the cohort; |
| • received all regular vaccines for their age group according to the Dutch NIP in the Netherlands; a copy of the vaccination booklet will be included in the participant's documents. If booklet is not available for cohorts A, B and C, vaccination status will be checked with regulatory agencies / GP. For cohort C and D this booklet might not be available due to their age; |
| • provision of written informed consent from the adult participants and parents or legal guardians of minors; |
| • willing to adhere to the protocol and be available during the study period. |

**Exclusion criteria**

Participants meeting any of the following criteria are excluded from participation in this study:

| |
|---|
| • present evidence of serious disease(s) within the last 3 months before inclusion requiring immunosuppressive or immune modulating medical treatment, such as systemic corticosteroids, that might interfere with the results of the study; |
| • chronic infection; |
| • known or suspected immune deficiency; |
| • history of any neurologic disorder, including epilepsy; |
| • previous administration of serum products (including immunoglobulins) within 6 months before vaccination and blood sampling; |
| • known or suspected allergy to any of the vaccine components (by medical history); |
| • occurrence of serious adverse events (SAEs) after primary DTwP-IPV vaccination, DTaP-IPV vaccination or any other vaccination (by medical history); |
| • vaccination with any pertussis containing vaccine other than those described in the inclusion criteria (i.e. only according to NIP); |
| • adult pertussis vaccination according to the NIP in the last 5 years (i.e. maternal vaccination); |
| • vaccination with any other diphtheria, tetanus or polio containing vaccine in the last 5 years, other than described in the NIP; |
| • children between 8 and 10 years of age eligible for cohort A who have already received the dT-IPV booster vaccination according to the Dutch NIP around 9 years of age; |
| • mixed wP and aP priming within a participant; |
| • pregnancy. |

**Supplementary Table 2.** Composition of the EuroFlow B-cell panel and technical information on the reagents for the IMI-2 PERISCOPE BERT study.

| **Marker** | CD27 | IgM | CD62L | CD24 | CD21 | CD19 | IgD | IgH-isotype panel – Subclasses (CYT-IGS-1) | CD20 | CD5 | CD138 | IgD | CD45 | CD38 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Fluorochrome** | BV421 | BV510 | BV605 | BV650 | BV711 | BV786 | FITC | - | PE CF594 | PE Cy7 | PE Cy7 | APC | Alexa Fluor 700 | APC H7 |
| **Manufacturer** | BD | Biolegend | Biolegend | BD | BD | BD | Biolegend | Cytognos | BD | BD | Biolegend | BD | BD | BD |
| **Clone** | M-T271 | MHM-88 | DREG-56 | ML5 | B-Ly4 | SJ25C1 | IA6-2 | n/a | 2H7 | L17F12 | MI15 | IA6-2 | HI30 | HB7 |
| **Volume (µl)** | 2 | 2 | 5 | 5 | 5 | 4 | 1.25 | 25 | 0.25 | 6 | 5 | 4 | 10 | 3 |
| **Membrane stain** | All markers are used for membrane stain. | | | | | | | | | | | | | |
| **Intracellular stain** | IgM BV510, IgD FITC, IgH-isotype panel- subclasses (CYT-IGS-1), IgD APC are used for additional intracellular stain. | | | | | | | | | | | | | |

**Supplementary Table 3**. Phenotypic descriptions used to define B-cell subsets stained with the EuroFlow B-cell panel by manual analysis. Of note: in this study, the automated gating and identification (AGI) module in the Infinicyt Software was used. The results obtained by the AGI-module are highly similar to, but more reproducible than, the results obtained by manual analysis. This AGI module makes use of clustering algorithms and comparison with fully annotated reference flow cytometry (FCS) data files of healthy individuals to assign clusters of events to a population. The removal of debris and doublets is not indicated in the analysis strategy below but should be performed to ensure high quality data.

| Stepwise approach (gating in 2D plots) | Phenotypic description |
|---|---|
| #1. Identification of total plasma cells | CD45+CD19dimCD38highCD21-CD24-<br>Light scatter properties are low/medium (between lymphocytes and monocytes). |
| #2. Definition of maturation stage | Least mature plasma cells: CD20+CD138-<br>Intermediate mature plasma cells: CD20-CD138-<br>Most mature plasma cells: CD20-CD138+ |
| #3. Classification of plasma cells based on isotype | IgM+, no expression of other isotype Igs<br>IgG1+, no expression of other isotype Igs<br>IgG2+, no expression of other isotype Igs<br>IgG3+, no expression of other isotype Igs<br>IgG4+, no expression of other isotype Igs<br>IgA1+, no expression of other isotype Igs<br>IgA2+, no expression of other isotype Igs<br>IgD+, no expression of other isotype Igs<br>IgH-, no Ig expression of any isotype Igs |
| #4. Classification of plasma cells based on CD62L expression | CD62L-<br>CD62L+ |
| #5. Identification of total B cells | CD45+CD19+CD20+ B cells show low light scatter characteristics (lymphocyte range) |
| #6. Identification of switched memory B-cell (MBC) subsets based on isotype. Switched MBCs express only one isotype | IgG1+, no expression of other isotype Igs<br>IgG2+, no expression of other isotype Igs<br>IgG3+, no expression of other isotype Igs<br>IgG4+, no expression of other isotype Igs<br>IgA1+, no expression of other isotype Igs<br>IgA2+, no expression of other isotype Igs |
| #7. Subclassification based on maturation/ functional CD markers | CD20+CD21+<br>Homogenous CD24 staining<br>CD20++CD21-/dim<br>CD24+<br>CD24- |
| #8. Subclassification based on CD62L/CD27 positivity | CD27+CD62L+<br>CD27+CD62L-<br>CD27-CD62L-<br>CD27-CD62L+ |
| #9. Identification of non-switched MBCs | CD27+IgM++IgD+<br>Of note, a minor subset of IgD+IgM- MBCs may be found as well. These can be classified separately. |
| #10. Subclassification based on maturation/functional CD markers | CD20+CD21+<br>Homogenous CD24 staining<br>CD20++CD21-/dim<br>CD24+<br>CD24-<br>No further subclassification in these populations. |

| Stepwise approach (gating in 2D plots) | Phenotypic description |
|---|---|
| #11. Classification of pre-germinal center (preGC) B cells | CD27-IgM+IgD+ |
| #12. Subclassification based on maturation/functional CD markers | Immature preGC B cells: CD38+CD24+CD5+CD21-/+<br>Naive CD5+ B cells: CD38-/dim CD24-/dimCD5+<br>Naive CD5- B cells:CD38-/CD24-/dimCD5- |
| #13. Subclassification of naive B cells based on maturation/functional CD markers | CD20+CD21+<br>Homogenous CD24 staining<br>CD20++CD21-/dim<br>CD24+<br>CD24- |

**Supplementary Table 4.** Baseline distribution of leukocytes, lymphocytes, T cells and NK cells (cells/µL, median (min-max)) in donor groups.

| Cohort | Children | | | Adolescents | | | Young adults | | | Older adults | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| age | 7-10 y/o | | | 11-15 y/o | | | 20-34 y/o | | | 60-70 y/o | | |
| priming background | aP | | | mixed aP and wP | | | wP | | | no data available | | |
| N= | 12 | | | 12 | | | 12 | | | 12 | | |
| Cohort data | median | min. | max. | median | min. | max. | median | min. | max. | median | min. | max. |
| Leukocytes | 5795 | 3397 | 14263 | 6148 | 3296 | 7837 | 5054 | 3224 | 6501 | 5234 | 3373 | 7547 |
| Lymphocytes | 2551 | 2128 | 3902 | 2389 | 1077 | 3312 | 2017 | 1699 | 3057 | 2003 | 1150 | 3080 |
| T cells | 1806 | 1132 | 3308 | 1674 | 814 | 2466 | 1481 | 1204 | 2405 | 1481 | 794 | 2312 |
| NK cells | 210 | 123 | 508 | 268 | 65 | 456 | 214 | 115 | 713 | 282 | 87 | 547 |
| Reference values† | Children | | | Adolescents | | | Young adults | | | Older adults | | |
| age | 5-10 y/o | | | 10-16 y/o | | | Adult cohort, no age range specified | | | | | |
| N= | 35 | | | 23 | | | 51 | | | | | |
| Reference data | median | 5th Perc. | 95th perc. | median | 5th Perc. | 95th perc. | median | | 5th Perc. | | 95th perc. | |
| Lymphocytes | 2800 | 1100 | 5900 | 2200 | 1000 | 5300 | 1800 | | 1000 | | 2800 | |
| T cells | 1900 | 700 | 4200 | 1500 | 800 | 3500 | 1200 | | 700 | | 2100 | |
| NK cells | 300 | 90 | 900 | 300 | 70 | 1200 | 30 | | 90 | | 600 | |

†Source publication for the reference values: Comans-Bitter et al., Journal of Pediatrics, 1997, doi: 10.1016/s0022-3476(97)70200-2. When using the here-presented reference values, please refer to the source publication of this data.

**Supplementary Table 5.** Spearman Ranking Correlation between IgG1+ plasma cell and memory B-cell kinetics and vaccine component-specific serum IgG.

| | | | **PT-specific IgG** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | D0 (IU/mL) | | D28 (IU/mL) | | D365 (IU/mL) | | ratio D28/D0 | | ratio D365/D0 | |
| | | | *Spearman r* | *p* | *Spearman r* | *p* | *Spearman r* | *p* | *Spearman r* | *p* | *Spearman r* | *p* |
| IgG1+ PCs | Total | D7-0 | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| | | ratio D7 | ns | ns | 0.3224 | 0.0289 | **0.3809** | **0.009** | ns | ns | ns | ns |
| | MS1 | D7-0 | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| | | ratio D7 | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| | MS2 | D7-0 | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| | | ratio D7 | ns | ns | **0.3895** | **0.0075** | **0.3884** | **0.0077** | ns | ns | ns | ns |
| | MS3 | D7-0 | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| | | ratio D7 | ns | ns | ns | ns | 0.3043 | 0.0446 | ns | ns | ns | ns |
| IgG1+ MBCS | | ratio D14/28 | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| | | | **Prn-specific IgG** | | | | | | | | | |
| | | | D0 (IU/mL) | | D28 (IU/mL) | | D365 (IU/mL) | | ratio D28/D0 | | ratio D365/D0 | |
| | | | *Spearman r* | *p* | *Spearman r* | *p* | *Spearman r* | *p* | *Spearman r* | *p* | *Spearman r* | *p* |
| IgG1+ PCs | Total | D7-0 | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| | | ratio D7 | ns | ns | ns | ns | ns | ns | **0.4347** | **0.0025** | **0.4628** | **0.0012** |
| | MS1 | D7-0 | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| | | ratio D7 | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| | MS2 | D7-0 | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| | | ratio D7 | ns | ns | ns | ns | ns | ns | **0.4384** | **0.0023** | **0.4277** | **0.003** |
| | MS3 | D7-0 | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| | | ratio D7 | ns | ns | ns | ns | ns | ns | ns | ns | 0.3718 | 0.013 |
| IgG1+ MBCS | | ratio D14/28 | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| | | | **FHA-specific IgG** | | | | | | | | | |
| | | | D0 (IU/mL) | | D28 (IU/mL) | | D365 (IU/mL) | | ratio D28/D0 | | ratio D365/D0 | |
| | | | *Spearman r* | *p* | *Spearman r* | *p* | *Spearman r* | *p* | *Spearman r* | *p* | *Spearman r* | *p* |
| IgG1+ PCs | Total | D7-0 | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| | | ratio D7 | ns | ns | ns | ns | 0.3589 | 0.0143 | ns | ns | ns | ns |
| | MS1 | D7-0 | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| | | ratio D7 | -0.3379 | 0.033 | ns | ns | ns | ns | 0.3276 | 0.0391 | 0.3664 | 0.02 |
| | MS2 | D7-0 | ns | ns | 0.2948 | 0.0442 | ns | ns | ns | ns | ns | ns |
| | | ratio D7 | ns | ns | 0.2911 | 0.0497 | 0.3527 | 0.0162 | ns | ns | 0.2929 | 0.0482 |
| | MS3 | D7-0 | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| | | ratio D7 | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| IgG1+ MBCS | | ratio D14/28 | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |

Tested were total IgG1+ Memory B cells (max. expansion at d14 or d28, expressed as ratio over baseline), IgG1+ plasma cells and the three defined maturation stages (MS1-2-3), both absolute increase in cell count (D7-D0) and ratio to baseline at D7 were correlated to IgG levels (IU/mL or ratio to baseline). All correlations with a *p*-value <0.05 are shown in the table. Correction for multiple testing was applied using Bonferroni to adjust for the correlation with 5 different serological readouts, therefore, only *p*-values <0.01 were considered significant. These values are indicated in **bold**. MS1: CD20+CD138- plasma cells, MS2: CD20-CD138- plasma cells, MS3: CD20-CD138+ plasma cells. D= Days post-vaccination.

**Supplementary Table 6.** Spearman Ranking Correlation between IgA1+ plasma cell and IgA memory B-cell kinetics and vacGcine component-specific serum IgA.

| | | | **PT-specific IgA** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | D0 (IU/mL) | | D28 (IU/mL) | | D365 (IU/mL) | | ratio D28/D0 | | ratio D365/D0 | |
| | | | *Spearman r* | *p* | *Spearman r* | *p* | *Spearman r* | *p* | *Spearman r* | *p* | *Spearman r* | *p* |
| IgA1+ PCs | Total | D7-0 | ns | ns | 0.3251 | 0.0275 | ns | ns | ns | ns | ns | ns |
| | | ratio D7 | ns | ns | **0.4047** | **0.0053** | 0.3691 | 0.0116 | ns | ns | ns | ns |
| | MS1 | D7-0 | ns | ns | ns | ns | ns | ns | -0.327 | 0.0265 | ns | ns |
| | | ratio D7 | ns | ns | -0.3111 | 0.0353 | ns | ns | ns | ns | ns | ns |
| | MS2 | D7-0 | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| | | ratio D7 | ns | ns | 0.3384 | 0.0214 | ns | ns | ns | ns | ns | ns |
| | MS3 | D7-0 | ns | ns | 0.3473 | 0.018 | ns | ns | ns | ns | ns | ns |
| | | ratio D7 | ns | ns | **0.4466** | **0.0027** | ns | ns | 0.3842 | 0.011 | ns | ns |
| IgA1+ MBCS | | ratio D14/28 | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| IgA2+ MBCs | | ratio D14/28 | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| | | | **Prn-specific IgA** | | | | | | | | | |
| | | | D0 (IU/mL) | | D28 (IU/mL) | | D365 (IU/mL) | | ratio D28/D0 | | ratio D365/D0 | |
| | | | *Spearman r* | *p* | *Spearman r* | *p* | *Spearman r* | *p* | *Spearman r* | *p* | *Spearman r* | *p* |
| IgA1+ PCs | Total | D7-0 | ns | ns | 0.3328 | 0.0238 | ns | ns | ns | ns | ns | ns |
| | | ratio D7 | ns | ns | 0.3451 | 0.0189 | 0.3702 | 0.0113 | ns | ns | 0.3295 | 0.0253 |
| | MS1 | D7-0 | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| | | ratio D7 | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| | MS2 | D7-0 | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| | | ratio D7 | ns | ns | 0.3364 | 0.0223 | 0.3037 | 0.0402 | ns | ns | ns | ns |
| | MS3 | D7-0 | ns | ns | 0.3203 | 0.03 | ns | ns | 0.3055 | 0.039 | ns | ns |
| | | ratio D7 | ns | ns | **0.434** | **0.0036** | 0.3792 | 0.0121 | 0.3844 | 0.0109 | 0.3309 | 0.0302 |
| IgG1+ MBCS | | ratio D14/28 | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| IgA2+ MBCs | | ratio D14/28 | 0.3688 | 0.0117 | ns | ns | ns | ns | ns | ns | ns | ns |
| | | | **FHA-specific IgA** | | | | | | | | | |
| | | | D0 (IU/mL) | | D28 (IU/mL) | | D365 (IU/mL) | | ratio D28/D0 | | ratio D365/D0 | |
| | | | *Spearman r* | *p* | *Spearman r* | *p* | *Spearman r* | *p* | *Spearman r* | *p* | *Spearman r* | *p* |
| IgA1+ PCs | Total | D7-0 | ns | ns | **0.5327** | **0.0001** | **0.4398** | **0.0022** | 0.3488 | 0.0175 | 0.3722 | 0.0109 |
| | | ratio D7 | ns | ns | **0.4831** | **0.0007** | **0.3845** | **0.0083** | 0.3728 | 0.0107 | 0.3665 | 0.0122 |
| | MS1 | D7-0 | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| | | ratio D7 | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| | MS2 | D7-0 | ns | ns | **0.4524** | **0.0016** | **0.378** | **0.0096** | 0.3154 | 0.0327 | 0.3162 | 0.0323 |
| | | ratio D7 | ns | ns | **0.4424** | **0.0021** | 0.3621 | 0.0134 | **0.3885** | **0.0076** | **0.4069** | **0.005** |
| | MS3 | D7-0 | ns | ns | **0.4328** | **0.0027** | 0.3375 | 0.0218 | ns | ns | 0.3191 | 0.0306 |
| | | ratio D7 | ns | ns | **0.5749** | **<0,0001** | **0.4787** | **0.0012** | ns | ns | **0.3947** | **0.0088** |
| IgG1+ MBCS | | ratio D14/28 | ns | ns | ns | ns | ns | ns | 0.3166 | 0.032 | **0.3783** | **0.0095** |
| IgA2+ MBCs | | ratio D14/28 | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |

Tested were total IgA1+ and IgA2+ memory B cells (max. expansion at d14 or d28 expressed as ratio over baseline), IgA1+ plasma cells and the three defined maturation stages (MS1-2-3), both absolute increase in cell count (D7-D0) and ratio to baseline at D7 were correlated to IgA levels (IU/mL or ratio to baseline). All correlations with a *p*-value <0.05 are shown in the table. Correction for multiple testing was applied using Bonferroni to adjust for the correlation with 5 different serological readouts, therefore, only *p*-values <0.01 were considered significant. These values are indicated in **bold**. MS1: CD20+CD138- plasma cells, MS2: CD20-CD138- plasma cells, MS3: CD20-CD138+ plasma cells. D= days post-vaccination.

# Chapter 7

# Long-term immunogenicity upon pertussis booster vaccination in young adults and children in relation to priming vaccinations in infancy

P. Versteegen, A. A. Bonačić Marinović, P. G. M. van Gageldonk, S. van der Lee, L. H. Hendrikx, E. A. M. Sanders, G. A. M. Berbers, and A. M. Buisman.

Vaccines 10, no. 5 (Apr 28 2022): 693.

## ABSTRACT

Booster vaccinations for pertussis are advised in many countries during childhood or adulthood. In a phase IV longitudinal interventional study, we assessed long-term immunity following an extra pertussis booster vaccination in children and adults. Children (9 years of age) were primed in infancy with either the Dutch whole cell pertussis (wP) vaccine (n = 49) or acellular pertussis (aP) vaccines (n = 59), and all children received a preschool aP booster. Adults (25–29 years, n = 86) were wP-primed in infancy and did not receive a preschool booster. All were followed-up for approximately 6 years. After the additional booster, antibody responses to pertussis were more heterogeneous but generally higher in adults compared with children, and additional modelling showed that antibody concentrations remained higher for at least a decade. Serologic parameters indicative of recent pertussis infection were more often found in aP-primed children (12%) compared with wP-primed individuals (2%) (p = 0.052). This suggests that the aP booster vaccination in aP-primed children offers less long-term protection against pertussis infection and consequently against transmission. Together, these data show that aP priming in combination with aP boosting may not be sufficient to prevent circulation and transmission, while wP-primed adults may benefit from enhanced long-lasting immunity.

## INTRODUCTION

Pertussis is an infectious disease that may have a severe course and can be life-threatening, particularly in infancy [1]. Older adults and individuals with pulmonary comorbidities are prone to its complications [2]. The introduction of a whole cell pertussis (wP) vaccine in the 1940–1950s almost completely eliminated pertussis cases in countries with high vaccination coverage during the first decades after implementation [3]. Although global pertussis incidence is still decreasing, a resurgence in pertussis has been seen in many countries over recent decades, despite high vaccine coverage [4]. In the Netherlands, an increase in pertussis reporting began in 1996, presumably following the introduction, in the early 1990s, of a Dutch wP vaccine with low effectiveness [5, 6]. Since then, several changes in the Dutch national immunisation programme (NIP) have been made (**Supplementary Table 1**). Currently, acellular pertussis (aP) immunisations are advised at 3, 5, and 11 months of age and at 4 years of age but not for school aged children or adults.

Because of the reactogenicity profile of wP vaccines, many high-income countries switched to aP vaccines when these became available in the 1990s [7, 8]. Next to the lower reactogenicity, the short-term immunogenicity based on humoral responses of the aP vaccines seemed superior compared with wP vaccines. Following the switch to aP vaccines, however, an increase in pertussis incidence was noted in many countries [9]. The Netherlands switched from wP to aP vaccines in 2005 and later observed an increase in reported disease incidence [6, 8]. The increase was observed in children, adolescents, and adults who can serve as an important reservoir of pertussis circulation and may be the source of transmission to infants who need to be protected to avoid severe disease or death [10]. For this reason, many countries added aP booster doses not only at preschool age, but also for school-aged children, adolescents, adults, and older adults or specific target groups such as pregnant women, healthcare workers, and military conscripts [11-14].

Long-term immunogenicity studies that compare adolescent and adult immune responses to aP booster vaccinations are rare and in general show similar antibody kinetics [15, 16]. However, some differences have been reported, with initially higher humeral responses against pertussis toxin (Ptx) in adolescents, while follow-up antibody concentrations against pertactin (Prn) were higher in adults. In both adolescents and adults, Ptx-specific antibody concentrations declined to almost pre-vaccination levels within 5 years, although in a prediction model, adult long-term antibody concentrations would remain above a presumed protective level of 20 international units (IU)/mL for over 9 years after aP booster vaccination in the case of wP-primed young adults [17]. In paediatric immunogenicity studies, aP- and wP-priming in infancy was compared after an aP booster vaccination at 4 years and an additional booster at 9 years of age. Higher Ptx and filamentous haemagglutinin (FHA) immunoglobulin (Ig) G antibody concentrations one month post the

additional aP booster vaccination in wP-primed children at age 9 years were reported [18]. The differences in Ptx and FHA IgG had disappeared one year post-booster vaccination.

