TURUN
YLIOPISTO
UNIVERSITY
OF TURKU

CHARACTERISTICS OF ANTI-PERTUSSIS TOXIN ANTIBODIES AFTER VACCINATION AND INFECTION

Aapo Knuutila

TURUN YLIOPISTON JULKAISUJA – ANNALES UNIVERSITATIS TURKUENSIS
SARJA – SER. D OSA – TOM. 1780 | MEDICA – ODONTOLOGICA | TURKU 2024

TURUN
YLIOPISTO
UNIVERSITY
OF TURKU

CHARACTERISTICS OF ANTI-PERTUSSIS TOXIN ANTIBODIES AFTER VACCINATION AND INFECTION

Aapo Knuutila

TURUN YLIOPISTON JULKAISUJA – ANNALES UNIVERSITATIS TURKUENSIS
SARJA – SER. D OSA – TOM. 1780 | MEDICA – ODONTOLOGICA | TURKU 2024

University of Turku

Faculty of Medicine
Institute of Biomedicine
Medical Microbiology and Immunology
Doctoral Programme in Clinical Research

Supervised by

Professor Qiushui He, MD, PhD
Institute of Biomedicine
University of Turku
Turku, Finland

Reviewed by

Adjunct Professor Hanna Jarva, MD, PhD
Department of Bacteriology and Immunology
University of Helsinki and
Helsinki University Hospital
Helsinki, Finland

Adjunct Professor Minna Hankaniemi, PhD
Faculty of Medicine and Health Technology
Tampere University
Tampere, Finland

Opponent

Professor Mika Rämet, MD, PhD
Faculty of Medicine and Health Technology
Tampere University
Tampere, Finland

ISBN 978-951-29-9628-5 PRINT
ISBN 978-951-29-9629-2 PDF
ISSN 0355-9483 (Painettu/Print)
ISSN 2343-3213 (Sähköinen/Online)
Painosalama, Turku, Finland 2024

Failure to show a correlation does not mean that a correlate does not exist.

UNIVERSITY OF TURKU
Faculty of Medicine
Institute of Biomedicine
Medical Microbiology and Immunology
AAPO KNUUTILA: Characteristics of anti-pertussis toxin antibodies after vaccination and infection
Doctoral Dissertation, 138 pp.
Doctoral Programme in Clinical Research
March 2024

ABSTRACT

Pertussis or whooping cough is a human respiratory tract infection caused by *Bordetella pertussis* bacteria. Despite extensive vaccinations, pertussis incidence has increased during the last decades. The reasons behind this resurgence are not entirely understood. Multiple factors may influence the resurgence, such as improved diagnostic methods, the adaptation of circulating *B. pertussis* strains, the waning of vaccination-induced immunity, and asymptomatic pathogen transmission.

The shift from whole-cell to acellular pertussis vaccines (aPV) has been speculated as one of the reasons for the quickly waning vaccination-induced immunity. The reduced reactogenicity of the aPVs is achieved by chemically treating the vaccine components. The treatments alter the structure of the vaccine antigens and may affect the functional properties of antibodies generated after vaccination.

Currently, no internationally established immunological correlates of protection exist for evaluating pertussis vaccine-induced