Here we investigate differences in the long-term pertussis antibody concentrations after an additional aP booster vaccination at 9 years of age comparing aP- and wP-primed children. With respect to age, wP-primed children and wP-primed adults 25–29 years of age were followed-up until 6 years post the additional aP booster vaccination. These types of data provide information for vaccine policy concerning additional booster vaccinations in the current Dutch NIP to reduce infection, transmission, and the disease burden of *Bordetella pertussis* in the Netherlands.

Our data implies that extra aP pertussis booster vaccinations for aP-primed children offer only limited protection against infection and circulation of *B. pertussis*.

## MATERIALS AND METHODS

### Study design and participants

Two groups of healthy children and one group of adult participants included in this immunology study originated from three different interventional cohort studies in the Netherlands as previously described, with the flow scheme presented in **Figure 1** [17-19]. For the present long-term follow-up study, a single additional sampling timepoint around 6 years post the study aP booster vaccination was added in all three study cohorts. Paediatric study participants were routinely primed in infancy with either the Dutch wP vaccine or different aP vaccines at 2, 3, 4 and 11 months of age. All children were aP-boosted at 4 years, and at 9 years of age received the additional study booster vaccine containing diphtheria toxoid, tetanus toxoid, pertussis toxoid, filamentous haemagglutinin, pertactin, and inactivated poliovirus (dTap3-IPV, Boostrix-IPV, GlaxoSmithKline (GSK), Rixensart, Belgium). This was in 2009 for wP-primed children and in 2013 for aP-primed children. Results of previous studies at baseline, 28 days post-vaccination and one-year post-vaccination are reported [18, 19]. For the present study, a single additional blood sample was drawn by venepuncture 5 years and 8 months post-vaccination in wP-primed children (study number 2013-001864-50/NTR4089) and by fingerprick 6 years and 9 months post-vaccination in the aP-primed children (study numberISRCTN644117538) [18, 19]. In addition, young adults, who were primed in infancy with the Dutch wP vaccine at 3, 4, 5 and 11 months without a booster at 4 years, received the study dTap3 booster (Boostrix, GSK) in 2014 at age 25–29 years. Data at baseline, day 14, day 28, 1 year, and 2 years post the aP booster vaccination are published [17]. For the current study, a single additional blood sample was collected by fingerprick 6 years and 3 months post the booster vaccination in these adults (study number 2013-005355-32/NTR4494) [17]. An overview of timelines and recruitment regions is provided in **Supplementary Figure 1**, and the cumulative local disease notifications in the included regions at times of the studies can

be viewed in **Supplementary Figure 2**. All participants filled out a questionnaire at the last timepoint concerning information regarding self-reported, laboratory confirmed and/or clinically treated pertussis infection during the study and regarding extra pertussis vaccinations (i.e., a maternal aP booster) during the follow-up period (in addition to the study booster vaccination).

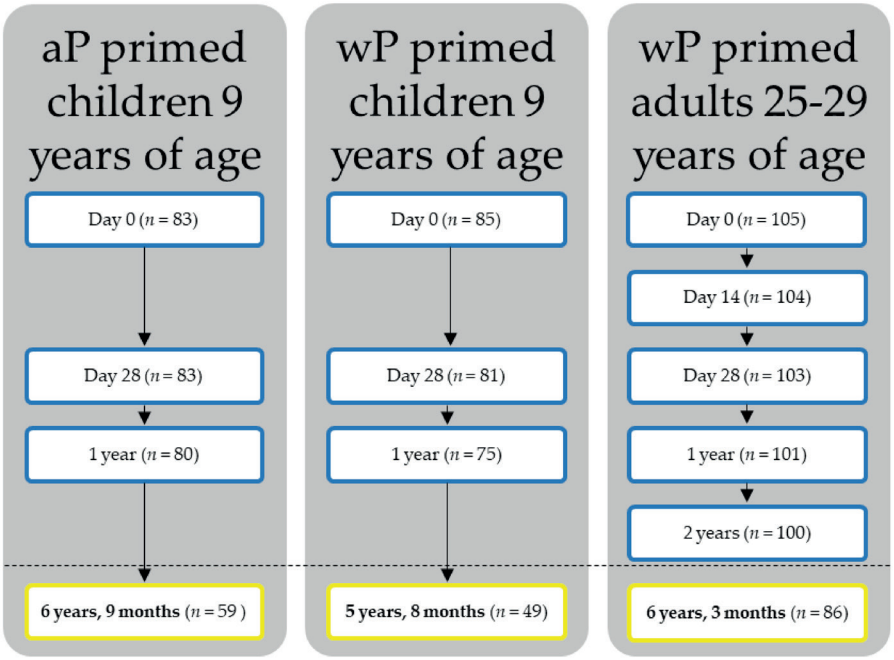



**Figure 1.** Flow scheme. aP: acellular pertussis; wP: whole cell pertussis.

### Serological analysis

The small volumes obtained per participant were sufficient to measure serum IgG concentrations against Ptx (Netherlands Vaccine Institute (NVI)), Bilthoven, Netherlands), FHA (Kaketsuken, Kumamoto, Japan), Prn [20], diphtheria toxoid (Dtxd) (NVI), and tetanus toxin (Ttx) (T3194, Sigma Aldrich, Saint Louis, MO, USA) and were quantified using a fluorescent-bead-based multiplex immunoassay (MIA) with independent duplicates, as previously described [21, 22].

Briefly, the conjugated fluorescent microbeads were incubated with plasma or serum samples in two dilutions (200 and 4000), and a reference serum in a dilution series, and control sera on each plate. The measurement was performed using a BioPlex LX 200 combined with BioPlex Manager 6.2 (Bio-Rad Laboratories, Hercules, CA, USA). To express

antibody concentrations in international units (IU)/mL, an in-house standard, calibrated on the pertussis antiserum (human) 1st WHO International Standard, was used. For each analyte, the mean fluorescent intensity was converted to IU/mL by interpolation from a five-parameter logistic standard curve. The lower limit of quantification (LLOQs) was 0.85 IU/mL for Ptx, 0.82 IU/mL for FHA, 1.0 IU/mL for Prn, 0.001 IU/mL for Dtxd, and 0.001 IU/mL for Ttx. For Dtxd and Ttx, 0.1 IU/mL was defined as the cut-off of protection [23, 24]. For IgG-Ptx, 20 IU/mL was defined as arbitrary cut-off for protection against clinical disease [25-27]. Pertussis infection at baseline was defined as IgG-Ptx ≥100 IU/mL in the absence of a recent vaccination which conforms to the Dutch pertussis infection diagnostic criterion using a single serum/plasma sample [28]. Infection during the study was defined as a three-fold increase in IgG-Ptx between the 1-year timepoint and the last timepoint, combined with IgG-Ptx ≥20 IU/mL at the last timepoint, which conforms to the Dutch pertussis infection diagnostic criterion using a paired serum/plasma sample [28].

### Stastical analyses

The primary outcome is the difference in IgG antibody concentration kinetics between the age groups and between the different priming vaccination backgrounds for pertussis toxin (Ptx), filamentous haemagglutinin (FHA), pertactin (Prn), diphtheria toxoid (Dtxd), and tetanus toxin (Ttx).

At all study timepoints, geometric mean concentrations (GMCs) were calculated for the three cohorts. Additionally, the GMCs at the last timepoints were separately calculated for (1) participants who developed a pertussis infection during the study based on antibody concentrations as indicated in the serological analysis, (2) participants who received an extra vaccination during the study (in addition to the study intervention) indicated by the questionnaire, and (3) participants who did not get infected nor received an extra pertussis vaccine during the study. One participant was excluded from all analyses because antibody concentrations on multiple antigens did not match the questionnaire nor the patterns of the different antigens measured.

Predictions from a bi-exponential IgG antibody decay model was used to compare antibody decay rates among the cohorts [29-31]. This model gave insight in kinetics and offered us the possibility to calculate the estimated duration of protection. The model was fitted under a Bayesian statistical framework to the participant antibody data by using Markov chain Monte Carlo simulations run with the sampling software JAGS, and interfaced with the R Statistical Software [32, 33]. Pertussis antigen data from participants with a possible recent pertussis infection at baseline were excluded from the decay model because of potential recent infection at the start of the study (diagnostic criterion based on a single serum/plasma sample) [28]. The final datapoints from participants who became infected during the study and participants who received an extra vaccination during the study

(based on the questionnaire) were also excluded from the decay model. Additionally, the last datapoint from participants who showed an increase in antibody concentration between the 1-year timepoint and the last timepoint were excluded from the decay model for the concerning antigen. Although they might not have met the criteria for pertussis infection using a paired serum/plasma sample, natural boosting with *B. pertussis* could not be excluded; an increase in diphtheria toxoid (Dtxd) and tetanus toxin (Ttx) could have been caused by a Dtxd and/or tetanus toxoid (Ttxd) containing vaccination, since this was not specifically asked in the questionnaire.

The proportion of individuals with protective antibody concentrations was calculated based on the decay model per study cohort based on a Ttx and Dtxd IgG-antibody concentration cut-off of 0.1 IU/mL against tetanus and diphtheria respectively, and an arbitrary cut-off of 20 IU/mL for pertussis toxin (Ptx) for the proportion with protective antibody concentrations against pertussis disease [23-27].

Serum/plasma infection prevalence during the study was also studied, including a 2-step risk analysis for contracting pertussis. Step 1: we included all participants to calculate a crude odds ratio to determine whether there were risk factors independently associated with an increased chance of contracting pertussis, using logistic regression analysis. All variables were first tested in a univariate model and variables with a p value < 0.1 were included in the multivariable model. By stepwise backward selection, variables independently associated with infection were identified. Step 2: to avoid the influence of age or priming vaccination background, significances found in the multivariable model were analysed using an appropriate statistical test comparing two out of three cohorts: either the acellular pertussis (aP) primed children to the whole cell pertussis (wP) primed children or the wP-primed children to the wP-primed adults.



## RESULTS

### Geometric mean antibody concentrations (GMCs) per timepoint

To investigate differences in the long-term immunogenicity of an aP booster between age groups and vaccination backgrounds, we measured antibody GMCs in aP-primed children, wP-primed children, and wP-primed adults at baseline, 28 days, 1 year, and approximately 6 years post the aP booster vaccination. For adults, there were additional timepoints at 14 days and 2 years post the aP booster vaccination. Antibody GMC data are presented in **Table 1** and the individual responses in **Supplementary Figure 3**. Antibody concentrations in adults were highest 14 days post-vaccination for all antigens. Children showed the highest antibody concentrations 28 days post-vaccination; no blood was drawn at day 14. Approximately 6 years post-vaccination, GMCs still tended to be higher compared with baseline in all three study groups. Infection during the study was defined as a three-fold increase in IgG-Ptx between the one-year timepoint and the last follow-up timepoint after

approximately 6 years, combined with IgG-Ptx ≥20 IU/mL at the last timepoint. Based on this definition, 12% (7/59) of the aP-primed paediatric cohort contracted pertussis between 1 year and 6–7 years post the booster vaccination in contrast to 2% (1/49) in the wP-primed paediatric cohort between 1 year and 5–6 years post the booster vaccination. No adults met the criterion for pertussis infection between 1 year and 6 years post the booster vaccination, while 21% (18/85) of adults received an extra pertussis vaccination during the study (in addition to the study intervention). This included vaccination during pregnancy, or incidentally was as a work-related requirement. In both paediatric cohorts, no additional pertussis vaccinations were reported, though in contrast to adults, all had been routinely aP-boosted at 4 years of age.

**Table 1.** Geometric mean concentrations calculated per cohort per timepoint.

| | N= | GMC Ptx (95% CI) | GMC FHA (95% CI) | GMC Prn (95% CI) | GMC Dtxd (95% CI) | GMC Ttx (95% CI) |
|---|---|---|---|---|---|---|
| Children aP primed[1] | | | | | | |
| Baseline | 83 | 7 (5-9) | 27 (23-33) | 36 (29-45) | 0.04 (0.03-0.05) | 0.35 (0.29-0.44) |
| 28 days | 83 | 68 (57-81) | 158 (140-179) | 307 (267-352) | 0.61 (0.49-0.75) | 7.44 (6.31-8.76) |
| 1 year | 80 | 19 (15-24) | 60 (50-71) | 99 (84-117) | 0.15 (0.12-0.19) | 1.94 (1.65-2.30) |
| 6 years 9 months | 59 | 14 (10-19) | 46 (38-57) | 46 (36-60) | 0.05 (0.04-0.07) | 3.69 (2.95-4.61)* |
| 6y 9m infected | 7 (12%) | 57 (37-87) | 128 (80-202) | 84 (22-319) | N/A | N/A |
| 6y 9m non-infected | 52 (88%) | 12 (8-16) | 41 (33-50) | 43 (33-55) | N/A | N/A |
| Children wP primed[2] | | | | | | |
| Baseline | 83 | 7 (5-10) | 34 (26-44) | 13 (10-18) | 0.05 (0.04-0.06) | 0.46 (0.38-0.56) |
| 28 days | 81 | 112 (91-139) | 282 (244-325) | 343 (275-429) | 0.88 (0.69-1.12) | 9.10 (7.90-10.5) |
| 1 year | 79 | 24 (18-30) | 105 (92-121) | 93 (70-124) | 0.19 (0.14-0.25) | 2.09 (1.78-2.45) |
| 5 years 8months | 49 | 13 (9-18) | 34 (27-43) | 34 (24-47) | 0.07 (0.05-0.10) | 0.66 (0.52-0.82) |
| 5y 8m infected | 1 (2%) | 41 (-) | 183 (-) | 43 (-) | N/A | N/A |
| 5y 8m non-infected | 48 (98%) | 13 (9-18) | 33 (27-41) | 33 (23-47) | N/A | N/A |
| Adults wP primed[3] | | | | | | |
| Baseline | 104 | 5 (4-7) | 11 (8-13) | 11 (8-14) | 0.09 (0.07-0.11) | 1.28 (1.07-1.53) |
| 14 days | 103 | 130 (94-179) | 408 (343-485) | 369 (277-492) | 1.56 (1.30-1.89) | 11.7 (10.1-13.5) |
| 28 days | 102 | 122 (93-161) | 341 (289-402) | 364 (275-483) | 1.27 (1.07-1.52) | 9.44 (8.36-10.7) |
| 1 year | 100 | 43 (33-55) | 133 (110-160) | 186 (136-253) | 0.35 (0.29-0.43) | 2.97 (2.63-3.34) |
| 2 years | 99 | 34 (26-44) | 109 (90-132) | 146 (107-199) | 0.24 (0.20-0.29) | 2.15 (1.90-2.43) |
| 6 years 3 months | 85 | 32 (25-43) | 72 (59-89) | 132 (97-180) | 0.23 (0.19-0.29) | 2.47 (2.13-2.86) |
| 6y 3m extra vaccinated | 18 (21%) | 84 (48-146) | 131 (92-187) | 214 (114-401) | 0.38 (0.22-0.65) | 3.69 (2.86-4.77) |
| 6y 3m not extra vaccinated | 67 (79%) | 25 (19-34) | 62 (49-78) | 116 (81-166) | 0.20 (0.17-0.25) | 2.21 (1.88-2.61) |

If relevant, the group was additionally split up to represent infected individuals and/or participants who received an extra vaccination during the study next to the study intervention. [1] aP priming vaccinations at 2, 3, 4, and 11 months of age and aP boosted at 4 years of age. [2] wP priming vaccinations at 2, 3, 4, and 11 months of age and aP boosted at 4 years of age. [3] wP priming vaccinations at 3, 4, 5 and 11 months of age, no preschool booster. *Antibody concentrations went up because of meningococcal A,C,W,Y catch-up vaccination as the concerning vaccine contained tetanus toxoid conjugate. N: number; GMC: geometric mean concentration, Ptx: pertussis toxin; CI: confidence interval; FHA: filamentous haemagglutinin; Prn: pertactin; Dtxd: diphtheria toxoid; Ttx: tetanus toxin; aP: acellular pertussis; wP: whole cell pertussis; N/A: not applicable.

## Antibody kinetics

In **Figure 2**, we present model predicted antibody concentrations up to 10 years (120 months) post-vaccination for the five vaccine antigens. This was based on the majority of the participants' individual datapoints over time until approximately 6 years post-vaccination, as presented in **Supplementary Figure 3**. For all antigens, we observed an increase in antibody concentrations caused by vaccination which peaked within the first month post-vaccination, followed by a biphasic antibody decay with a rapid decline in the first phase, as described in previous studies, with 1-year follow-up and a slower decline in the second phase between 1 year and 6 years [17-19].

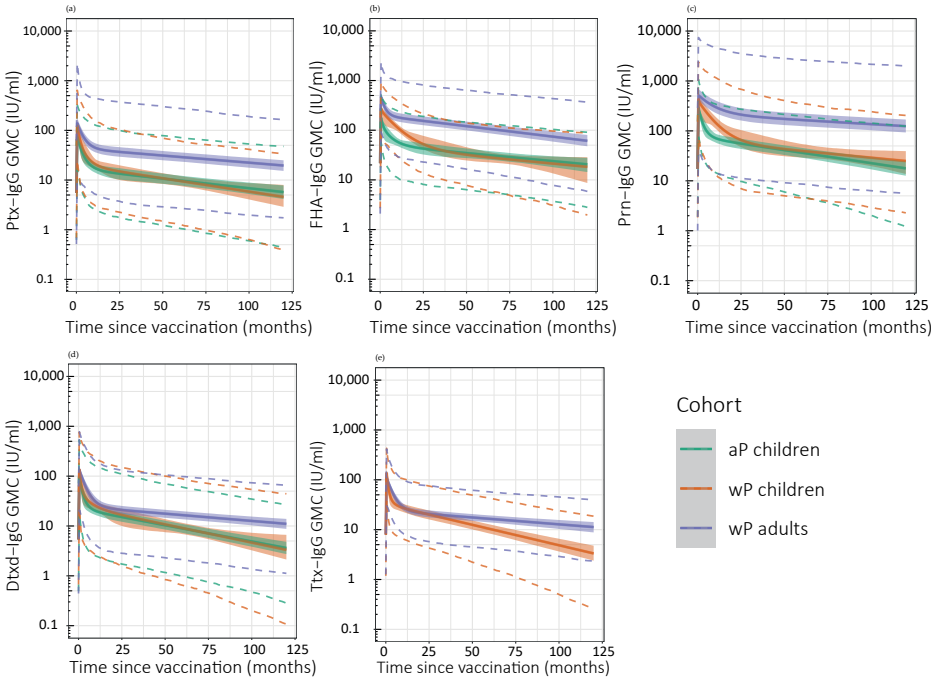



**Figure 2.** Modelled IgG antibody concentrations from time of vaccination (0 months) up to 10 years (120 months) post-vaccination in acellular pertussis (aP) primed children, whole cell pertussis (wP) primed children, and wP-primed young adults, as the colours in the legend indicate. Modelled geometric mean IgG concentrations are shown (solid lines) with their respective 95% confidence interval (ribbons). Dashed lines show the range where 95% of most probable predictions lie. Datapoints underlying this model can be viewed in **Supplementary Figure 3**. Acellular primed children are missing in the tetanus toxin model because of interference from another (non-pertussis) vaccine in between the last two timepoints. IU: international units; Ptx: pertussis toxin; FHA: filamentous haemagglutinin; Prn: pertactin; Dtxd; diphtheria toxoid; Txt: tetanus toxin; IgG: immunoglobulin G. Modelled IgG antibody concentrations for (**a**): pertussis toxin, (**b**): filamentous haemagglutinin; (**c**): pertactin; (**d**): diphtheria toxoid; and (**e**): tetanus toxin.

For Ptx, the initial peak was higher in wP-primed children and adults compared with aP-primed children (**Figure 2a**). The initial decline in both wP- and aP-primed children started early and resulted in similar antibody concentrations and antibody kinetics in both groups within a year after vaccination. Adults tended to have a slower decline in the second phase leading to a higher upkeep of Ptx antibodies over time, however, the distribution of antibody concentrations was more heterogeneous in adults at all times compared to children.

For FHA, the highest peak in antibody concentrations was seen in wP-primed adults, followed by wP-primed children, and was lowest in aP-primed children (**Figure 2b**). Antibody kinetics for wP- and aP-primed children were initially different. Children with a wP-priming background tended to have a slower initial decline, but the duration of decline seemed to be over a longer period compared with aP-primed children, resulting in similar concentrations between both groups from approximately 30 months (2.5 years) post the booster vaccination onwards. Adults had similar FHA antibody kinetics compared to aP-primed children, but since they started with higher peak concentrations, their antibody concentrations remain higher for a longer period.

The Prn antibody peak concentrations were similar for the three groups, but the initial and second decay phases were much slower in the adults compared to both paediatric groups (**Figure 2c**). Similar to the responses against FHA, the initial decline in Prn-specific antibody concentrations in wP-primed children was slower compared to aP-primed children with a longer duration, resulting in similar kinetics between wP- and aP-primed children from approximately 30 months (2.5 years) post-vaccination onwards.



The peak concentrations for Dtxd antibodies were slightly higher in adults, but the main difference between wP-primed adults and both paediatric groups is the extremely slow second decay phase leading to higher upkeep of antibodies in adults over time (**Figure 2d**).

For Ttx, antibody concentrations of only the wP-primed adults and children were compared since the aP-primed children received a Ttxd conjugated meningococcal vaccine during the study, which boosted their immune response to Ttxd (**Figure 2e**). Antibody decay differences between the adults and the children were similar to that of Dtxd, again showing an extremely slow second decay phase in the adult cohort compared to the children.

### Proportion with protective antibody concentrations

**Figure 3** shows the modelled proportion of individuals protected against pertussis, diphtheria, and tetanus per cohort. The presumed protection against clinical pertussis (arbitrary cut-off of IgG-Ptx ≥20 IU/mL) over 10 years (120 months) is illustrated in **Figure 3a**. The height of the peak concentration as well as the speed of the initial fast decay and

the second slow decay phase influenced the proportion of participants with protective antibody concentrations at a certain timepoint. This resulted in 50% of children with antibody concentrations already below the limit of protection against clinical pertussis in both paediatric cohorts around 1 year (12 months) post-vaccination, whereas in the wP-primed adults this 50% point was only reached around 7.5 years (90 months) post-vaccination.

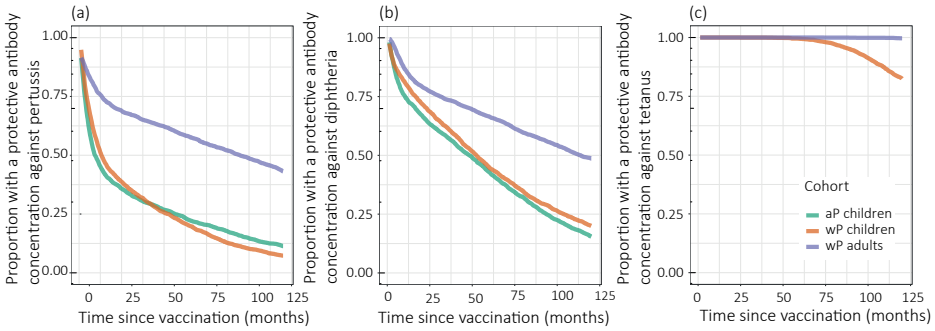



**Figure 3.** The modelled proportion with protective antibody concentrations is based on the antibody decay model (**Figure 2**). For pertussis toxin, an arbitrary cut-off of protection of ≥20 IU/mL was used. For both diphtheria toxoid and tetanus toxin, the WHO cut-off for clinical protection of 0.1 IU/mL was used. Acellular primed children are missing in the tetanus toxin model because of interference from another (non-pertussis) vaccine in between the last two timepoints. aP: acellular pertussis; wP: whole cell pertussis. The modelled proportion with protective antibody concentrations for (**a**): pertussis; (**b**): diphtheria; and (**c**): tetanus.

The Dtxd antibodies illustrated in **Figure 3b** wane more slowly compared to the Ptx antibodies, and 50% of children (both aP- and wP-primed) were clinically protected (IgG-Dtxd ≥ 0.1 IU/mL) for approximately 4 years (48 months), while adults reach this 50% protection point after approximately 9 years (108 months).

The Ttx antibodies illustrated in **Figure 3c** barely declined in adults and started to decline after 5 years (60 months) in wP-primed children, resulting in almost 100% of adults still protected even after 10 years (120 months) and more than 75% of wP-primed children still protected. From the decay model, it can be calculated that the GMC of the wP-primed children reached the limit of clinical protection (IgG-Ttx ≥ 0.1 IU/mL) after 14 years and for adults only after 38 years.

### Risk factors to contract pertussis

From the questionnaires, it appeared that there were no clinically symptomatic cases diagnosed as pertussis reported in any of the three cohorts during approximately 6 years

follow-up time. However, there appeared to be multiple participants who showed a three-fold increase in IgG-Ptx antibodies reaching at least 20 IU/mL during the study, pointing to pertussis infection according to the Dutch criterion based on a paired serum/plasma sample. Based on this definition, one participant became infected in the cohort of wP-primed children in contrast to seven in the cohort of aP-primed children, and no adults became infected. In **Table 2**, we performed a 2-step risk analysis. In step 1, a number of risk factors for contracting pertussis is listed and was used for logistic regression analysis on the entire cohort. Univariately tested aP priming, Ptx antibody concentrations ($p = 0.052$), though we cannot correct for differences in the follow-up time and the degree of exposure to *B. pertussis*.

**Table 2.** Risk factors to contract pertussis infection.

| | N (%) | N recent pertussis infection (%) | Univariate crude OR (95% CI) | p-value | Multivariate adjusted OR (95% CI) | p-value |
|---|---|---|---|---|---|---|
| Step 1: multivariable model with all participants combined | | | | | | |
| Sex | | | | 0.322 | | |
| Male | 79 (45%) | 5 (6.3%) | Ref. | | | |
| Female | 96 (55%) | 3 (3.1%) | 0.477 (0.110-2.063) | | | |
| Age at inclusion | | | | 0.997 | | |
| 9 y | 108 (62%) | 8 (7.4%) | Ref. | | | |
| 25-29 y | 67 (38%) | 0 (0.0%) | <0.001 (N/A) | | | |
| Priming vaccinations | | | | 0.011 | | 0.028 |
| wP | 116 (66%) | 1 (0.9%) | Ref. | | Ref. | |
| aP | 59 (34%) | 7 (12%) | 15.481 (1.857-129.067) | | 11.061 (1.293-94.604) | |
| Ptx < 20 IU/ml at 1y | | | | 0.025 | | 0.063 |
| no | 102 (59%) | 1 (1.0%) | Ref. | | Ref. | |
| yes | 70 (41%) | 7 (10%) | 11.222 (1.349-93.380) | | 7.701 (0.895-66.294) | |
| Ptx Ab at 1 month* | 173 | 8 (4.6%) | 0.440 (0.123-1.577) | 0.208 | | |
| FHA Ab at 1 month* | 173 | 8 (4.6%) | 0.069 (0.005-0.877) | 0.039 | | |
| Prn Ab at 1 month* | 173 | 8 (4.6%) | 0.618 (0.146-2.627) | 0.515 | | |
| Ptx Ab at 1 year* | 172 | 8 (4.7%) | 0.316 (0.087-1.146) | 0.080 | | |
| FHA Ab at 1year* | 172 | 8 (4.7%) | 0.088 (0.011-0.736) | 0.025 | | |
| Prn Ab at 1 year* | 172 | 8 (4.7%) | 0.582 (0.168-2.019) | 0.148 | | |
| Step 2: Pearson Chi-square test on priming vaccinations between aP and wP primed children (adults excluded) | | | | | | |
| wP primed children | 49 (45%) | 1 (2.0%) | N/A | 0.052 | | |
| aP primed children | 59 (55%) | 7 (12%) | | | | |

* Added as continuous variable. N: number; OR; odds ratio; CI: confidence interval; aP: acellular pertussis; wP: whole cell pertussis; Ptx: pertussis toxin; FHA: filamentous haemagglutinin; Prn: pertactin; Ab: antibody concentrations; N/A: not applicable.

## DISCUSSION

We set out to determine the long-term humoral immunity after an aP booster vaccination in both children and adults and compared the initial priming vaccinations in infancy in children. We found that pertussis antibody concentrations after an aP booster vaccination are higher in adults compared with children and antibody concentrations persist at a higher level for over a decade. Serological evidence of recent pertussis infection was more often found after aP-priming vaccinations in infancy compared with wP priming vaccinations.

Differences in antibody kinetics between wP-primed children and wP-primed adults with ultimately higher antibody concentrations in the young adults, are probably caused by the exposure to *B. pertussis* during life. Cumulative local pertussis disease notification data obtained from the RIVM indicates that the degree of exposure to *B. pertussis* during the study was highest in the wP-primed children cohort and lowest in the wP-primed adults. Nevertheless, the young adults had a longer life with more opportunity to become infected considering the high pertussis infection prevalence in the Netherlands [34]. These possible infections are likely to have boosted their immune systems and might explain the slower waning in antibodies in the young adults. The slow waning in Ptx antibody concentrations in adults has been previously described in a Danish study by Dalby et al. where they used a study vaccine that contained 20 µg Ptx, instead of 8 µg as in our study [35]. In contrast, Pool et al. observed fast waning in Ptx antibody concentrations in adolescents as well as in adults that were already almost back to pre-vaccination levels 5 years post-vaccination in a study in the USA [15]. From this we can assume that the antibody decay rate is multifactorially determined. The dose of the booster vaccine, but possibly also the dose and number of previous vaccinations, in combination with the frequency of natural boosting, will probably influence the antibody response and the decay rate.

Antibodies rapidly waned in the wP-primed paediatric cohort with ultimately comparable kinetics to aP-primed children. However, most of the wP-primed children, unlike aP-primed children, appeared to still be protected against infection during the follow-up, based on the serological defined cut-off of infection. In the aP-primed paediatric cohort, 12% met our serological criterion of pertussis infection after six years compared with 2% in the wP-primed paediatric cohort. The observed difference in the proportion of pertussis infected individuals in aP-primed children compared to the wP-primed children is close to significance ($p = 0.052$). This is in line with findings described in non-human primates, where aP priming compared with wP priming led to higher infectious susceptibility [36]. Therefore, protection in the long term is likely to be dependent not only on humoral immunity but also on cellular immunity, which has been suggested before, however, a correlate of protection against pertussis has not yet been established [37]. Previous reports regarding our two paediatric cohorts showed that aP-primed children had a higher proportion of IgG4 antibodies following aP boosting, indicating more Th2 skewing

compared with wP-primed children [38, 39]. One year post-booster vaccination, the difference in the proportion of IgG4 still existed. The higher proportion of IgG4 might be caused by the relatively lower T helper (Th) 1/Th2 ratio in aP-primed children [39]. A Th2-skewed response has frequently been described in aP-primed individuals in contrast to a more Th1/Th17-skewed immune response in wP-primed individuals [18, 40]. In baboon studies, it has been shown that Th2 responses protect against clinical disease, but Th1 and Th17 responses are needed to protect against colonisation and transmission [36]. The Th2 skewing in our aP-primed children in combination with rapidly waning antibodies after the booster might have increased their vulnerability to infection.

A limitation of the study is the fact that other aspects may have affected the number of pertussis infections, such as a longer observation period in the aP-primed children compared to the wP-primed children (13 months later) and wP-primed adults (6 months later) (**Supplementary Figure 1**). Humoral and cellular immunity may have waned more in the aP-primed paediatric cohort after a longer time compared to the wP-primed cohorts, possibly resulting in higher susceptibility to infection. We cannot discern the time of infection, either early or later, after the aP booster since we have only a single blood sample at the end of the follow-up. Furthermore, the cyclic pattern of pertussis outbreaks as well as the studies not being executed in the same period nor in the same municipalities, may have caused differences in pertussis exposure among the cohorts. In 2012 and 2014, the Netherlands experienced pertussis outbreaks where most disease cases were observed in adolescents [22]. Cumulative local pertussis disease notification data obtained from the RIVM indicates that exposure to *B. pertussis* during the study period was highest in the wP-primed children cohort, which is expected considering the most recent outbreaks, followed by the aP-primed children, and was lowest in the wP-primed adults. Therefore, the degree of pertussis circulation is unlikely to explain the serological differences associated with the proportion of infected participants in the three study cohorts. Taken together, not only priming vaccination background but potentially also in combination with the longer post-booster follow-up time, may have resulted in a higher proportion of infected individuals in the aP-primed paediatric cohort.

Some of the individuals that are aP-primed might form a reservoir for *B. pertussis* and transmit the bacterium to others. Since the proportion of aP-primed individuals in the population is increasing, additional population-wide boosters are unlikely to substantially increase the overall herd immunity to pertussis. If herd immunity is insufficient to achieve protection to vulnerable individuals such as infants and other risk groups such as individuals with pulmonary co-morbidity and older adults, these risk groups might benefit from an extra aP booster at the individual level [2]. Furthermore, certain groups who are prone to transmit infection, such as health care personnel and professionals working with very young children or vulnerable older adults, might benefit from an extra

booster [36]. Since most professionals in the Netherlands are currently still wP-primed in infancy, an aP booster may offer reduced transmission by protecting professionals against infection. However, little is known regarding repeated boosters and their advantages or disadvantages [41].

Although some individuals in our study became infected, we had no reported pertussis cases in any of our cohorts. In the Netherlands, the number of reported cases in adolescents and young adults is quite low [6, 34]. This might be an underestimation because pertussis generally presents less severe symptoms in adolescents and adults [42]. From a nationwide serosurveillance study in the Netherlands we know that serum infection prevalence in the studied age groups is approximately 200 times higher compared to the number of reported cases. Considering that only eight individuals in total became infected, it was not surprising that we did not find any symptomatic pertussis cases. Therefore, we cannot conclude from our data if an aP booster protects against the disease, however, from the literature it is known that aP boosters do protect against the disease in the short term in aP and wP-primed individuals [43]. In the long term, aP-primed individuals are less protected against the disease compared to their wP-primed peers [44].

Antibody kinetics of our young adult cohort might be translatable to maternal vaccination since Huygen et al. found that pregnant and non-pregnant women respond comparably to an aP booster vaccine up to at least one year post-vaccination [45]. This means that we could expect the same major inter-individual variability in antibody responses between pregnant women as we have seen between our young adult participants. The current recommendation is to boost expectant women every pregnancy to induce high antibody concentrations necessary for sufficient transplacental transmission [46]. However, considering the slow decline in antibodies, it is questionable if all pregnant women would benefit from a booster at subsequent pregnancies, as some might still have very high antibody concentrations from a previous (maternal) booster. Determination of IgG-Ptx antibody concentrations early in pregnancy for every subsequent pregnancy after the first could help to substantiate whether expectant mothers would benefit from a subsequent booster. Since blood is already drawn early in pregnancy for several other (immunological) measurements such as lupus, this is not difficult to implement in the Netherlands.

Pertussis vaccines are always part of a multi-component combination vaccine, including at least Dtxd and Ttxd. Therefore, not only immunity against pertussis but also immunity against diphtheria and tetanus will be boosted. In line with our data and other studies, a decennial booster in adults seems adequate for diphtheria [47]. However, Ttx antibody concentrations decrease much more slowly and IgG-Ttx concentration seems sufficient for a much longer period. Furthermore, tetanus hyper-immunisation could lead to hypersensitivity responses [48, 49]. Our results seem to substantiate a tetanus booster approximately every 35 years for adult individuals who participated in the NIP, in line with

Hammarlund et al. who also found protective immunity against tetanus for more than 30 years [50]. Current recommendation in the Netherlands is to boost after possible exposure to tetanus if the last vaccination is more than 10 years ago [51]. Antibody concentrations of Ttx seem to decrease faster among children compared to young adults, and therefore, it seems reasonable to retain a 10-year interval after the booster at 9 years of age before the first possible exposure.

In conclusion, in this phase IV longitudinal interventional study, where we assessed long-term immunity following a pertussis booster vaccination in children and adults, we found heterogeneous but high antibody concentrations in adults. Children primed with aP vaccines had the highest prevalence of serologic parameters indicative of recent pertussis infection. Therefore, an extra aP booster vaccine does not seem to sufficiently protect against infection for more than 6 years after a booster vaccination in aP-primed school aged children. Since the proportion of aP-primed individuals is increasing in the population, it is not likely that the implementation of additional aP boosters after the previous preschool booster would reduce transmission of *B. pertussis*. Protecting risk groups seems most important, with the maternal vaccination as the most essential measure.

## AUTHOR CONTRIBUTIONS

Conceptualization, A.-M.B., G.A.M.B. and E.A.M.S., with input from L.H.H., S.v.d.L. and P.V.; methodology, A.A.B.M. and P.G.M.v.G., with input from L.H.H., S.v.d.L. and P.V.; software, A.A.B.M. and P.V.; validation, L.H.H., S.v.d.L., P.V. and P.G.M.v.G.; formal analysis, A.A.B.M. and P.V.; investigation, L.H.H., S.v.d.L., P.V. and P.G.M.v.G.; resources, L.H.H. and S.v.d.L.; data curation, P.V., S.v.d.L. and L.H.H.; writing—original draft preparation, P.V.; writing—review and editing, G.A.M.B., A.-M.B., A.A.B.M., S.v.d.L., L.H.H. and P.V.; visualization, A.A.B.M. and P.V.; supervision, A.-M.B., G.A.M.B. and E.A.M.S.; project administration, L.H.H., S.v.d.L. and P.V.; funding acquisition, A.-M.B. and G.A.M.B. All authors have read and agreed to the published version of the manuscript.

## FUNDING

This research was funded by the National Institute for Public Health and the Environment of the Ministry of Health, Welfare, and Sport, The Netherlands.

## INSTITUTIONAL REVIEW BOARD STATEMENT

The studies were conducted in accordance with the Declaration of Helsinki and approved by the Medical Research Ethics Committees United (NL44640.100.13, 22 July 2013, and NL47382.094.13, 6 March 2014) and the Central Committee in Research Involving Human Subjects (NL23149.000.08, 1 February 2009).

## INFORMED CONSENT STATEMENT

Informed consent was obtained from all participants and/or parents involved in the study.

## DATA AVAILABILITY STATEMENT

The data presented in this study are available upon request from the corresponding author.

## ACKNOWLEDGMENTS

We are grateful to all the participants who participated at the additional timepoint 6 years post-vaccination. We also want to thank Natasha Kaagman for her help in the clinical trial management and the sample processing.

## CONFLICTS OF INTEREST

The authors declare no conflict of interest.

## REFERENCES


1. Cherry, J.D., Pertussis in Young Infants Throughout the World. Clin Infect Dis, 2016. 63(suppl 4): p. S119-S122. doi: 10.1093/cid/ciw550.
2. Kandeil, W., et al., The burden of pertussis in older adults: what is the role of vaccination? A systematic literature review. Expert Rev Vaccines, 2019. 18(5): p. 439-455. doi: 10.1080/14760584.2019.1588727.
3. Cherry, J.D., Historical review of pertussis and the classical vaccine. J Infect Dis, 1996. 174 Suppl 3: p. S259-63.
4. Jackson, D.W. and P. Rohani, Perplexities of pertussis: recent global epidemiological trends and their potential causes. Epidemiol Infect, 2014. 142(4): p. 672-84.
5. Health Council of the Netherlands, Vaccination against pertussis. Vol. 2004. publication no. 2004/04, The Hague: Health Council of the Netherlands, 2004.
6. Schurink-van 't Klooster, T. and H. de Melker, The National Immunisation Programme in the Netherlands : Surveillance and developments in 2019-2020. 2020, DOI: 10.21945/rivm-2020-0077, Rijksinstituut voor Volksgezondheid en Milieu RIVM.
7. Lambert, L.C., Pertussis vaccine trials in the 1990s. J Infect Dis, 2014. 209 Suppl 1(Suppl 1): p. S4-9.
8. David, S., P.E. Vermeer-de Bondt, and N.A.T. van der Maas, Reactogenicity of infant whole cell pertussis combination vaccine compared with acellular pertussis vaccines with or without simultaneous pneumococcal vaccine in the Netherlands. Vaccine, 2008. 26(46): p. 5883-5887, DOI: 10.1016/j.vaccine.2008.07.105.
9. Clark, T.A., Changing pertussis epidemiology: everything old is new again. J Infect Dis, 2014. 209(7): p. 978-81.
10. Wiley, K.E., et al., Sources of pertussis infection in young infants: a review of key evidence informing targeting of the cocoon strategy. Vaccine, 2013. 31(4): p. 618-25.
11. Zoldi, V., et al., Decreased incidence of pertussis in young adults after the introduction of booster vaccine in military conscripts: Epidemiological analyses of pertussis in Finland, 1995-2015. Vaccine, 2017. 35(39): p. 5249-5255.
12. Cassimos, D.C., et al., Vaccination Programs for Adults in Europe, 2019. Vaccines (Basel), 2020. 8(1):34(1).
13. Haviari, S., et al., Vaccination of healthcare workers: A review. Hum Vaccin Immunother, 2015. 11(11): p. 2522-37.
14. Voysey, M., et al., The Influence of Maternally Derived Antibody and Infant Age at Vaccination on Infant Vaccine Responses : An Individual Participant Meta-analysis. JAMA Pediatr, 2017. 171(7): p. 637-646.
15. Pool, V., et al., Humoral immunity 10years after booster immunization with an adolescent and adult formulation combined tetanus, diphtheria, and 5-component acellular pertussis vaccine in the USA. Vaccine, 2018. 36(17): p. 2282-2287. doi: 10.1016/j.vaccine.2018.03.029.
16. Squeri, R. and C. Genovese, Immunogenicity and antibody persistence of diphteria-tetanus-acellular pertussis vaccination in adolescents and adults: a systematic review of the literature showed different responses to the available vaccines. J Prev Med Hyg, 2020. 61(4): p. E530-e541.
17. van der Lee, S., et al., Robust Humoral and Cellular Immune Responses to Pertussis in Adults After a First Acellular Booster Vaccination. Front Immunol, 2018. 9: p. 681.

**18.** van der Lee, S., et al., Whole-Cell or Acellular Pertussis Primary Immunizations in Infancy Determines Adolescent Cellular Immune Profiles. Front Immunol, 2018. 9: p. 51. doi: 10.3389/fimmu.2018.00051.

**19.** Schure, R.M., et al., Pertussis circulation has increased T-cell immunity during childhood more than a second acellular booster vaccination in Dutch children 9 years of age. PLoS One, 2012. 7(7): p. e41928.

**20.** Hijnen, M., et al., The Bordetella pertussis virulence factor P.69 pertactin retains its immunological properties after overproduction in Escherichia coli. Protein Expr Purif, 2005. 41(1): p. 106-112, DOI: 10.1016/j.pep.2005.01.014.

**21.** van Gageldonk, P.G.M., et al., Development and validation of a multiplex immunoassay for the simultaneous determination of serum antibodies to Bordetella pertussis, diphtheria and tetanus. J Immunol Methods, 2008. 335(1-2): p. 79-89, DOI: 10.1016/j.jim.2008.02.018.

**22.** van der Lee, S., et al., Enhanced Bordetella pertussis acquisition rate in adolescents during the 2012 epidemic in the Netherlands and evidence for prolonged antibody persistence after infection. Euro Surveillance, 2017. 22(47): p. 17-00011. doi: 10.2807/1560-7917.ES.2017.22.47.17-00011.

**23.** World Health Organization, Module 3: tetanus, in The immunological basis for immunization series. 2018: Geneva.

**24.** World Health Organization, Module 2: diphtheria, in The immunological basis for immunization series. 2009: Geneva.

**25.** Long, S.S., C.J. Welkon, and J.L. Clark, Widespread silent transmission of pertussis in families: antibody correlates of infection and symptomatology. J Infect Dis, 1990. 161(3): p. 480-6.

**26.** Sato, Y. and H. Sato, Anti-pertussis toxin IgG and anti-filamentous hemagglutinin IgG production in children immunized with pertussis acellular vaccine and comparison of these titers with the sera of pertussis convalescent children. Dev Biol Stand, 1985. 61: p. 367-72.

**27.** Olin, P., et al., How to make sense of pertussis immunogenicity data. Clin Infect Dis, 2001. 33 Suppl 4: p. S288-91.

**28.** Mollers M., R.W.L.M. Kinkhoest richtlijn. 2018 23 July 2020 [cited 2020 19-8-2020].

**29.** Slifka, M.K., et al., Humoral immunity due to long-lived plasma cells. Immunity, 1998. 8(3): p. 363-72.

**30.** Teunis, P.F., et al., Linking the seroresponse to infection to within-host heterogeneity in antibody production. Epidemics, 2016. 16: p. 33-9.

**31.** van Ravenhorst, M.B., et al., Long-term persistence of protective antibodies in Dutch adolescents following a meningococcal serogroup C tetanus booster vaccination. Vaccine, 2016. 34(50): p. 6309-6315.

**32.** Plummer, M. JAGS: A program for analysis of Bayesian graphical models using Gibbs sampling. in Proceedings of the 3rd international workshop on distributed statistical computing. 2003. Vienna, Austria.

**33.** R Core Team, R: A language and environment for statistical computing. R Foundation for Statistical Computing. 2021: Vienna, Austria.

**34.** Versteegen, P., et al., More than 10 years after introduction of an acellular pertussis vaccine in infancy: a cross-sectional serosurvey of pertussis in the Netherlands. The Lancet Regional Health – Europe, 2021. 10: p. 100196.

**35.** Dalby, T., et al., 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(Pt 9): p. 1029-1036, DOI: 10.1099/jmm.0.020826-0.

**36.** Warfel, J.M., L.I. Zimmerman, and T.J. Merkel, Acellular pertussis vaccines protect against disease but fail to prevent infection and transmission in a nonhuman primate model. Proc Natl Acad Sci U S A, 2014. 111(2): p. 787-792, DOI: 10.1073/pnas.1314688110.

**37.** PERISCOPE Consortium, PERISCOPE: road towards effective control of pertussis. Lancet Infect Dis, 2019. 19(5): p. e179-e186, DOI: 10.1016/s1473-3099(18)30646-7.

**38.** van der Lee, S., et al., Whole-cell or acellular pertussis vaccination in infancy determines IgG subclass profiles to DTaP booster vaccination. Vaccine, 2018. 36(2): p. 220-226.

**39.** Aalberse, R.C., et al., Immunoglobulin G4: an odd antibody. Clin Exp Allergy, 2009. 39(4): p. 469-77.

**40.** da Silva Antunes, R., et al., Th1/Th17 polarization persists following whole-cell pertussis vaccination despite repeated acellular boosters. J Clin Invest, 2018. 128(9): p. 3853-3865.

**41.** McAlister, S.M., et al., An observational study of antibody responses to a primary or subsequent pertussis booster vaccination in Australian healthcare workers. Vaccine, 2021. 39(11): p. 1642-1651.

**42.** Macina, D. and K.E. Evans, Bordetella pertussis in School-Age Children, Adolescents and Adults: A Systematic Review of Epidemiology and Mortality in Europe. Infect Dis Ther, 2021. 10(4): p. 2071-2118.

**43.** Wilkinson, K., et al., Pertussis vaccine effectiveness and duration of protection - A systematic review and meta-analysis. Vaccine, 2021. 39(23): p. 3120-3130.

**44.** Sheridan, S.L., et al., Waning vaccine immunity in teenagers primed with whole cell and acellular pertussis vaccine: recent epidemiology. Expert Rev Vaccines, 2014. 13(9): p. 1081-1106. doi: 10.1586/14760584.2014.944167.

**45.** Huygen, K., et al., Humoral and cell mediated immune responses to a pertussis containing vaccine in pregnant and nonpregnant women. Vaccine, 2015. 33(33): p. 4117-23.

**46.** National Institute for Public Health and the Environment (RIVM), RVP-richtlijn Uitvoering Rijksvaccinatieprogramma. 2022: Bilthoven.

**47.** Berbers, G., et al., Circulation of pertussis and poor protection against diphtheria among middle-aged adults in 18 European countries. Nat Commun, 2021. 12(1): p. 2871.

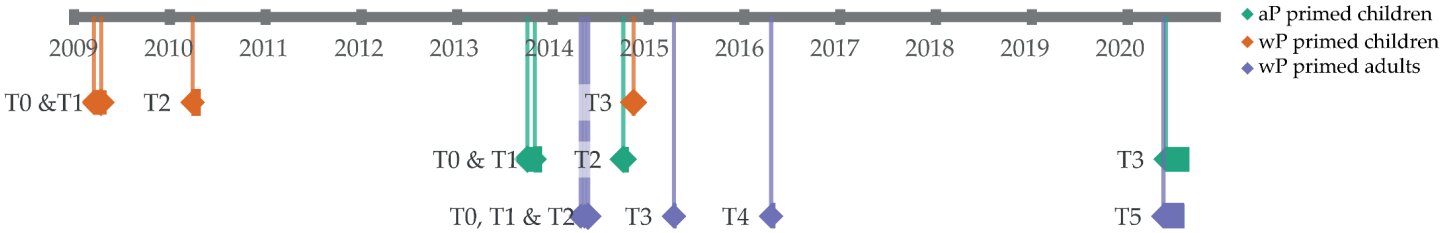
**48.** Aboud, S., et al., Antibodies to tetanus toxoid in women of childbearing age in Dar es Salaam and Bagamoyo, Tanzania. Trop Med Int Health, 2001. 6(2): p. 119-25.

**49.** Gentili, G., et al., Prevalence of hyperimmunization against tetanus in Italians born after the introduction of mandatory vaccination of children with tetanus toxoid in 1968. Infection, 1993. 21(2): p. 80-2.

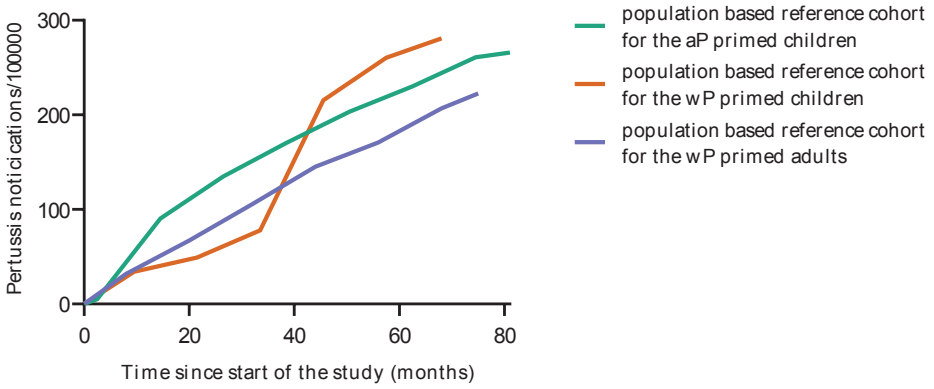
**50.** Hammarlund, E., et al., Durability of Vaccine-Induced Immunity Against Tetanus and Diphtheria Toxins: A Cross-sectional Analysis. Clin Infect Dis, 2016. 62(9): p. 1111-1118.

**51.** National Coordination Centre for Communicable Disease Control (LCI). Tetanus - Richtlijn. Available from: https://lci.rivm.nl/richtlijnen/tetanus.

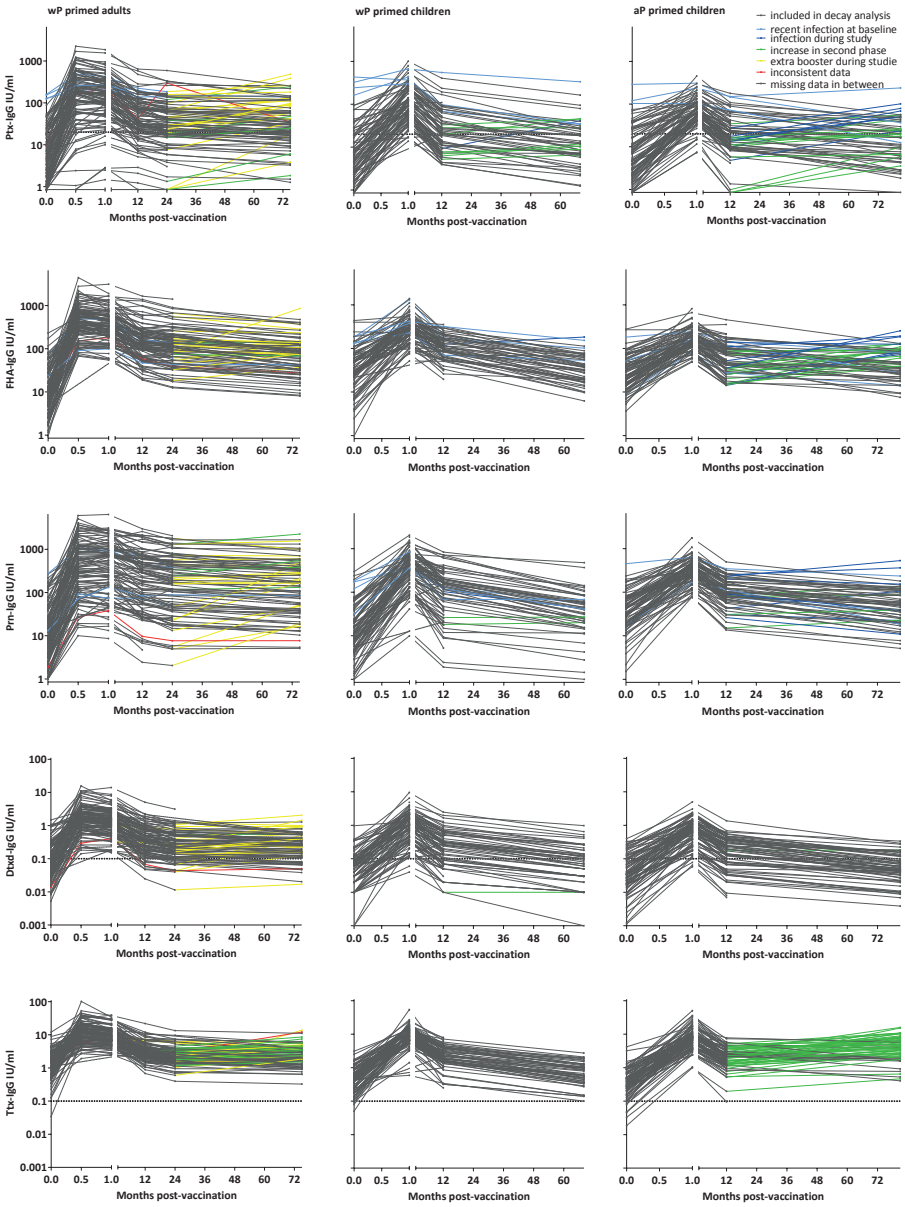
## SUPPLEMENTARY MATERIALS




**Supplementary Figure 1.** Timelines for the different study cohorts. T0-T5 represent the study visits; aP primed children were recruited in the municipalities of Almere, Breda, Eindhoven, Ede, and Utrecht; wP primed children were recruited in the city of Hoofddorp; wP primed adults were recruited in the municipality of Amersfoort. aP: acellular pertussis; wP: whole cell pertussis ; T: time point.




**Supplementary Figure 2.** Cumulative local pertussis disease notifications per 100,000 inhabitants. Pertussis disease notification data were obtained from the Dutch National Institute for Public Health and the Environment (RIVM) based on the postal code matched regions at times of inclusion for the three study cohorts, covering the period from inclusion to final blood draw per cohort. Incidence is plotted against the amount of inhabitants in the concerning region. aP: acellular pertussis; wP: whole cell pertussis.




**Supplementary Figure 3.** Individual responses. aP: acellular pertussis; wP: whole cell pertussis IU: international units; Ptx: pertussis toxin; FHA: filamentous haemagglutinin; Prn: pertactin; Dxtd; diphtheria toxoid; Txt: tetanus toxin.

**Supplementary Table 1.** *B. Pertussis* vaccine schedules and vaccines in the Netherlands.

| Year | Scheme | Vaccine composition | Change | Pertussis vaccine name (Company) |
|---|---|---|---|---|
| before | | | | No vaccine available |
| 1954 | | DTwP | Combination vaccine became available | DTwP (RIVM) |
| 1957 | 3,4,5,11 m | DTwP | Start of NIP | DTwP (RIVM) |
| 1962 | 3,4,5,11 m | DTwP-IPV | Addition of IPV | DTwP-IPV (RIVM) |
| 1997 | 3,4,5,11 m | DTwP-IPV + Hib | Addition of Hib tot schedule | DTwP-IPV (RIVM) |
| 1999 | 2,3,4,11 m | DTwP-IPV + Hib | Accelerated vaccination | DTwP-IPV (RIVM) |
| 2001 | 2,3,4,11 m<br>4y | DTwP-IPV + Hib<br>DT-IPV + aP3 | Introduction aP booster, 3 Bp components | DTwP-IPV (RIVM)<br>monovalent aP (GSK) |
| 2003 | 2,3,4,11 m<br>4y | DTwP-IPV-Hib<br>DT-IPV + aP3 | Combination vaccine | DTwP-IPV/Hib (NVI)<br>monovalent aP (GSK) |
| 2005 | 2,3,4,11 m<br>4y | DTaP3-IPV-Hib<br>DT-IPV + aP3 | Introduction aP priming, 3 Bp components | Infanrix IPV + Hib (GSK)<br>monovalent aP (GSK) |
| 2006 (Jan) | 2,3,4,11 m<br>4y | DTaP5-IPV-Hib<br>DT-IPV + aP3 | 5 Bp components | Pediacel (SP MSD)<br>monovalent aP (GSK) |
| 2006 (Jun) | 2,3,4,11 m<br>4y | DTaP5-IPV-Hib + Pneu<br>DT-IPV + aP3 | Addition of Pneu to schedule | Pediacel (SP MSD)<br>monovalent aP (GSK) |
| 2006 (Jul) | 2,3,4,11 m<br>4y | DTaP5-IPV-Hib + Pneu<br>DTaP5-IPV | Combination vaccine, 5 Bp components | Pediacel (SP MSD)<br>Triaxis Polio (SP MSD) |
| 2008 (Feb) | 2,3,4,11 m<br>4y | DTaP5P-IPV-Hib + Pneu<br>DTaP3-IPV | 3 Bp components | Pediacel (SP MSD)<br>Infanrix-IPV (GSK) |
| 2008 (Jul) | 2,3,4,11 m<br>4y | DTaP3-IPV-Hib + Pneu<br>DTaP3-IPV | 3 Bp components | Infanrix-IPV + Hib (GSK)<br>Infanrix-IPV (GSK) |
| 2009 | 2,3,4,11 m<br>4y | DTaP3/5-IPV-Hib + Pneu<br>DTaP3-IPV | 3 or 5 Bp components | Pediacel (SP MSD)/Infanrix-IPV + Hib (GSK)<br>Infanrix-IPV (GSK) |
| 2010 | 2,3,4,11 m<br>4y | DTaP5-IPV-Hib + Pneu<br>DTaP3-IPV | 5 Bp components | Pediacel (SP MSD)<br>Infanrix-IPV (GSK) |
| 2011 (Oct) | 2,3,4,11 m<br>4y | DTaP3-IPV-Hib-HepB + Pneu<br>DTaP3-IPV | Addition of HepB to schedule, 3 Bp components | Infanrix hexa (GSK)<br>Infanrix-IPV (GSK) |

| Year | Scheme | Vaccine composition | Change | Pertussis vaccine name (Company) |
|---|---|---|---|---|
| 2017 (Jan) | 2,3,4,11 m<br>4y | DTaP3-IPV-Hib-HepB + Pneu<br>Tdap3-IPV | <br>Reduced dose Bp components | Infanrix hexa (GSK)<br>Boostrix-IPV (GSK) |
| 2018 (Dec) | 2,3,4,11 m<br>4y | DTaP5-IPV-Hib-HepB + Pneu<br>Tdap3-IPV | 5 Bp components | Vaxelis (SP MSD)<br>Boostrix-IPV (GSK) |
| 2019 (Dec) | Maternal (22 weeks gestational age) | Tdap3 | Booster for pregnant women, 3 reduced dose Bp components | Boostrix (GSK) |
| 2020 | 3, 5, 11m<br>4y | DTaP3-IPV-Hib-HepB + Pneu<br>Tdap3-IPV | Revised priming schedule | Vaxelis (SP MSD)<br>Boostrix-IPV (GSK) |

Supplemantary Table 1. m=months; y= years; D=diphtheria; T=tetanus; wP: whole cell pertussis; aP: acellular pertussis; IPV: inactivated poliovirus; Pneu: pneumococcal; Hib: Haemophilus influenzae type B; HepB: Hepatitis B; Bp: *Bordetella pertussis*; NVI: Netherlands Vaccine Institute; GSK: GlaxoSmithKline; SP: Sanofi Pasteur; MSD: Merck Sharp & Dohme. This table is a modified from Versteegen P, Berbers GAM, Smits G, et al. More than 10 years after introduction of an acellular pertussis vaccine in infancy: a cross-sectional serosurvey of pertussis in the Netherlands. *The Lancet Regional Health – Europe* 2021; **6**(10): 1016. doi: 10./j.lanepe.2021.100196.

# Chapter 8

# Thesis summary and general discussion

## SUMMARY OF MAIN FINDINGS

In **chapter 2** prevalence of *B. pertussis* infections based on serosurveillance in 2016/2017 in the Netherlands was described. An immunoglobulin (Ig) G concentration against Ptx ≥ 100 IU/ml was used as indicator of a recent infection. Results were compared to previously performed similar serosurvey studies. Serum infection prevalence had increased from 1.0% in 1995/1996 to 3.5% in 2006/2007, and to 5.9% in 2016/2017. The age group with the highest pertussis serum infection prevalence in 2016/2017 was the adolescent group of 12-18 years. This was also the group with the largest increase compared to the survey in 2006/2007. In addition to seroprevalence studies, clinically confirmed pertussis notifications were monitored, since pertussis is a vaccine preventable notifiable disease. Despite the increase in seroprevalence, there was no increase in reported pertussis incidence. The serum infection prevalence in 2006/2007 was 100 times higher than the number of reported pertussis notifications in the Dutch population in the same period and this increased up to 200 times in 2016/2017, and even up to 650 times in 80+ older adults.

**Chapters 3, 4, 5, and 6** made use of samples of the BERT study. In the BERT study participants from Finland, the Netherlands, and the United Kingdom (UK) were included and divided in four different age categories: children aged 7-11 years (n=109), all primed in infancy by an acellular pertussis (aP) vaccine; adolescents aged 12-15 years (n=121) half of them primed by an aP vaccine, half of them by a whole cell pertussis (wP) vaccine; young adults aged 20-34 years (n=74) all wP primed; and older adults aged 60-70 years (n=75) either wP primed or unvaccinated. All participants received Boostrix-IPV at the start of the study, a vaccine containing diphtheria toxoid (Dtxd), tetanus toxoid (Ttxd), pertussis toxoid (Ptxd), filamentous haemagglutinin (FHA), pertactin (Prn), and three types of inactivated poliovirus (dT3ap-IPV).

In **chapter 3,** IgG and IgA antibody responses against the three *B. pertussis* vaccine antigens (Ptx, FHA, Prn) were described pre- and post-vaccination. All age groups showed an increase in geometric mean IgG and IgA antibody concentrations in the first month post-booster, followed by a subsequent decrease with antibody concentrations still exceeding baseline after one year. IgG-Ptx antibody concentrations were lowest among the young adults compared to the other age groups, both at baseline and post booster vaccination, but IgG-Prn absolute increase from baseline was highest among these young adults. Older adults had the lowest IgG-Prn concentrations at all study timepoints. Considering all three pertussis vaccine antigens, IgG concentrations were not consistently related to age, in contrast to IgA specific antibody concentrations that consistently increased with age at all measured study timepoints. No significant difference in antibody concentrations at any timepoint was found between adolescents primed with either aP or wP vaccines in infancy.

In **chapter 4** Ptx neutralising antibodies (PTNAs) were described pre and post-pertussis booster vaccination from the Dutch and Finnish participants (n=258). By measuring the

PTNAs, the levels of all Ptx specific antibodies together (potentially all Ig classes) that are able to neutralise Ptx were assessed. PTNAs increased in the first month upon dTap booster vaccination in all age groups, and remained above baseline one year post-booster. The lowest PTNA responses were observed in young adults, in line with the lowest IgG-Ptx antibody concentrations in young adults as described in **chapter 3**. PTNAs correlated very well with the total IgG-Ptx antibody concentrations (Pearson R = 0.829). In older adults, this correlation was also found for Ptx-IgA and the PTNAs responses, but not in the other age groups. This can correspond to the higher IgA-Ptx concentrations with increasing age. Another finding was the better neutralising responses in participants with IgG-Ptx ≥ 10 IU/ml at baseline. Differences in PTNAs between aP and wP primed adolescents were only noticed at baseline, with higher PTNAs in the wP group despite similar pertussis vaccine specific IgG concentrations.

In **chapter 5** we described the amount of circulating memory B cells specific for IgG-Ptx, -FHA, and -Prn from a subset of all age cohorts of the Finnish, Dutch, and UK participants (n=268), both pre and post vaccination. Like vaccine-antigen specific IgG concentrations, also memory B cell frequencies were still above baseline one year post-booster vaccination. In general, the highest memory B cell frequencies for IgG against Ptx, FHA, and Prn were observed at all study time points in adolescents, and the lowest frequencies in older adults. In case frequencies of memory B cells per antigen (Ptx, FHA, Prn) were above 1/100,000 peripheral blood mononuclear cells (PBMCs) at baseline, both memory B cell and antigen specific antibody responses were higher compared with the group with less or undetectable memory B cells specific for that vaccine antigen in all age groups together. The number of peripheral circulating memory B cells per antigen (Ptx, FHA, Prn) at baseline could therefore possibly be indicative of the magnitude of the booster memory B cell and IgG response. Correlations between memory B cell frequencies and antibody concentrations were best for Prn at all study timepoints. Memory B cell analysis in adolescents with different vaccination backgrounds (aP versus wP), showed a minor trend of higher memory B cell IgG Ptx, FHA, and Prn responses upon booster vaccination among wP primed individuals compared to aP primed individuals, although not significant.

Next to memory B cells, plasma cells specific to Ptx, FHA, and Prn were evaluated 7 days post-booster vaccination (**chapter 5, supplement 3**). Correlation between plasma cell frequencies at day 7, and memory B cell frequencies and antibody concentrations one month and one year post-booster were again highest for Prn and showed good correlation.

In **chapter 6,** the presence of over a hundred different circulating B cell subsets was described in a smaller subset of the Dutch participants (n=48). B cell subsets were differentiated by maturation stage and expressed Ig subclass as measured by flowcytometry. In all age groups, the most prominent change in B cell subsets upon booster vaccination, was the expansion of plasma cells of all maturation stages 7 days

post-vaccination, with the most marked increase of the circulation of IgG1 plasma cells. The highest absolute increase in numbers of IgG1 plasma cells from baseline to day 7 was observed in children, the highest fold-increase was found in older adults. Numbers of IgA1 plasma cells upon booster vaccination increased with age, both for absolute numbers as for fold-increase over baseline. A weak correlation (r=0.39) between absolute increase in (non-antigen specific) IgA plasma cells and the vaccine specific IgA antibody concentration of the pertussis vaccine antigens at day 28 was observed, but no such correlation was found for IgG. In contrast, at day 7 a higher correlation was found between frequencies of all vaccine antigen specific plasma cells together (as measured in **chapter 5, Supplementary Figure 3**) and the increase in total IgG and IgA plasma cell numbers found by flowcytometry (r=0.59 and r=0.60 respectively). Therefore it is likely that the observed plasma cell increase at day 7 after vaccination is largely directed against the vaccine antigens. A significant difference between aP and wP primed individuals in the B cell compartment in our flowcytometric analysis was observed post booster, with IgG1 plasma cell expansion more pronounced in the wP primed participants compared to the aP primed participants, in line with the observed trend in pertussis vaccine specific memory B cell frequencies described in **chapter 5**.

In **chapter 7**, immunological follow-up data up till approximately 6 years after Boostrix(-IPV) vaccination, from three other studies than the BERT study were described: the KIM study concerned aP primed children after an aP booster at age 9 years (n=59), the Booster study included wP primed children after aP booster at 9 years (n=49), and the VIKING study included wP primed young adults 25-29 years of age (n=86). A model to predict antibody kinetics up to 10 years post-vaccination was applied. In this model, young adults had higher antigen specific responses and showed a slower decay of IgG concentrations against pertussis vaccine antigens up to 10 years post-booster compared to the aP and wP primed children. In addition, children who were wP primed had a slightly slower initial antibody decay compared to the aP primed children, but the decline continued over a longer period in time, resulting in comparable antibody concentrations after 2.5 years. Children who were aP primed had more often experienced a recent *B. pertussis* infection, based on the proportion of children that showed a more than 3-fold increase of Ptx antibody concentration between the 1 year and the 6 year post booster follow-up period, combined with a Ptx IgG concentration of more than 20 IU/ml after 6 years (p=0.052). There was no clinically diagnosed and reported pertussis infection among the participants.

## GENERAL DISCUSSION

In this thesis, infection prevalence, and the humoral and B cell responses of an extra acellular pertussis (aP) booster vaccination were evaluated in the population with various vaccination backgrounds, and in various countries. The main study of this thesis is the BERT study which is part of the PERISCOPE project. Within the PERISCOPE project, studies and immunological assays on pertussis vaccinations are divided over different research groups. This thesis, as part of the PERISCOPE project, is mainly focussed on extensive studies of the humoral and B cell responses in participants of three countries, and in particular the participants of the BERT study in the Netherlands. Other participating research groups of the PERISCOPE project focused on T cell immunity in the BERT study or on other studies like a colonisation study. Together, these studies of the PERISCOPE project might provide more knowledge on potential immunological correlates of protection for pertussis.

In the general discussion of this thesis, the following issues are discussed:

- How did the infection prevalence in the Dutch population change over time?
- Do changes in infection prevalence have potential implications for the lifelong national immunisation programme (NIP)?
- What is the value of different assays on B cell and humoral immunity in evaluating aP booster vaccination responses?
- Are aP booster vaccination responses and hybrid immunity influenced by age?
- Are aP booster vaccination responses influenced by type of priming vaccination?



### How did the infection prevalence in the Dutch population change over time?

Upon the introduction of whole cell pertussis (wP) vaccines in the forties/fifties of the last century, the incidence of pertussis dropped significantly. However since 1990-2000, an increase in the global pertussis incidence has been observed, to which many factors have contributed, like increased awareness, improved diagnostic tools, evolution of the bacterium, and the type of pertussis priming vaccine for infants (wP switched to aP vaccination in the primary series) [1, 2]. By performing serosurveillance studies every ten years, as is current practice in the Netherlands since 1995, an increase in pertussis infection prevalence is observed, independent of clinical awareness or diagnostic tools. Between the serosurveillance studies performed in 1995/1996 and 2006/2007, the pertussis serum infection prevalence in the general population based on anti Ptx immunoglobulin (Ig) G levels ≥ 100 IU/ml, had increased from 1.0 to 3.5% [3]. In the latest serosurvey from 2016/2017, this level increased further to 5.9% (**chapter 2**). In several epidemiological studies on clinically diagnosed pertussis performed in other countries over approximately the same period in time, an increase in pertussis incidence based on notification rates has also been reported, like in Iran (during 2012-2018) and in Spain (during 2000-2015) [4, 5]. In the serosurveillance study in 2016/2017, the highest serum infection prevalence

in the population older than 7 years of age, was found in adolescents 12-18 years of age (**chapter 2**). This might be explained by the latest pertussis outbreaks in the Netherlands in 2012 and 2014, and is in line with the highest pertussis notification rates at the age of 10-14 years at the time [6]. The adolescent age group was likely to be more prone to pertussis infection compared with older age groups, because of waning immunity after the last vaccination against pertussis at 4 years of age, combined with little boosting of immunity due to natural infection over time [7]. In addition, since this adolescent age group tends to mix mostly with peers (schoolmates) of their own age, who all have become more susceptible at that age, pertussis infection can easily spread in this age group [8-11]. Pertussis infection prevalence also increased in the group of 7-11 year-olds. This increase, despite a recent aP booster vaccination against pertussis at 4 years of age, might be partially explained by the switch from wP to aP vaccines in the primary infant vaccination series in 2005 (3 doses before age 6 months and a booster at 11 months). It is described that protection against pertussis infection and disease following aP vaccines is of shorter duration compared to wP vaccines or following natural infection, and aP vaccines also protect less against infection and transmission [12, 13]. For this reason, this younger age group might also be less protected. In the adult age groups, where the source of pertussis infection is often relatives and workplace [10], the most outspoken increase of recent infections was observed in the 50-64-year-olds. A possible contribution to this increase is a more age-mixed contact pattern in 55-69 year-olds compared to younger and older adults, as described by Mossong et al., based on data of eight European countries together [8]. In another Dutch study, the age group that mixes most with other age groups is found to be younger, i.e. the groups of around 40 years of age, while children seem to mix mostly with peers of their own age and adults of their parents age [11]. Possibly, the peak in serum infection prevalence at age of 12-18 contributed to the peak of 50-64 year olds, assuming that indeed 50-64 year-olds frequently mix with adolescents. Also, the fact that nowadays children are fully aP vaccinated may contribute to more infection transmission by both children and adolescents to this 50-64 years group, since aP vaccine protects less against transmission of pertussis infection, while clinical symptoms may be reduced.

While an increase in serum infection prevalence in the population from 7 years onward was observed, the reported clinically confirmed pertussis incidence in the Netherlands did not increase [6], pointing in the direction of an increased proportion of subclinical, atypical and therefore not recognised, or even asymptomatic infections [14]. Not being aware of pertussis might also play a role, as well as the fact that diagnosis of pertussis does not have clinical implications for treatment. In older age groups, the gap between serum infection prevalence and reported clinical pertussis amounted even to over a 650 fold difference between the proportion of individuals with high serum anti Ptx IgG concentrations and reported pertussis infections, presumably also due to lack in awareness by physicians of pertussis infections in older adults who present with or without typical clinical symptoms

[15]. Based on these findings, the question arises whether we should booster vaccinate age groups of 7-11-year-olds, 12-18-year-olds and 50-64-year-olds, in order to increase protection against pertussis infection, while considering also the relatively low efficacy of aP vaccines against transmission, and the limited duration of protection post-vaccination, which would require repeated boosting.

**Do changes in infection prevalence have potential implications for the lifelong national immunisation programme (NIP)?**

On a population level, the most crucial group that needs protection against clinical pertussis are newborns, too young to be fully vaccinated. Nowadays, better protection is offered by maternal pertussis vaccination. But what about the older primary school children, adolescents, (older) adults, and patients with chronic diseases like cardiovascular or lung disease or immunocompromised persons? Do we need to offer extra pertussis booster vaccinations to these groups? To increase protection against pertussis infection and disease in the 7-11-year-olds, the aP booster in 4-year-olds can safely be delayed to 5-6 years of age, as recently advised by the Dutch Health Council [16]. Protection against pertussis infection of this 7-11 year old age group can also contribute to the protection against pertussis transmission within households to infants too young to be fully vaccinated. This change of aP booster vaccination from 4 to 6 years however needs monitoring to detect potential new susceptible groups, and more so since various changes in the national immunisation schedule for infants have been introduced over the last 2 decades that may impact susceptibility for pertussis. In 2005, the infant wP vaccinations (3 before 6 months of age and a booster at 11 months) were replaced by aP vaccinations. The aP booster at 4 years was already implemented in the wP era in 2001 in the Netherlands. Studies from the last decades have shown that priming by aP vaccinations causes higher anti pertussis antibody concentrations compared to wP vaccines, and this difference is still significant at 4 years of age [17]. Also T cell responses are still higher in aP compared to wP primed children at age 4 years. Upon booster vaccination at age 4 years, T cell responses only increased in wP primed, but remained at the same high level in aP primed 4-year-olds [18]. However, these studies were performed at the time when infants received three instead of the current two priming aP vaccines in the first 6 months of life, when mother was dTap vaccinated during pregnancy. Maternal dTap vaccinations causes some blunting post primary and post booster of the offspring's immune response to vaccination [19]. It is an interesting question how T cell responses will be following these changes in the vaccination schedule, and whether T cell responses will change upon vaccination in 4 or 6 year old children who previously received a 2 dose aP priming schedule with a booster at 12 months. For all these reasons, it is required to perform new studies to compare with results of previous studies in 4 and 6 year old children. This will inform whether booster doses would potentially improve protection against pertussis infection and transmission

in 7-11 year old children. Apart from new immunological data, the important question is how the new schedule with a maternal pertussis vaccinations and two primary infant doses with an aP booster at 12 months, and at 6 years will impact pertussis infection prevalence, transmission, and protection against clinical disease in both vaccinees as well as infants too young to be vaccinated and other age and risk groups. For this reason, also regular serosurveillance studies needs to be continued.

Comparing the age of pertussis booster vaccinations between European countries and the current Dutch schedule with a 2-dose priming schedule and a booster at 11 months and at four years of age (new advice of the Dutch Health Council [16] not yet taken into account), four countries including the Netherlands currently advise a 3 and 5 month priming schedule, with booster vaccination at 4 years of age, and the other six countries boost at 5, 6, 7 or even 8 years of age [20]. Infant and young child notification rates do not seem to correspond to the timing of this booster vaccination [6, 20], which also favours to delay the booster in 4-year-olds with two years as currently advised [16].

The next question is whether we should offer another aP booster vaccination to adolescents 12-18 years of age, to prevent infection, transmission, and disease. The highest increase in serum infection prevalence was observed in this age group, however, without an increase in the national notification rate. For this age group one has to weigh the benefit of reduction of transmission for a limited period of time to peers and to other age groups versus the improvement of longer lasting immunity due to boosting immunity by natural infection. We know that natural infection offers the longest protection against re-infection and transmission [12, 13]. With respect to the recent COVID-19 pandemic, hybrid immunity based on both infection and vaccination also offers the longest protection against reinfection and hospitalisation [21]. Many Dutch inhabitants are likely to have built up hybrid immunity against pertussis during lifetime, since most have received childhood vaccinations against pertussis, but got infected with *B. pertussis* later in life due to the endemic circulation. The current aP vaccines are parenterally administered and contain a subset of 1 to 5 *B. pertussis* antigens, thereby inducing a different immune response compared to natural infection, where individuals encounter the entire bacterium via the mucosal route in the upper respiratory tract [22]. Natural infection after vaccination will broaden the immune response and will induce a mucosal and systemic IgA response next to an IgG response, and one that is longer lasting. Since adolescents have few clinical problems in case of pertussis infection due to previous vaccinations, natural infection instead of aP booster vaccination may therefore be the preferred strategy for adolescents who will reach childbearing age within less than a decade in a country like the Netherlands, where *B. pertussis* is still highly endemic [23]. This at the cost of transmission to other age groups who may be more vulnerable. Future serosurveillance studies as well as close

monitoring of clinical pertussis cases and transmission patterns to vulnerable groups, need to remain warranted.

The third age group with a significant increase in pertussis infection prevalence over the last decade are the 50-64-year-olds. In the context of lifelong vaccinations and ongoing endemic pertussis, revaccination of this age group might be considered, since at this age pertussis infection may cause serious clinical problems. This age group is more at risk of complications like pneumonia, more at risk of hospitalisation due to age and comorbidity, and pertussis may cause for instance exacerbation of pre-existing COPD symptoms or heart failure [24]. Severe coughing in older adults can also cause complications like encephalopathy, intracranial haemorrhage, stroke, or carotid artery dissection [15]. Furthermore, the mismatch between reported clinical pertussis incidence in older adults and the serum infection prevalence is found highest in older adults (**chapter 2)**. Since cough is not a very specific symptom, especially not in older adults or individuals with cardiopulmonary comorbidities, pertussis diagnosis is easily missed, even in case of hospitalisation. Although vaccination coverage in studies with adults are scare to absent, the limited available data indicate sub-optimal coverage uptake [25]. Regular boosting from the age of 50 years onwards and in high risk individuals with cardiopulmonary comorbidities or other vulnerable groups, can increase protection against disease at an individual level. In the BERT study as described in **chapter 3**, IgG antibody responses after an aP booster vaccination were not inferior in 50-60-year-olds compared with younger adults, and showed stronger IgA responses, likely due to previous encounters with circulating *B. pertussis*. Also, as described in **chapter 4**, pertussis toxin neutralisation capacity did not seem to be less in the 50-60-year-olds. Older adults and individuals with cardiopulmonary comorbidities are already targeted in pertussis vaccination schedules of many other countries [20]. Although costs-of-illness vary from country to country, the economic burden of pertussis disease in older adults is high and vaccination against pertussis in this age group seems cost-effective [26, 27]. This type of prevention therefore needs to be considered in life long vaccination strategies and vaccination schedules for patient groups.

In summary, higher infection prevalence in 7-11-years-old needs to be followed by new studies after changes in the pertussis vaccination schedule. For adolescents, not booster vaccination but hybrid immunity might offer better protection on the longer term when previously vaccinated, though some higher risk groups might benefit from extra aP booster vaccinations. In older adults, revaccination should be considered. The duration of protection of the currently available aP booster vaccines is however limited and requires repeated boosting. New vaccines inducing longer lasting immunity and improving the reduction of transmission are required.

**What is the value of different assays on B cell and humoral immunity in evaluating aP booster vaccination responses?**

To get more insight in B cell responses and humoral immunity following aP booster vaccination at different ages and with different previous vaccines, several assays studying B cell development and Ig levels were explored.

Classically, IgG antibodies against Ptx are measured as an indication of pertussis vaccine responsiveness. In addition to measuring the quantity of antibody concentrations, diverse functionalities of anti-pertussis antibodies can be measured using different assays [28-32]. In the BERT study we measured both specific IgG and IgA antibodies against the three pertussis vaccine antigens, and in addition Ptx neutralising antibodies, IgG memory B cells against the three pertussis vaccine antigens, IgG plasma cells against the three pertussis vaccine antigens, and the total number of circulating plasma cells shortly post vaccination. An initial increase of IgG and IgA antibodies against the three pertussis vaccine antigens during the first month, followed by a decrease in the period between the first month and a year post booster vaccination was found (**chapter 3**), as has been described many times before [33]. A similar pattern was seen for the Ptx neutralising antibodies as measured in **chapter 4**, and of antigen specific IgG memory B cells against the three pertussis vaccine antigens (**chapter 5**). The quantity of the pertussis toxin neutralising antibodies (dependent on potentially all subclasses together) in serum correlated well with the total IgG-Ptx antibody concentrations (Pearson R = 0.829), which is in agreement with earlier findings [34]. This seems to indicate that IgG-Ptx might be a good proxy for neutralising capacity of antibody concentrations post booster vaccination. However on the individual level, PTNAs could not predict individual Ptx-IgG antibody concentrations, or the other way around. This may be partly explained by other factors. For instance, PTNAs of individuals with high IgA-Ptx, are likely to be influenced not only by anti Ptx-IgG, but also by IgA antibodies directed against Ptx. Therefore PTNAs might present a more complete picture of the total functional anti-Ptx antibodies compared with just IgG-Ptx antibody concentrations, highlighting PTNAs as a better candidate for evaluation of pertussis vaccination responses. On the other hand, PTNAs are only directed against the antigen Ptx, whereas antibody concentrations against other vaccine antigens may also be of interest, and can be measured simultaneously in the MIA assay as applied in the studies in this thesis.

The correlation between the frequency of antigen specific IgG memory B cells and the IgG antibody concentrations turned out to be antigen dependent. For Prn, this correlation was moderate to strong both at one month and one year post booster vaccination, for pertussis toxin these correlations were moderate, and for filamentous haemagglutinin these correlations were low, which is in line with previous studies [35, 36]. Differences in correlations for the *B. pertussis* vaccine antigens might be explained by the circulation in

the population of *B. pertussis* that variously express the vaccine antigens. *B. pertussis* is still endemic in many countries, and various immune responses will be boosted by natural infection. The vaccine antigen Ptx is specific for the *B. pertussis* bacterium and IgG-Ptx will only be boosted by *B. pertussis* infection or pertussis vaccination. In contrast, FHA is not only expressed by Bordetella species, but also by other species and will therefore potentially be boosted more often and by this lose the correlation with anti-FHA IgG concentrations upon vaccination [37]. Pertactin, specific to Bordetella species, is very immunogenic and induces serum bactericidal antibodies [38, 39]. However, by vaccine pressure Prn negative *B. pertussis* strains are circulating more often, and constituted already 43% of all strains in the Netherlands in 2019 [40, 41]. This may have led to less boosting of Prn-specific immunity by natural infection, in contrast to that of Ptx [42, 43]. Consequently, this may contribute to the explanation why Prn is likely to show the best correlation between Prn-specific IgG antibody concentrations, memory B cells frequencies, and plasma cell frequencies upon booster vaccination. (**chapter 5, supplement 3**). However, while measuring Prn B-cell responses might be most specific to measure the height of the vaccine response, it may contribute less to clinical protection in view of the increasing proportion of Prn-negative strains of *B. pertussis* that circulate.

At day 7 post booster vaccination, IgG and low IgA plasma cell frequencies against the three pertussis vaccine antigens correlated well with the total IgG and IgA plasma cell increase at day 7 (r = 0,59 and r = 0.60 respectively) as measured by flowcytometry (**chapter 6**). Therefore, it is likely that the circulating plasma cells observed shortly after vaccination are largely directed against vaccine antigens. This has previously been demonstrated for influenza vaccination, showing 80% of the IgG secreting plasma cells to be influenza vaccine specific [44] as analysed by cell sorting 7 days post vaccination.



Although results of the different assays show similar directions, each assay is providing additional insight in features of the immune responses. In the PERISCOPE project T cells [45] and mucosal responses [46] are also investigated. Ultimately, together with preclinical studies, a human challenge model [47], and epidemiological studies as part of the PERISCOPE project [48], and other studies, a better understanding of immunological correlates of protection will be feasible, in order to guide new vaccine development in the future. At this moment it is not yet possible to pinpoint which immunological data are most predictive for protection against pertussis. Since antibody concentrations are classically measured to evaluate vaccine responses, it is currently recommended to measure at least IgG specific antibody concentrations against Ptx, because this assay is not too labour intensive and enables a good comparison between different studies.

*Baseline antibody concentrations and memory B cell frequencies related to the magnitude of vaccination response*

The magnitude of the booster vaccine responses turned out to be correlated with antigen specific baseline IgG antibody concentrations and baseline memory B cell frequencies. Individuals with baseline IgG-Ptx antibody concentrations < 10 IU/ml generally had lower PTNA responses post-booster (**chapter 4**). For antigen-specific memory B cell frequencies, we observed a similar pattern. Individuals with memory B cells ≤ 1/100,000 PBMCs for Ptx, FHA or Prn generally had lower memory B cell frequencies, and lower specific IgG antibody concentrations post-booster for the corresponding antigen (**chapter 5**). The importance of baseline antibody concentrations against the three pertussis vaccine antigens on the magnitude of the response has been described before by Kaml et al. who also described a correlation between pre and post booster antibody concentrations [49]. The implications of the presence of circulating memory B cells against pertussis antigens for protection against disease are still unclear, but suggests to be of importance. For Japanese encephalitis virus, memory B cells have been proposed as a correlate for evaluating vaccine efficacy [50]. If indeed baseline IgG concentrations and/or the presence of pertussis-specific memory B-cells would be indicative for a correlate of protection, then modelling of baseline antibody concentrations and/or baseline memory B cell frequencies might help to determine a booster interval by studying the decay of antibodies and/or memory B cells over time.

**Are aP booster vaccination responses and hybrid immunity influenced by age?**

In the BERT study, the most outstanding differences in the vaccine responses between various age groups were IgA related. Specific IgA responses were however generally much lower compared with specific IgG responses. The IgA antibody concentrations against Ptx, FHA, and Prn (**chapter 3**) increased with age at baseline, one month, and one year post vaccination and were much higher in the older adult group compared with children, adolescents, and young adults, as described previously [51-54]. In older adults, IgA antibody concentrations against Ptx correlated well (r = 0.76) with PTNAs, which was not found in the other age groups. The high correlation suggests a role of IgA-Ptx in the neutralisation assay in addition to the IgG-Ptx levels. Next to pertussis vaccine antigen specific IgA antibodies, also the increase in total numbers of IgA plasma cells as measured by flowcytometry were highest in older adults, both the absolute increase and the fold change from baseline. A combination of the exposure to (multiple) natural infections during lifetime and potentially also priming by natural infection instead of pertussis vaccination in infancy, are likely to contribute in these higher IgA responses upon aP booster vaccination in the older adults. Induction of IgA is mediated largely by the mucosal route and is therefore induced by infection rather than by intramuscular vaccination [55]. Once an IgA response against pertussis has been induced, parental vaccination will reactivate these IgA responses [55].

Age related differences concerning antigen specific IgG production post-vaccination were not observed in serum, but differences were observed at B cell level (**chapter 6)**. Generally, adolescents tended to show the highest vaccine specific memory B cell responses to all three pertussis vaccine antigens and the older adult group the lowest responses. With respect to the adolescent age group, higher vaccination responses in 9-15-year-olds compared to 16-25-year-olds have been previously described for HPV vaccines [56] and higher responses in 12 and 15 year-olds compared to 10 year-olds for meningococcal serogroup W [57]. These results might point to adolescence as a possible optimal age for vaccination, however this may be vaccine specific and is also dependent on the degree of circulation of the concerning pathogen. The high circulation of *B. pertussis* is also likely to contribute to the higher memory B cell frequencies in adolescence. From 2006 to 2019, notification rates of reported clinically diagnosed pertussis incidence were higher in this adolescent age group compared to primary school children and adults in all three study countries according to ECDC [6]. Therefore recent infection pressure might also be higher in this adolescent age group compared to other age groups. Also mucosal antibody concentrations directed to non-vaccine antigens in the Dutch aP primed participants imply higher exposure to *B. pertussis* in the aP primed adolescents compared to the aP primed children [46]. After infection a slow decay over time in memory B cell frequencies was previously found [58], resulting in higher baseline memory B cells against pertussis antigens in the adolescent age group. As noted earlier, higher memory B cell frequencies at baseline were associated with higher memory B cell frequencies post-vaccination. Together, this may all contribute to the trend of higher antigen specific memory B cells frequencies in the adolescents at all timepoints. On the other hand, the group of older adults showed a trend towards low frequencies of memory B cells both pre and post aP booster vaccination for all three pertussis vaccine antigens and a lower absolute increase in overall numbers of IgG plasma cells between baseline and day 7 post-booster. This might be explained by a lower immune function with age, called immunosenescence, that leads to less functional innate and adaptive immune responses [59, 60]. Immunosenescence is associated with low numbers of naive lymphocytes resulting in a lower induction of new memory B cells, reduced plasma cell generation, reduced levels of antibodies and reduced antibody functioning upon (booster) vaccination. However, while recirculating memory B cell responses seem to be affected by age in our study, we found no loss of quantitative IgG responses against the three vaccine antigens in the older age group. Also functional antibody responses seem not affected by age. Both the long-lived plasma cell population pool and the memory B cell pool residing in the bone marrow might play a role here. In an extensive review Kandeil et al. also reported good vaccine responses among older adults [15]. This is important in case booster vaccination for older age groups is considered.

*Influence of age differences in children and young adults on antibody concentrations on the long term*

In the antibody kinetics studies from **chapter 7** higher initial antibody concentrations of the pertussis vaccine antigens were observed in the young adults group (25-29 years) compared to the paediatric groups (9 years). After an increase within the first month, an initial fast decay was observed in the first 2,5 years, followed by a more gradual decay in the following years resulting in higher antibody concentrations in young adults compared to children with GMCs still exceeding baseline after 10 years in the young adults. Tomovici et al. did not observe any difference in any of the pertussis vaccine antigens between adolescents and adults during a 10 year follow-up study in the USA [61]. Pool et al., performed a long term follow-up study in Canada and also compared (wP primed) adolescents to (wP primed) adults [62]. IgG-Prn showed a comparable pattern to our study with a slower decay in adults compared to adolescents, however IgG-Ptx concentrations had already decreased back to baseline within 5 years in both groups. The exact antibody concentrations from the Canadian and USA study measured in EU/ml cannot be compared to the antibody concentrations in our study measured in IU/ml. In addition, a lot of variables are different between and within these studies like year of inclusion, primary immunisation programme and infection pressure preceding and during the study. It is likely that all these factors influence the vaccination responses, therefore results are difficult to translate from one country to another. In a country with high *B. pertussis* circulation like the Netherlands, antibody concentrations of young adults tend to remain higher post aP booster over a longer period compared to school aged children. Although differences in long-term vaccination responses between children and young adults were observed, for both age groups extra booster vaccinations seem not to be recommended since the pertussis disease burden is low and hybrid immunity seems favourable in terms of protection against reinfection and duration of protection. However, pertussis antibody kinetics in the young adults can be used to determine a booster interval for specific target groups like health care personnel.

**Are aP booster vaccination responses influenced by type of priming vaccination?**

Whole cell and acellular vaccines induce different types of B and T cell immunity [22]. In our study, the only significant difference in the B cell compartment between aP and wP primed participants (**chapter 6**) was a more pronounced IgG1 plasma cell expansion in the Dutch wP primed participants compared with the Dutch aP primed participants. This was most outspoken in the adolescent age group but observed also in all age groups together. Eberhard et al. suggested that aP vaccines lead to different germinal centre reactions compared to wP vaccines, resulting in an unsuccessful germinal centre-derived affinity maturation, selection and differentiation, and eventually in inefficient homing in the bone marrow, impacting survival and recall of B cell clones [63]. This is in line with the observed

vaccine antigen specific memory B cell results, as described in this thesis and earlier [17], with a non-significant trend towards higher pertussis vaccine antigen specific memory B cells in wP primed compared with aP primed children and adolescents (**chapter 5**). The lack of significance might be due to the small group size of participants in the BERT study. From previous studies it is known that after a first acellular booster dose, vaccine antigen specific B cell responses were higher in aP primed individuals [36], while after a second aP booster dose, B cell responses seemed higher in wP primed individuals [17]. In the BERT study, the aP booster vaccination was also a second booster, following the last aP booster at 4 years of age in the children and adolescent age groups.

The differences observed between the two adolescent groups with different vaccine background might also be explained by the interval since the last vaccination where the wP primed adolescents were not only older at the time of the aP booster compared with the aP primed individuals, but also experienced a longer interval since their last booster vaccination. In fact, the interval since the last vaccination in the aP cohort might have been too short with still high vaccine-specific antibody concentrations present in the circulation, causing a somewhat blunted memory B cell reactivation [64]. There was however no significant difference observed in the pre and post booster IgG and IgA antibody concentrations against the pertussis vaccine antigens between the aP and the wP groups. PTNA results however, did reveal differences at baseline between aP and wP primed adolescents, with higher values in the wP primed adolescents compared with the aP primed adolescents. Therefore, the influence from the type of priming vaccination seems to be more important than the interval since last vaccination. One year post aP booster, the Finnish wP primed adolescents showed a higher neutralisation capacity than their aP primed peers, but this difference was not observed in the Dutch adolescents. It should be noted that participating groups of adolescents were small with 48 Dutch and 37 Finnish children and adolescents, with many interfering factors like the number of priming vaccinations, time interval since last booster, and exposure to circulation of *B. pertussis* over time. This makes it not feasible to demonstrate the real impact of the type of priming vaccination at the adolescent age in this study.



*Influence of type of priming vaccine on antibody concentrations and infection prevalence on the long term*

In the antibody kinetics studies (**chapter 7**) higher initial antibody responses of the pertussis vaccine antigens in the first month were observed in the wP primed children at age 9 years compared to the aP primed children. After the increase of vaccine antigen specific IgG antibody concentrations post aP booster vaccination within the first month, an initial faster antibody decay in the aP primed children compared with the wP primed children was observed, which is in line with other studies from the Netherlands and the USA [17, 65]. By

modelling of the antibody concentrations on the longer term, it was estimated that from approximately 2.5 years post-booster vaccination onwards both aP and wP primed children had similar IgG pertussis vaccine antibody concentrations. The meaning of the observed lower increase followed by an initial faster antibody decay in aP primed children compared to wP primed children in relation to protection is unclear. After approximately six years we did find a higher percentage of aP primed participants meeting the serological criteria of infection (seroconversion), though none of the participants has been clinically diagnosed with pertussis disease during the study. The different vaccine antibody responses in the aP primed children compared to the wP primed children might have contributed to this seroconversion following infection. This difference in seroconversion might be best explained by the different T cell immune profiles. Pertussis infection and wP vaccination in infancy leads to a more Th1 and Th17 dominant response, where aP vaccination in infancy leads to a more Th2 dominant response, as previously described in other studies as well as in the BERT study cohort [12, 17, 45, 66, 67]. Both Th1 and Th17 mediated immune responses are required for protection against transmission and colonisation [12, 13, 22]. Interestingly, the Th2 polarisation in aP primed young adults is specific for the vaccine antigens. Non-vaccine antigens are similarly Th1/Th2 balanced in both aP and wP primed young adults suggesting repeated natural infection to counteract the Th1/Th2 polarisation [68]. This again favours hybrid immunity over booster vaccination in aP primed individuals in adolescence and young adult age.

**Future perspectives and recommendations**

To prevent clinical pertussis, infant protection against infection and disease is of the utmost importance because they are most vulnerable. Newborns in their first months of life are best protected by maternal vaccination since maternal antibodies and possibly other transferred immune cells [19] protect them against severe clinical pertussis immediately after birth until they have received the primary vaccination series themselves. Maternal vaccination is for the moment the keystone in protection of newborns, since the disease remains endemic and herd protection is not achieved with current aP vaccines. Currently, in the Netherlands and many other countries, pregnant women are advised to get a maternal dTap vaccination during every pregnancy [69]. Although successive dTap vaccinations are described to be safe [70], immunogenicity of multiple dTap vaccinations with relatively short time intervals in case of frequent pregnancies, need further investigation. Our longer term data on the non-pregnant young adults at childbearing age (**chapter** 7) indicate generally high antibody responses and slow antibody decay. In the first year post-booster, antibody kinetics of dTap vaccinated pregnant and non-pregnant women are similar [71]. It would be interesting to investigate if the antibody kinetics will remain similar between the pregnant and non-pregnant women over a longer period. Also data on cellular immunity is scarce. In addition, more research is required on the immunogenicity of a

dTap booster related to previous vaccination history, since women of child bearing age nowadays are potentially primed in infancy with aP vaccines instead of wP vaccines. In the final analysis of all data obtained within the PERISCOPE project, more conclusions on potential immunological correlates of protection for pertussis will follow which together with epidemiological data can contribute to the (maternal) vaccination recommendations.

Recently, during the COVID-19 pandemic, the reported pertussis incidence declined dramatically with the introduction of non-pharmaceutical interventions (NPI) e.g. face masks and physical distancing as public health measures aimed to prevent SARS-CoV-2 transmission in the community [72, 73]. NPIs were shown not only to prevent COVID-19 infections, but also other airborne infections like pertussis. As was noticed for RSV, easing of restrictions led to delayed outbreaks because the immunity in the population against the pathogen was decreased [74]. In case of pertussis, also more people have become vulnerable because of the absence of boosting due to natural infection over the recent years. When the circulation of *B. pertussis* increases again, this will cause new outbreaks, as is currently reported in Denmark [75]. Also in the Netherlands and Belgium pertussis disease incidence is increasing considerably this winter of 2023/24 and is much higher compared with the past few years [76]. A new serosurveillance study in the Netherlands is planned for 2026 to evaluate the vulnerability of the population to pertussis infection and specifically that of the different risk and age groups.

Based on the studies in this thesis, aP booster vaccination in several age and risk groups are to be considered. Older adults and especially those above 50 years of age with pre-existing chronic obstructive pulmonary disease or asthma seem more at risk for pertussis disease and may benefit from aP booster vaccination [77, 78]. In contrast, adolescents, might benefit from hybrid immunity via natural infection, since they show mostly few disease symptoms and hybrid immunity may offer longer and better protection compared with aP booster vaccine induced immunity. However, the solution for pertussis is to develop vaccines with longer lasting immunity against infection as well as prevention of colonisation and transmission. When these new better pertussis will enter the market, population wide booster vaccinations should be reconsidered to try creating herd protection in order to protect vulnerable groups that cannot be vaccinated. The live attenuated pertussis vaccine 'BPZE1' currently tested in phase 2 trails seems to be a promising candidate [79].

For the pertussis research into new vaccines the establishment of some CoPs for pertussis disease and infection would be helpful, because once we discover the key of protective immunity against pertussis, recommendations about both primary, booster, and maternal vaccinations can be optimised. For the evaluation of herd protection, we also need potential CoPs against colonisation and transmission. Once these CoPs will be established and improved vaccines are available, pertussis outbreaks might belong to the past.

## REFERENCES


1. Nieves, D.J. and U. Heininger, Bordetella pertussis. Microbiol Spectr, 2016. 4(3): p. EI10-0008-2015. doi: 10.1128/microbiolspec.EI10-0008-2015.
2. Cherry, J.D., Pertussis: challenges today and for the future. PLoS Pathog, 2013. 9(7): p. e1003418. doi: 10.1371/journal.ppat.1003418.
3. de Greeff, S.C., et al., Seroprevalence of pertussis in The Netherlands: evidence for increased circulation of Bordetella pertussis. PLoS One, 2010. 5(12): p. e14183, DOI: 10.1371/journal.pone.0014183.
4. Alimohamadi, Y., et al., Spatio-temporal analysis of Pertussis using geographic information system among Iranian population during 2012-2018. Med J Islam Repub Iran, 2020. 34: p. 22.
5. Brugueras, S., et al., Does the economic recession influence the incidence of pertussis in a cosmopolitan European city? BMC Public Health, 2019. 19(1): p. 144.
6. ECDC. Disease data from ECDC Surveillance Atlas for pertussis. 2019 [cited 2019 20 September]; Available from: http://ecdc.europa.eu/en/pertussis/surveillance-and-disease-data/atlas].
7. van der Lee, S., et al., Enhanced Bordetella pertussis acquisition rate in adolescents during the 2012 epidemic in the Netherlands and evidence for prolonged antibody persistence after infection. Euro Surveillance, 2017. 22(47): p. 17-00011. doi: 10.2807/1560-7917.ES.2017.22.47.17-00011.
8. Mossong, J., et al., Social contacts and mixing patterns relevant to the spread of infectious diseases. PLoS Med, 2008. 5(3): p. e74, DOI: 10.1371/journal.pmed.0050074.
9. Wallinga, J., P. Teunis, and M. Kretzschmar, Using data on social contacts to estimate age-specific transmission parameters for respiratory-spread infectious agents. Am J Epidemiol, 2006. 164(10): p. 936-44.
10. De Serres, G., et al., Morbidity of pertussis in adolescents and adults. J Infect Dis, 2000. 182(1): p. 174-179, DOI: 10.1086/315648.
11. van de Kassteele, J., J.C. van Eijkeren, and J. Wallinga, Efficient estimation of age-specific social contact rates between men and women. Ann. Appl. Stat., 2017. 11(1): p. 320-339.
12. Sheridan, S.L., et al., Waning vaccine immunity in teenagers primed with whole cell and acellular pertussis vaccine: recent epidemiology. Expert Rev Vaccines, 2014. 13(9): p. 1081-1106. doi: 10.1586/14760584.2014.944167.
13. Warfel, J.M., L.I. Zimmerman, and T.J. Merkel, Acellular pertussis vaccines protect against disease but fail to prevent infection and transmission in a nonhuman primate model. Proc Natl Acad Sci U S A, 2014. 111(2): p. 787-792, DOI: 10.1073/pnas.1314688110.
14. Craig, R., et al., Asymptomatic Infection and Transmission of Pertussis in Households: A Systematic Review. Clin Infect Dis, 2020. 70(1): p. 152-161.
15. Kandeil, W., et al., The burden of pertussis in older adults: what is the role of vaccination? A systematic literature review. Expert Rev Vaccines, 2019. 18(5): p. 439-455. doi: 10.1080/14760584.2019.1588727.
16. Gezondheidsraad, Evaluatie schema Rijksvaccinatieprogramma, W.e.S. Volksgezondheid, Editor. 2022, Gezondheidsraad 2022: Den Haag.
17. van der Lee, S., et al., Whole-Cell or Acellular Pertussis Primary Immunizations in Infancy Determines Adolescent Cellular Immune Profiles. Front Immunol, 2018. 9: p. 51. doi: 10.3389/fimmu.2018.00051.

**18.** Schure, R.M., et al., Differential T- and B-cell responses to pertussis in acellular vaccine-primed versus whole-cell vaccine-primed children 2 years after preschool acellular booster vaccination. Clin Vaccine Immunol, 2013. 20(9): p. 1388-1395. doi: 10.1128/cvi.00270-13.

**19.** Callender, M. and E.T. Harvill, Maternal vaccination: shaping the neonatal response to pertussis. Front Immunol, 2023. 14: p. 1210580.

**20.** ECDC. Pertussis: Recommended vaccinations. 2020 [cited 2020 16 September]; Available from: https://vaccine-schedule.ecdc.europa.eu/Scheduler/ByDisease?SelectedDiseaseId=3&SelectedCountryIdByDisease=-1.

**21.** Nordström, P., M. Ballin, and A. Nordström, Risk of SARS-CoV-2 reinfection and COVID-19 hospitalisation in individuals with natural and hybrid immunity: a retrospective, total population cohort study in Sweden. The Lancet Infectious Diseases, 2022. 22(6): p. 781-790.

**22.** Kapil, P. and T.J. Merkel, Pertussis vaccines and protective immunity. Curr Opin Immunol, 2019. 59: p. 72-78.

**23.** The Lancet Infectious, D., Why hybrid immunity is so triggering. Lancet Infect Dis, 2022. 22(12): p. 1649.

**24.** Choi, J.H., et al., Improving vaccination rates in older adults and at-risk groups: focus on pertussis. Aging Clin Exp Res, 2022. 34(1): p. 1-8.

**25.** Kanitz, E.E., et al., Variation in adult vaccination policies across Europe: an overview from VENICE network on vaccine recommendations, funding and coverage. Vaccine, 2012. 30(35): p. 5222-8.

**26.** McGirr, A., D.N. Fisman, and A.R. Tuite, The health and economic burden of pertussis in Canada: A microsimulation study. Vaccine, 2019. 37(49): p. 7240-7247.

**27.** an-Willem, V., J. Nicolas, and R. Ken, Cost of illness due to pertussis in adults ≥50 years of age in the United Kingdom. Vaccine, 2023.

**28.** Hovingh, E.S., et al., Detection of opsonizing antibodies directed against a recently circulating Bordetella pertussis strain in paired plasma samples from symptomatic and recovered pertussis patients. Sci Rep, 2018. 8(1): p. 12039.

**29.** Weiss, A.A., et al., Acellular pertussis vaccines and complement killing of Bordetella pertussis. Infect Immun, 2004. 72(12): p. 7346-51.

**30.** Zanaboni, E., et al., A novel high-throughput assay to quantify the vaccine-induced inhibition of Bordetella pertussis adhesion to airway epithelia. BMC Microbiol, 2016. 16: p. 215.

**31.** Hewlett, E.L., et al., Induction of a novel morphological response in Chinese hamster ovary cells by pertussis toxin. Infect Immun, 1983. 40(3): p. 1198-203.

**32.** Blanc, P., et al., The role of bactericidal and opsonic activity in immunity against Bordetella pertussis. Expert Rev Vaccines, 2022. 21(12): p. 1727-1738.

**33.** Gao, H., E.H.Y. Lau, and B.J. Cowling, Waning Immunity After Receipt of Pertussis, Diphtheria, Tetanus, and Polio-Related Vaccines: A Systematic Review and Meta-analysis. J Infect Dis, 2022. 225(4): p. 557-566.

**34.** Barkoff, A.M., A. Knuutila, J. Mertsola, and Q. He, Evaluation of Anti-PT Antibody Response after Pertussis Vaccination and Infection: The Importance of Both Quantity and Quality. Toxins (Basel), 2021. 13(8).

**35.** van der Lee, S., et al., Robust Humoral and Cellular Immune Responses to Pertussis in Adults After a First Acellular Booster Vaccination. Front Immunol, 2018. 9: p. 681.

**36.** Hendrikx, L.H., et al., Impact of infant and preschool pertussis vaccinations on memory B-cell responses in children at 4 years of age. Vaccine, 2011. 29(34): p. 5725-5730. doi: 10.1016/j.vaccine.2011.05.094.

**37.** Vincent, J.M., et al., Prolonged afebrile nonproductive cough illnesses in American soldiers in Korea: a serological search for causation. Clin Infect Dis, 2000. 30(3): p. 534-539. doi: 10.1086/313707.

**38.** Storsaeter, J., H.O. Hallander, L. Gustafsson, and P. Olin, Levels of anti-pertussis antibodies related to protection after household exposure to Bordetella pertussis. Vaccine, 1998. 16(20): p. 1907-16.

**39.** Hellwig, S.M., et al., Crucial role of antibodies to pertactin in Bordetella pertussis immunity. J Infect Dis, 2003. 188(5): p. 738-742. doi: 10.1086/377283.

**40.** Schurink-van 't Klooster, T. and H. de Melker, The National Immunisation Programme in the Netherlands : Surveillance and developments in 2019-2020. 2020, DOI: 10.21945/rivm-2020-0077, Rijksinstituut voor Volksgezondheid en Milieu RIVM.

**41.** Lesne, E., et al., Acellular Pertussis Vaccines Induce Anti-pertactin Bactericidal Antibodies Which Drives the Emergence of Pertactin-Negative Strains. Front Microbiol, 2020. 11: p. 2108.

**42.** Barkoff, A.M., et al., Pertactin-deficient Bordetella pertussis isolates: evidence of increased circulation in Europe, 1998 to 2015. Euro Surveill, 2019. 24(7): p. 1700832. doi: 10.2807/1560-7917.Es.2019.24.7.1700832.

**43.** Hegerle, N., et al., Evolution of French Bordetella pertussis and Bordetella parapertussis isolates: increase of Bordetellae not expressing pertactin. Clin Microbiol Infect, 2012. 18(9): p. E340-E346. doi: 10.1111/j.1469-0691.2012.03925.x.

**44.** Wrammert, J., et al., Rapid cloning of high-affinity human monoclonal antibodies against influenza virus. Nature, 2008. 453(7195): p. 667-71.

**45.** Lambert, E.E., et al., Uncovering Distinct Primary Vaccination-Dependent Profiles in Human Bordetella Pertussis Specific CD4+ T-Cell Responses Using a Novel Whole Blood Assay. Vaccines (Basel), 2020. 8(2): p. 225. doi: 10.3390/vaccines8020225.

**46.** van Schuppen, E., et al., Prior exposure to B. pertussis shapes the mucosal antibody response to acellular pertussis booster vaccination. Nat Commun, 2022. 13(1): p. 7429.

**47.** de Graaf, H., et al., Investigating Bordetella pertussis colonisation and immunity: protocol for an inpatient controlled human infection model. BMJ Open, 2017. 7(10): p. e018594.

**48.** PERISCOPE Consortium, PERISCOPE: road towards effective control of pertussis. Lancet Infect Dis, 2019. 19(5): p. e179-e186, DOI: 10.1016/s1473-3099(18)30646-7.

**49.** Kaml, M., et al., Booster vaccination in the elderly: their success depends on the vaccine type applied earlier in life as well as on pre-vaccination antibody titers. Vaccine, 2006. 24(47-48): p. 6808-11.

**50.** Larena, M., et al., JE-ADVAX vaccine protection against Japanese encephalitis virus mediated by memory B cells in the absence of CD8(+) T cells and pre-exposure neutralizing antibody. J Virol, 2013. 87(8): p. 4395-402.

**51.** Fumimoto, R., et al., Seroprevalence of IgA and IgM antibodies to Bordetella pertussis in healthy Japanese donors: Assessment for the serological diagnosis of pertussis. PLoS One, 2019. 14(7): p. e0219255.

**52.** May, M.L., et al., Pertussis toxin IgA testing over-diagnoses recent pertussis infection. Pathology, 2017. 49(7): p. 770-775.

**53.** Prince, H.E., J.M. Lieberman, and J.D. Cherry, Age-related differences in patterns of increased Bordetella pertussis antibodies. Clin Vaccine Immunol, 2012. 19(4): p. 545-550, DOI: 10.1128/cvi.05725-11.

**54.** Le, T., et al., Immune responses and antibody decay after immunization of adolescents and adults with an acellular pertussis vaccine: the APERT Study. J Infect Dis, 2004. 190(3): p. 535-44.

**55.** Hendrikx, L.H., et al., Serum IgA responses against pertussis proteins in infected and Dutch wP or aP vaccinated children: an additional role in pertussis diagnostics. PLoS One, 2011. 6(11): p. e27681.

**56.** Van Damme, P., et al., Immunogenicity and Safety of a 9-Valent HPV Vaccine. Pediatrics, 2015. 136(1): p. e28-39.

**57.** van Ravenhorst, M.B., et al., Adolescent meningococcal serogroup A, W and Y immune responses following immunization with quadrivalent meningococcal A, C, W and Y conjugate vaccine: Optimal age for vaccination. Vaccine, 2017. 35(36): p. 4753-4760.

**58.** van Twillert, I., et al., Age related differences in dynamics of specific memory B cell populations after clinical pertussis infection. PLoS One, 2014. 9(1): p. e85227. doi: 10.1371/journal.pone.0085227.

**59.** Crooke, S.N., I.G. Ovsyannikova, G.A. Poland, and R.B. Kennedy, Immunosenescence and human vaccine immune responses. Immun Ageing, 2019. 16: p. 25. doi: 10.1186/s12979-019-0164-9.

**60.** Gustafson, C.E., C. Kim, C.M. Weyand, and J.J. Goronzy, Influence of immune aging on vaccine responses. J Allergy Clin Immunol, 2020. 145(5): p. 1309-1321. doi: 10.1016/j.jaci.2020.03.017.

**61.** Tomovici, A., et al., Humoral immunity 10 years after booster immunization with an adolescent and adult formulation combined tetanus, diphtheria, and 5-component acellular pertussis vaccine. Vaccine, 2012. 30(16): p. 2647-53.

**62.** Pool, V., et al., Humoral immunity 10years after booster immunization with an adolescent and adult formulation combined tetanus, diphtheria, and 5-component acellular pertussis vaccine in the USA. Vaccine, 2018. 36(17): p. 2282-2287. doi: 10.1016/j.vaccine.2018.03.029.

**63.** Eberhardt, C.S. and C.A. Siegrist, What Is Wrong with Pertussis Vaccine Immunity? Inducing and Recalling Vaccine-Specific Immunity. Cold Spring Harb Perspect Biol, 2017. 9(12): p. a029629. doi: 10.1101/cshperspect.a029629.

**64.** Amanna, I.J. and M.K. Slifka, Contributions of humoral and cellular immunity to vaccine-induced protection in humans. Virology, 2011. 411(2): p. 206-15.

**65.** Decker, M.D., D.P. Greenberg, D.R. Johnson, and V. Pool, Randomized study of immune responses to two Tdap vaccines among adolescents primed with DTaP and comparison with results among adolescents primed with DTwP. Vaccine, 2019. 37(35): p. 5003-5008.

**66.** da Silva Antunes, R., et al., Th1/Th17 polarization persists following whole-cell pertussis vaccination despite repeated acellular boosters. J Clin Invest, 2018. 128(9): p. 3853-3865.

**67.** Schure, R.M., et al., T-cell responses before and after the fifth consecutive acellular pertussis vaccination in 4-year-old Dutch children. Clin Vaccine Immunol, 2012. 19(11): p. 1879-86.

**68.** da Silva Antunes, R., et al., T cell reactivity to Bordetella pertussis is highly diverse regardless of childhood vaccination. Cell Host Microbe, 2023. 31(8): p. 1404-1416.e4.

**69.** National Institute for Public Health and the Environment (RIVM), RVP-richtlijn Uitvoering Rijksvaccinatieprogramma. 2022: Bilthoven.

**70.** D'Heilly, C., C. Switzer, and D. Macina, Safety of Maternal Immunization Against Pertussis: A Systematic Review. Infect Dis Ther, 2019. 8(4): p. 543-568.

**71.** Huygen, K., et al., Humoral and cell mediated immune responses to a pertussis containing vaccine in pregnant and nonpregnant women. Vaccine, 2015. 33(33): p. 4117-23.

**72.** Tessier, E., et al., Impact of the COVID-19 pandemic on Bordetella pertussis infections in England. BMC Public Health, 2022. 22(1): p. 405.

**73.** He, H., et al., The decline in immunity and circulation of pertussis among Chinese population during the COVID-19 pandemic: A cross-sectional sero-epidemiological study. Vaccine, 2022. 40(48): p. 6956-6962.

**74.** den Hartog, G., et al., Decline of RSV-specific antibodies during the COVID-19 pandemic. Lancet Infect Dis, 2023. 23(1): p. 23-25.

**75.** Bagcchi, S., Pertussis cases rise in Denmark. The Lancet Infectious Diseases, 2023. 23(11): p. e469.

**76.** Nivel. Cijfers ziekten per week - actueel: wat heerst er nu in Nederland? 2023 05-12-2023]; Available from: https://www.nivel.nl/nl/resultaten-van-onderzoek/actuele-cijfers-ziekten-per-week.

**77.** Chen, J., et al., Incidence and Healthcare Burden of Pertussis among Older Adults with and without Pre-Existing Chronic Obstructive Pulmonary Disease or Asthma in South Korea. Copd, 2023. 20(1): p. 126-134.

**78.** Bahar, E., D. Shamarina, Y. Sergerie, and P. Mukherjee, Descriptive Overview of Pertussis Epidemiology Among Older Adults in Europe During 2010-2020. Infect Dis Ther, 2022. 11(5): p. 1821-1838.

**79.** Locht, C., The Path to New Pediatric Vaccines against Pertussis. Vaccines (Basel), 2021. 9(3).

8

# Appendices

# Nederlandse samenvatting

# Dankwoord

# List of publications

# About the author

## NEDERLANDSE SAMENVATTING

Kinkhoest is een infectie van de luchtwegen die wordt veroorzaakt door de gram negatieve bacterie *Bordetella pertussis*. Kinkhoest begint als een milde verkoudheid, maar kan een ernstig beloop krijgen. Volgens de WHO definitie spreken we van kinkhoest als er sprake is van minimaal twee weken hoesten in combinatie met krampachtige hoestaanvallen, gierend naar lucht happen na lang hoesten (het typische kinken), of braken na hoesten. Ook spreken we van kinkhoest als laboratorium diagnostiek positief is voor de aanwezigheid van *Bordetella pertussis* bij mensen met klachten. Kinkhoest is vooral voor nog ongevaccineerde zuigelingen erg gevaarlijk omdat het kan leiden tot ademstops (apneus) en een hoge bloeddruk in de longen die vrijwel niet te behandelen is (refractaire pulmonale hypertensie). Dit kan zelfs leiden tot de dood. Bij ouderen kan kinkhoest verergering van onderliggend lijden geven, zoals bijvoorbeeld een longaanval (exacerbatie COPD) of hartfalen (decompensatio cordis). Gevaccineerde personen en mensen die eerder kinkhoest hebben doorgemaakt, worden vaak opnieuw geïnfecteerd, maar hebben dan meestal een atypisch, mild of zelfs asymptomatisch infectie beloop. In de jaren vijftijd werd vaccinatie tegen kinkhoest voor jonge zuigelingen ingevoerd, met een vaccin gebaseerd op de hele *B. pertussis* bacterie (whole cell pertussis (wP) vaccin). Hierdoor is de kinkhoest incidentie sterk afgenomen en is het reproductiegetal sterk gedaald van 15-17 ($R_0$) naar 5,5.

Zowel kinkhoestvaccinatie als natuurlijke infectie leiden tot opbouwen van specifieke immuniteit tegen kinkhoest, door antistoffen en door immuuncellen, het adaptieve immuunsysteem genaamd. De B- en T-cellen zijn de belangrijkste cellen van het immuunsysteeem die betrokken zijn bij de ontwikkeling van immuniteit. De B-cellen zijn belangrijk voor de productie van antistoffen en worden in hun ontwikkeling geholpen door specifieke T-helpercellen. De meest ontwikkelde B-cellen zijn de plasmacellen, die de meeste antistoffen uitscheiden in het bloed. Wat betreft antistoffen worden na vaccinatie vooral immunoglobuline (Ig) G antistoffen tegen eiwitten van het vaccin in het bloed gevonden. In geval van een natuurlijke infectie zijn zowel IgG als IgA antistoffen in het bloed aantoonbaar en worden IgA antistoffen ook uitgescheiden op de slijmvliezen van de luchtwegen, waar de infectie plaats vindt. Als iemand eerder een infectie heeft doorgemaakt, wordt door een vaccinatie daarna zowel de IgG als de IgA respons gereactiveerd. Antistoffen hebben verschillende functies waaronder het neutraliseren van toxinen. In geval van kinkhoest is het neutraliseren van het pertussis toxine belangrijk, want dit voorkomt de typische kinkhoest symptomen zoals hoesten, leukocytose, immuunonderdrukking en ontsteking van weefsels. Daarom zijn antistoffen tegen pertussis toxine (anti-Ptx) ook een centrale component in de preventie van kinkhoest. Naast het maken van antistoffen zijn B-cellen ook belangrijk voor de vorming van immunologisch geheugen, dit betreffen de B-geheugencellen en de langlevende plasmacellen, die bij een nieuw kinkhoest contact snel kunnen reageren.

Bij de invoer van het wP vaccin in de jaren vijftig werd in Nederland vier keer een wP vaccinatie toegediend in het eerste levensjaar. Wegens bijwerkingen van wP vaccins zoals koorts kwamen in de jaren negentig acellulaire pertussis (aP) vaccins op de markt, die een tot vijf gezuiverde eiwitcomponenten van de kinkhoestbacterie bevatten. Alle aP vaccins bevatten een aangepast pertussis toxine (Ptx), pertussistoxoïd (Ptxd)(aP1), de meeste vaccins bevatten daarnaast filamenteus hemagglutinine (FHA) en pertactine (Prn)(aP3) en een aantal aP vaccines bevatten ook nog fimbriale agglutinogenen types 2 en 3 (Fim2/3) (aP5). Deze aP vaccins geven beduidend minder bijwerkingen, maar sturen de immuniteit ook anders aan dan wP vaccins, vooral wat betreft de T-cel immuniteit. T-helper (Th) cellen zijn belangrijk in de activatie van andere immuuncellen en zijn te onderscheiden in verschillende types afhankelijk van de signaalstoffen die ze produceren. Th1 en Th17 cellen zijn belangrijk voor de directe bescherming en klaring van de *B. pertussis* bacterie en Th2 cellen richten zich meer op activatie van de B-cellen. In vergelijking met wP vaccins die vooral een beschermende Th1 en in mindere mate ook een Th17 respons induceren, geven de aP vaccins meer een gemengd Th1/Th2 beeld. Hoewel ziektesymptomen goed worden voorkomen door aP vaccinatie, is het effect op de klaring van de *B. pertussis* bacterie veel minder en daarmee wordt het overdragen van de infectie van de ene naar de andere persoon minder goed tegengegaan. In Nederland is in 2001 een aP boostervaccinatie ingevoerd voor 4-jarigen, voordat ze naar school gaan. In 2005 werd het Nederlandse wP vaccin in de primaire serie vervangen door een aP vaccin. Recent, in 2019, is daarnaast een maternale aP vaccinatie ingevoerd om vooral nog ongevaccineerde zuigelingen in de eerste levensmaanden te beschermen tegen ernstige kinkhoest. Indien moeder tijdens de zwangerschap tijdig is gevaccineerd, is de baby voor een belangrijk deel beschermd na de geboorte en kan de eerste vaccinatie tegen kinkhoest bij gezonde, op tijd geboren zuigelingen pas bij drie maanden worden gegeven. Bovendien zijn twee vaccinaties voor de leeftijd van zes maanden in plaats van drie vaccinaties voldoende, met een booster rond twaalf maanden. De baby wordt na de geboorte vooral beschermd door antistoffen tegen kinkhoest van de moeder die via de placenta overgedragen worden naar de foetus. Deze antistoffen verdwijnen geleidelijk uit het bloed, maar bieden de eerste maanden goede bescherming. Daarnaast geeft moeder antistoffen via de borstvoeding door.

Ondanks de hoge vaccinatiegraad kwam er toch weer een toename van het aantal kinkhoestgevallen wereldwijd, deze toename viel veelal samen met de wissel van wP naar aP vaccins. Naast kinkhoest bij nog ongevaccineerde baby's wordt nu ook vaker kinkhoest gezien bij adolescenten en volwassenen. Behalve de wissel van het type vaccin, zijn er andere factoren die aan de gerapporteerde stijging kunnen hebben bijgedragen, zoals verbeterde registratie, verbeterde diagnostiek, verhoogde aandacht voor kinkhoest en ook de evolutie van de bacterie in de tijd.

De belangrijkste studie van dit proefschrift is de BERT-studie, die deel uitmaakt van een groot EU project: PERISCOPE. Binnen het PERISCOPE-project werken verschillende onderzoeksgroepen aan diverse kinkhoest gerelateerde (immunologische) onderzoeken. Dit proefschrift, als onderdeel van het PERISCOPE-project, is voornamelijk gericht op onderzoek naar antistoffen en B-celresponsen bij deelnemers uit drie landen (Nederland, Finland en het Verenigd Koninkrijk) en vooral gericht op uitgebreid en diepgaand onderzoek bij de Nederlandse deelnemers van de BERT-studie in Nederland na een boostervaccinatie met o.a. aP componenten (difterie, tetanus, aP3, en polio (dTaP3-IPV) vaccin). Andere deelnemende onderzoeksgroepen van het PERISCOPE-project concentreerden zich op T-cel immuniteit en mucosale immuniteit. De verschillende studies van het PERISCOPE-project zullen meer kennis opleveren over potentiële immunologische correlaten van bescherming tegen kinkhoest. Dit is relevant voor toekomstige vaccinatiestrategieën.

## KINKHOEST INFECTIE IN DE POPULATIE

Om de prevalentie van *B. pertussis* infecties (seruminfectieprevalentie) in Nederland te monitoren, werd in 2016/2017 bloed afgenomen in een dwarsdoorsnede van de Nederlandse bevolking en werden antistoffen gemeten tegen kinkhoest, serosurveillance genoemd, dit werd beschreven in hoofdstuk 2. Een IgG antistof concentratie ≥ 100 IU/ml gericht tegen het belangrijkste toxine van de kinkhoestbacterie, pertussis toxine (Ptx), werd gebruikt als indicatie voor een recente infectie met *B. pertussis*. De resultaten werden vergeleken met eerdere soortgelijke serosurveillance-onderzoeken. De seruminfectieprevalentie bleek gestegen van 1,0% in 1995/1996 naar 3,5% in 2006/2007 en naar 5,9% in 2016/2017. De leeftijdsgroep met de hoogste seruminfectieprevalentie in 2016/2017 was de adolescentengroep van 12-18 jaar, met tevens de grootste toename ten opzichte van het onderzoek in 2006/2007. Ook bij kinderen van 7-11 jaar en volwassenen van 50-64 jaar werd een toename van kinkhoestinfecties op basis van de antistoffen tegen Ptx gezien. Ondanks deze toename van de seruminfectieprevalentie, was er geen gelijktijdige stijging van meldingen van gediagnostiseerde kinkhoest gevallen door artsen in de praktijk. Het verschil tussen kinkhoestinfectie op basis van hoge antistoffen en gemelde gevallen in de praktijk was hoog; de seruminfectieprevalentie in 2006/2007 was 100 keer hoger dan het aantal gemelde gevallen in dezelfde periode. Dit verschil liep op tot 200 keer in 2016/2017, en zelfs tot 650 keer bij 80-plussers. Omdat de ziekte incidentie van kinkhoest met symptomen niet lijkt toegenomen, is momenteel een extra booster ter preventie van infectie niet geïndiceerd voor adolescenten en jong volwassenen. Echter oudere volwassenen vanaf 50 jaar, zeker met bijkomende medische risicofactoren, zouden wel gebaat kunnen zijn bij extra kinkhoest vaccinaties, omdat de diagnose kinkhoest in deze leeftijdsgroep waarschijnlijk vaker gemist wordt en vooral omdat ook veel vaker ernstige complicaties kunnen optreden.

## IMMUUNRESPONS NA EEN ACELLULAIRE BOOSTER VACCINATIE

Hoofdstukken 3, 4, 5 en 6 beschrijven immunologische gegevens van deelnemers van de BERT-studie. Kinderen van 7-11 jaar, adolescenten (12-15 jaar), jong volwassenen (20-34 jaar) en oudere volwassenen (50-60 jaar) uit Finland, Nederland en het Verenigd Koninkrijk ontvingen allen een aP booster vaccinatie aan het begin van de studie.

In hoofdstuk 3 werden IgG en IgA-antistofresponsen tegen drie *B. pertussis*-vaccin eiwitten (Ptx, FHA en Prn) beschreven. Alle leeftijdsgroepen vertoonden in de eerste maand na de boostervaccinatie een toename in de gemiddelde IgG en IgA antistofconcentraties, gevolgd door een afname, waarbij de antistof concentraties na een jaar nog steeds hoger lagen dan vooraf aan de booster vaccinatie. Opmerkelijk was dat de jong volwassenen de laagste absolute IgG-Ptx-antistofconcentraties in hun bloed hadden, zowel vóór als na de boostervaccinatie, maar wel de hoogste absolute toename in IgG-Prn antistoffen vertoonden. Bij oudere volwassenen waren de IgG-Prn-antistof concentraties het laagst op alle meetmomenten. De IgG antistof kinetiek van de drie afzonderlijke pertussis-vaccin eiwitten liet geen consistente relatie met leeftijd zien, in tegenstelling tot die van IgA antistoffen in het bloed, die zowel pre- als postvaccinatie toenamen met leeftijd. Dit heeft zeer waarschijnlijk direct relatie met het doormaken van pertussis infecties gedurende het leven. Er werden geen significante verschillen in antistof concentraties gevonden tussen kinderen en adolescenten die als zuigeling met aP of wP gevaccineerd waren. In alle leeftijdsgroepen werd een goede vaccinatierespons waargenomen, die niet afnam met de leeftijd. Vaccinatie van oudere volwassenen zou daarom kunnen worden overwogen om de ziektelast en ziektekosten die gepaard gaan met kinkhoestinfecties, te verminderen.

In hoofdstuk 4 wordt nader ingegaan op de neutraliserende functie van antistoffen tegen Ptx. Hierbij kunnen zowel IgG als IgA antistoffen en ook andere Ig's een rol spelen. Bij Nederlandse en Finse deelnemers zijn de Ptx neutraliserende antistoffen (PTNA's) geanalyseerd voor en na boostervaccinatie. In lijn met de toename van antistoffen tegen Ptx, namen ook PTNA's toe in de eerste maand na de dTap-boostervaccinatie bij alle leeftijdsgroepen en ook deze bleven een jaar na de boostervaccinatie op een hoger niveau dan prevaccinatie. De laagste PTNA-responsen werden waargenomen bij jongvolwassenen, in lijn met de laagste IgG-Ptx-antistofconcentraties bij jongvolwassenen, zoals beschreven in hoofdstuk 3. PTNA's vertoonden een zeer goede correlatie met de totale IgG-Ptx-antistofconcentraties. Alleen bij oudere volwassenen en niet bij de andere leeftijdsgroepen, werd ook een correlatie gevonden tussen Ptx-IgA antistoffen en de PTNA-respons. Dit past bij de hoge IgA-Ptx-concentraties op oudere leeftijd. Een andere bevinding was dat de neutraliserende responsen hoger waren bij deelnemers die vooraf aan de boostervaccinatie al een IgG-Ptx antistof concentratie hadden van ≥ 10 IU/ml. Er waren verschillen in PTNA's tussen adolescenten met aP en wP-achtergrond, maar alleen prebooster vaccinatie, met hogere PTNA's in de wP-groep ondanks vergelijkbare

IgG concentraties tegen Ptx. Concluderend namen op alle leeftijden de PTNA's toe door de dTap booster vaccinatie en was de toename van PTNA's gerelateerd aan de hoogte ≥ 10 IU/ml van de Ptx antistof concentraties voorafgaand aan de booster.

In hoofdstuk 5 werd de hoeveelheid circulerende geheugen B-cellen beschreven die specifiek IgG antistoffen produceren tegen de pertussis vaccin eiwitten (Ptx, FHA en Prn). Dit onderzoek is gedaan bij een subset van deelnemers aan de BERT studie, binnen alle leeftijdsgroepen in de drie deelnemende landen. Een jaar na de boostervaccinatie bleken de frequenties van IgG-geheugencellen tegen Ptx, FHA en Prn nog steeds hoger dan prevaccinatie. Over het algemeen werden op alle meetmomenten de hoogste frequenties van deze geheugen B-cellen waargenomen bij adolescenten en de laagste frequenties geheugen B-cellen bij oudere volwassenen. Als er prevaccinatie al geheugen B-cellen aangetoond konden worden, was het aantal geheugen B-cellen post dTap vaccinatie hoger in vergelijking met diegenen die geen aantoonbare geheugencellen hadden voorafgaand aan de vaccinatie. De correlaties tussen de frequenties van geheugen B-cellen en IgG-antistofconcentraties waren het sterkst voor het eiwit Prn op alle meetmomenten. Nadere analyse van de geheugen B-cellen bij adolescenten met verschillende vaccinatieachtergronden (aP versus wP), toonde een lichte maar niet significante trend met hogere responsen voor alle drie de eiwitten bij de adolescenten die als zuigeling het wP vaccin hadden gekregen. De hogere geheugen B-cel frequenties een jaar na de dTap vaccinatie impliceren een toegenomen immunologisch geheugen. Dit lijkt echter minder uitgesproken te zijn bij oudere volwassenen, wat mogelijk verklaard kan worden door veroudering van het immuunsysteem. Daarnaast lijkt de aanwezigheid van circulerende kinkhoest specifieke geheugen B-cellen vooraf aan de vaccinatie een positief effect te hebben op de hoogte van de vaccinatierespons.

Behalve geheugen B-cellen werden ook plasmacellen tegen de drie vaccineiwitten geëvalueerd 7 dagen na de dTap vaccinatie (in hoofdstuk 5 supplement 3). De correlatie tussen de frequenties van plasmacellen op dag 7 met zowel de frequenties van geheugen B-cellen als de IgG antistofconcentraties na een maand en een jaar volgend op de dTap vaccinatie, was opnieuw het sterkst voor Prn.

In hoofdstuk 6 werd in een kleinere groep van Nederlandse deelnemers aan de BERT studie, de aanwezigheid van meer dan honderd verschillende circulerende B-celsubsets, gemeten met flowcytometrie, verder geanalyseerd. Deze B-celsubsets werden gedifferentieerd op basis van celmarkers voor de verschillende ontwikkelingsstadia en de expressie van verschillende Ig-(sub)typen op het celoppervlak. In alle leeftijdsgroepen was de meest opvallende verandering in B-celsubsets de sterke verhoging van verschillende ontwikkelingsstadia van plasmacellen 7 dagen na dTap vaccinatie. Vooral plasmacellen die het subtype IgG1 produceren waren het meest opvallend toegenomen. Die expansie was significant hoger in adolescenten die als zuigeling wP-vaccinaties hadden gekregen

in vergelijking met die het aP vaccin hadden gekregen. Dit is in lijn met de frequenties van pertussis specifieke geheugen B-cellen in hoofdstuk 5. Net als de IgA antistoffen (hoofdstuk 3), nam het aantal plasmacellen dat het subtype IgA1 antistoffen produceert toe met de leeftijd. Er werd een zwakke correlatie (r=0,39) waargenomen tussen de absolute toename in (niet-kinkhoest specifieke) IgA-plasmacellen en de kinkhoest specifieke IgA-antistofconcentraties een maand na de dTap vaccinatie, maar de aantallen IgA producerende plasmacellen waren veel lager dan het aantal IgG producerende plasmacellen. Een dergelijke correlatie tussen totaal aantal IgG producerende plasmacellen en vaccin specifieke IgG concentratie werd niet gevonden. Wel werd 7 dagen na de vaccinatie een redelijke correlatie gevonden tussen de frequenties van alle plasmacellen voor de vaccin eiwitten samen en de toename van het totale aantal plasmacellen die IgG en IgA produceren (r=0,59 en r=0,60 respectievelijk). Tevens betekent dit dat de hoeveelheid plasmacellen in het bloed 7 dagen na de vaccinatie potentieel een vroege marker zou kunnen zijn voor een vaccinatie respons. De gevonden verschillen tussen de leeftijdsgroepen en de vaccinatieachtergrond van deelnemers (aP versus wP) dragen bij aan het begrijpen van verschillen in immuun responsen tussen leeftijdsgroepen en vaccinatieachtergronden.

In hoofdstuk 7 werden IgG antistof concentraties tegen kinkhoest zes jaar na een dTap(-IPV) booster vaccinatie beschreven in 9 jarige kinderen, die als zuigeling aP of wP vaccinaties hadden gekregen en in jong volwassenen van 25-29 jaar met een wP vaccinatie achtergrond. Deelnemers kwamen uit drie andere studies dan de BERT-studie. Er werd een model toegepast om de antistofkinetiek tot 10 jaar postvaccinatie te voorspellen. In dit model vertoonden jongvolwassenen kort na de dTap vaccinatie de hoogste IgG antistof concentraties gevolgd door een langzamere afname over de tijd voor alle drie de pertussis vaccin eiwitten vergeleken met de 9-jarige kinderen. Bij de kinderen werden ook verschillen gezien tussen de aP en wP vaccinatie achtergrond, waarbij kinderen na de wP vaccinaties als zuigeling een iets langzamere initiële afname van antistoffen vertoonde ten opzichte van kinderen die aP vaccinaties als zuigeling hadden gekregen. Echter, de afname van de antistof concentratie zette langer door bij de wP-groep, waardoor na 2,5 jaar de antistofconcentraties ongeveer vergelijkbaar waren. Bovendien bleek dat op basis van hele hoge toename van IgG-Ptx antistofconcentraties na 6 jaar, de groep kinderen die als zuigeling aP vaccinaties hadden gekregen, vaker een recente *B. pertussis*-infectie hadden doorgemaakt in die zes jaar tijd dan de wP groepen kinderen en jong volwassenen. Dit alles zonder dat een van hen klinisch gediagnostiseerde kinkhoest had doorgemaakt. Dit fenomeen kan worden verklaard door waarschijnlijk atypische symptomen of zelfs vrijwel geen symptomen bij *B. pertussis* infectie en door de eerdere vaccinaties. Deze data suggereren opnieuw dat een aP booster vaccinatie bij kinderen die als zuigeling aP vaccinatie hebben gekregen, wel beschermt zijn tegen ziekte, maar niet tegen infectie met *B. pertussis* en waarschijnlijk dan ook niet tegen transmissie van *B. pertussis* van deze

kinderen naar anderen. Bij wP gevaccineerden en dan met name de jongvolwassenen lijkt een aP booster wel langdurig betere bescherming te geven tegen ziektesymptomen en infectie.

## AANBEVELINGEN

Het voorkomen van symptomatische kinkhoest is vooral cruciaal voor pasgeborenen omdat zij het meest kwetsbaar zijn voor een ernstige beloop met complicaties. Maternale vaccinatie beschermt pasgeborenen in de eerste levensmaanden door overdracht van antistoffen en immuuncellen van de gevaccineerde moeder naar het kind. Het advies voor zwangere vrouwen is om tijdens iedere zwangerschap opnieuw een dTap-vaccinatie te halen. Hoewel herhaalde dTap-vaccinaties als veilig worden beschouwd, moet de immunogeniciteit en antistofoverdracht van moeder naar kind van meerdere dTap-vaccinaties na elkaar, met relatief korte tijdsintervallen bij frequente zwangerschappen, verder worden onderzocht. De lange termijn gegevens in dit proefschrift laten hoge antistofresponsen zien bij jongvolwassenen na een dTap vaccinatie, gevolgd door een trage afname in antistofconcentraties. Het eerste jaar na de booster lijkt de antistofkinetiek van dTap-gevaccineerde zwangere en niet-zwangere vrouwen vergelijkbaar, maar dit is nog niet onderzocht over een langere periode. Meer onderzoek is ook nodig naar cellulaire immuniteit en de immunogeniciteit van een dTap-booster in relatie tot het type vaccin (acellulair of whole cell) in de voorgeschiedenis. Uiteindelijke conclusies uit het PERISCOPE-project zullen ook bijdragen aan aanbevelingen voor maternale vaccinatie tegen kinkhoest.

Tijdens de COVID-19 pandemie daalde de gerapporteerde kinkhoest incidentie aanzienlijk omdat interventies zoals het dragen van gezichtsmaskers en fysiek afstand houden niet alleen transmissie van COVID-19 voorkwamen, maar ook van andere luchtweginfecties, waaronder kinkhoest. Na het opheffen van deze maatregelen worden nieuwe uitbraken van deze luchtweginfecties verwacht, doordat de immuniteit in de bevolking tegen de betreffende pathogenen is afgenomen. Momenteel is in Denemarken een kinkhoest uitbraak gaande en zien we ook in Nederland en België een toename van de gerapporteerde kinkhoest incidentie. Er worden nieuwe serosurveillance onderzoeken gepland in Nederland om de vatbaarheid van de bevolking voor kinkhoest en andere infecties te blijven evalueren.

Op basis van de onderzoeken in dit proefschrift kan een herhalingsvaccinatie voor oudere volwassenen, medische en andere risicogroepen worden overwogen, zeker zolang kinkhoest rondwaart. Oudere volwassenen, vooral die boven de 50 jaar met cardiopulmonaal lijden lijken meer risico te lopen op het doormaken van kinkhoest met complicaties en kunnen baat hebben bij herhaalde aP vaccinaties. Adolescenten en jong volwassenen hebben weinig symptomen in geval van een van een nieuwe kinkhoestinfectie. Voor hen zou in tegenstelling tot een herhaalde aP-vaccinatie, juist het doormaken van een

infectie gunstig kunnen zijn omdat een infectie bijdraagt aan een bredere en langduriger opbouw van de immuniteit tegen kinkhoest.

Uiteindelijk is de oplossing voor het doorbreken van kinkhoestinfecties in de bevolking het ontwikkelen van vaccins die niet alleen langdurige immuniteit tegen symptomen geven, maar ook de transmissie van de bacterie weten te voorkomen. Een veelbelovend fase 2 kandidaat vaccin is het levend verzwakte kinkhoestvaccin 'BPZE1'. Voor toekomstig onderzoek naar nieuwe vaccins kunnen correlaten van bescherming leidend zijn voor het vaststellen van een gewenste immuunrespons. Meer inzicht in mogelijke correlaten van bescherming en nieuwe verbeterde vaccins zijn nodig om kinkhoest uitbraken tot het verleden te laten behoren.

## DANKWOORD - ACKNOWLEDGEMENTS

Er zijn een heleboel mensen die ik wil bedanken voor hun bijdrage aan dit proefschrift.

Allereerst wil ik alle deelnemers van de verschillende klinische studies bedanken voor hun deelname. Zonder jullie was dit proefschrift er nooit geweest.

Lieke Sanders, wat ben ik blij met jou als promotor. Tijdens een sollicitatie werd mij gevraagd wie mijn rolmodel is, nou, dat ben jij dus. Wat jij allemaal bereikt hebt is voor mij een inspiratie. Ondanks dat je altijd heel druk bent, geef je me het gevoel dat ik er mag zijn en dat ik alles kan bereiken waar ik m'n zinnen op zet. Ik ben erg dankbaar dat ik jou als promotor heb mogen hebben.

Dan natuurlijk Guy en Annemarie. Er waren wel wat hobbels tijdens mijn promotie traject, gelukkig hebben we die altijd samen mogen nemen. Tijdens mijn jaren bij het RIVM, maar ook daarna nog maakten jullie altijd tijd om mijn concepten te lezen en te voorzien van feedback. Bedankt voor jullie begeleiding.

I would like to thank prof. dr. L.J. Bont, prof. dr. M.J.M. Bonten, prof. dr. C.A.C.M. van Els, prof. dr. M.I. de Jonge, and prof. dr. F. van Wijk from the assessment committee and the other members of doctoral examination committee prof. dr. A. Gorringe and dr. P.C.J.L. Bruijning-Verhagen for their time and willingness to assess this thesis and/or to oppose at my public defence.

Natuurlijk wil ik ook al m'n co-auteurs bedanken voor de analyses, voor het praktische werk en voor de kritische blik, het heeft de artikelen in mijn proefschrift stuk voor stuk beter gemaakt.

Kamergenoten van V0-west: Daan, Elsbeth, Bette en Marta. Ik weet eigenlijk niet eens meer hoe lang we kamergenoten zijn geweest, in mijn ervaring was het een groot deel van mijn promotie en daar ben ik blij mee. Het was een plek waar we samen konden nadenken over onze onderzoekresultaten, over hoe deze te analyseren en hoe ze op te schrijven en daarnaast ook een plek om m'n hart te kunnen luchten onder het genot van een kop thee. Nu nog steeds hebben we leuk contact, het is dan ook niet voor niets dat Daan en Elsbeth mijn paranimfen zullen zijn. Ook Hella wil ik niet vergeten, we hebben maar kort een kamer gedeeld, maar ook zonder een kamer te delen wisten wij het kamergenoten gevoel te behouden in de koffiecorner.

Op het lab heb ik gelukkig veel samen mogen werken met een fantastisch team van analisten. Een aantal hiervan wil ik met naam bedanken. Inge die naast me stond wat betreft de coördinatie van de BERT-studie. Pieter die altijd klaar stond als het ging om de MIA. Lia, Mary-Lène en Hinke met wie ik vele uren op de derde verdieping heb gestaan voor de ELISpot assay. En ook met Marjan en Marjan heb ik vele uren op het lab gestaan

om o.a. PBMC's te isoleren. Natascha mag ook niet vergeten worden, je was een ijverige student. Ook ben ik dankbaar voor de vele andere collega's van het RIVM die mij met raad en/of daad hebben bijgestaan.

I'm grateful to be part of the PERISCOPE consortium through which I have met many colleagues from all around the world (but mainly Europe). De belangrijkste hiervan is wel Nora. Jij als T-cel AIO, ik als B-cel AIO. Onze wegen hebben zich gesplitst, maar we hebben wat lief en leed gedeeld. Binnen het PERISCOPE project heb ik ook met veel andere collega's en onderzoeksgroepen mogen samenwerken. Het was fijn om samen te werken met mijn collega's van het Radboud in Nijmegen en het LUMC in Leiden. I'm also very grateful for our collaboration within the BERT study with my colleagues from Turku University (Finland) and Oxford University (United Kingdom). In particular I want to thank Alex who appeared to be a great sparring partner and of course Marta with input from a PID perspective.

Ik wil ook familie en vrienden bedanken. Soms was het moeilijk te begrijpen waarom ik eigenlijk door ging met mijn promotie traject terwijl ik er niet meer voor betaald werd. Toch voelde ik me keer op keer gesteund in de keuzes die ik maakte en waren jullie samen met mij trots als ik weer een publicatie uit had. Specifiek wil ik nog Carla bedanken, mijn schoonmoeder, die de prachtige omslag van dit proefschrift heeft geschilderd.

Al deze jaren heb ik mijn partner aan mijn zij gehad, Stefan, bedankt dat je er voor me was tijdens dit hobbelige avontuur met nu een mooi eind. Ondanks dat je niet in deze tak van sport werkt, kon je me vaak helpen een en ander in perspectief te zien. Toen we twee prachtige kinderen kregen, Floris en Ella, wisten we onze dagen vaak zo te plannen dat ik (letterlijk en figuurlijk) de ruimte had om verder te werken aan mijn proefschrift. Wat had ik zonder jou gemoeten?!

## LIST OF PUBLICATIONS

**This thesis:**

P. Versteegen, M. Valente Pinto, A. M. Barkoff, P. G. M. van Gageldonk, J. van de Kassteele, M. A. van Houten, E. A.M. Sanders, R. de Groot, D. A. Diavatopoulos, S. Bibi, R. Luoto, Q. He, A. M. Buisman, D. F. Kelly, J. Mertsola, and G. A..M. Berbers. "Responses to an Acellular Pertussis Booster Vaccination in Children, Adolescents, and Young and Older Adults: A Collaborative Study in Finland, the Netherlands, and the United Kingdom." EBioMedicine 65 (Feb 26 2021): 103247. https://dx.doi.org/10.1016/j.ebiom.2021.103247.

P. Versteegen, G. A. M. Berbers, G. Smits, E. A. M. Sanders, F. R. M. van der Klis, H. E. de Melker, and N. A. T. van der Maas. "More than 10 years after introduction of an acellular pertussis vaccine in infancy: a cross-sectional serosurvey of pertussis in the Netherlands." The Lancet Regional Health – Europe 10 (Sept 05 2021): 100196. https://dx.doi.org/10.1016/j.lanepe.2021.100196.

A.M. Diks, P. Versteegen, C. Teodosio, R. J. Groenland, B. de Mooij, A. M. Buisman, A. Torres-Valle, M. Pérez-Andrés, A. Orfao, G. A. M. Berbers, J. J. M. van Dongen, M. A. Berkowska, and on behalf of the IMI-2 PERISCOPE Consortium. "Age and Primary Vaccination Background Influence the Plasma Cell Response to Pertussis Booster Vaccination." Vaccines 10, no. 2 (Jan 18 2022): 136. https://dx.doi.org/10.3390/vaccines10020136.

A. Knuutila, P. Versteegen, A. M. Barkoff, P. G. M. van Gageldonk, J. Mertsola, G. A. M. Berbers, and Q. He. "Pertussis Toxin Neutralizing Antibody Response after an Acellular Booster Vaccination in Dutch and Finnish Participants of Different Age Groups." Emerging Microbes & Infections 11 (Mar 30 2022): 956-63. https://dx.doi.org/10.1080/22221751.2022.2053364.

P. Versteegen, A. A. Bonačić Marinović, P. G. M. van Gageldonk, S. van der Lee, L. H. Hendrikx, E. A. M. Sanders, G. A. M. Berbers, and A. M. Buisman. "Long-Term Immunogenicity Upon Pertussis Booster Vaccination in Young Adults and Children in Relation to Priming Vaccinations in Infancy." Vaccines 10, no. 5 (Apr 28 2022): 693. https://dx.doi.org/10.3390/vaccines10050693.

P. Versteegen*, A. M. Barkoff*, M. Valente Pinto*, J. van de Kasteele, A. Knuutila, S. Bibi, L. de Rond, J. Teräsjärvi, K. Sanders, M. L. de Zeeuw-Brouwer, R. Luoto, H. Ten Hulscher, E. A. Clutterbuck, E. A. M. Sanders, J. Mertsola, G. A. M. Berbers, Q. He, D. F. Kelly, and A. M. Buisman. "Memory B Cell Activation Induced by Pertussis Booster Vaccination in Four Age Groups of Three Countries." Front Immunol 13 (May 23 2022): 864674. https://dx.doi.org/10.3389/fimmu.2022.864674.

*equal contribution

**Other publications:**

L. M. van Leeuwen, P. Versteegen, S. D. Zaharie, S. L. van Elsland, A. Jordaan, E. M. Streicher, R. M. Warren, M. van der Kuip, and A. M. van Furth. "Bacterial Genotyping of Central Nervous System Tuberculosis in South Africa: Heterogenic Mycobacterium Tuberculosis Infection and Predominance of Lineage 4." J Clin Microbiol 57, no. 8 (July 26 2019): e00414-19. https://dx.doi.org/10.1128/jcm.00415-19.

E. E. Lambert, V. Corbière, J. A. M. van Gaans-van den Brink, M. Duijst, P. B. Venkatasubramanian, E. Simonetti, M. Huynen, D. A. Diavatopoulos, P. Versteegen, G. A. M. Berbers, F. Mascart, and C. A. C. M. van Els. "Uncovering Distinct Primary Vaccination-Dependent Profiles in Human Bordetella Pertussis Specific Cd4+ T-Cell Responses Using a Novel Whole Blood Assay." Vaccines 8, no. 2 (May 15 2020): 225. https://dx.doi.org/10.3390/vaccines8020225.

K. van der Pan, S. de Bruin-Versteeg, D. Damasceno, A. Hernández-Delgado, A. J. van der Sluijs-Gelling, W. B. L. van den Bossche, I. F. de Laat, P. Díez, B. A. E. Naber, A. M. Diks, M. A. Berkowska, B. de Mooij, R. J. Groenland, F. J. de Bie, I. Khatri, S. Kassem, A. L. de Jager, A. Louis, J. Almeida, J. A. M. van Gaans-van den Brink, A. M. Barkoff, Q. He, G. Ferwerda, P. Versteegen, G. A. M. Berbers, A. Orfao, J. J. M. van Dongen, and C. Teodosio. "Development of a Standardized and Validated Flow Cytometry Approach for Monitoring of Innate Myeloid Immune Cells in Human Blood." Front. Immunol. 13 (Sept 14 2022): 935879. https://dx.doi.org/10.3389/fimmu.2022.935879.

E. van Schuppen, J. Fröberg, P. B. Venkatasubramanian, P. Versteegen, H. de Graaf, J. Holubová, J. Gillard, P. G. M. van Gageldonk, I. Joosten, R. de Groot, P. Šebo, G. A. M. Berbers, R. C. Read, M. A. Huynen, M. I. de Jonge, and D. A. Diavatopoulos. "Prior Exposure to B. Pertussis Shapes the Mucosal Antibody Response to Acellular Pertussis Booster Vaccination." Nat Commun 13 (Dec 2 2022): 7429. https://dx.doi.org/10.1038/s41467-022-35165-w.

J. Gillard, M. Suffiotti, P. Brazda, P. B. Venkatasubramanian, P. Versteegen, M. I. de Jonge, D. Kelly, S. Bibi, M. V. Pinto, E. Simonetti, M. Babiceanu, A. Kettring, C. Teodosio, R. de Groot, G. Berbers, H. G. Stunnenberg, B. Schanen, C. Fenwick, M. A. Huynen, and D. A. Diavatopoulos. "Antiviral Responses Induced by Tdap-Ipv Vaccination Are Associated with Persistent Humoral Immunity to Bordetella Pertussis." Nat Commun 15 (Mar 8 2024): 2133. https://dx.doi.org/10.1038/s41467-024-46560-w.

## ABOUT THE AUTHOR

Pauline Versteegen was born December 27th, 1987 in Boxmeer, the Netherlands. In 2008, she finished secondary school at Merlet College in Cuijk. Subsequently she performed a propaedeutic year in nursing at the Hogeschool Amsterdam. In 2009 she started her studies Medicine at the Vrije Universiteit (VU) in Amsterdam. In the last year of her study, she performed her final clinical rotation at the paediatrics department of the Deventer Ziekenhuis and her scientific rotation on 'Genotyping of *Mycobacterium tuberculosis* in formalin-fixed paraffin embedded human brain tissue' at the Tijgerberg Hospital in Cape Town South Africa, under the supervision of prof. Marceline van Furth (VUmc). In December 2015, she obtained her medical degree and went back to Cape Town to finalise the research project that was started during the scientific rotation. In January 2017, she started as a PhD candidate at the Centre for Infectious Disease Control of the National Institute for Public Health and the Environment (RIVM). Her supervisors were dr. Guy Berbers, dr. Anne-Marie Buisman and prof. dr. Lieke Sanders. The research of her PhD focused on the vaccine-induced humoral and B cell immunity against pertussis. The results obtained during her PhD are described in this thesis.

In December 2021 she started as a medical doctor at the department of corona vaccinations at the Public Health Service (GGD) of Utrecht. In May 2022 she switched to the department of internal medicine at St. Jansdal Ziekenhuis in Harderwijk from where she will go to the paediatrics department of the Gelre Ziekenhuis in Apeldoorn in June.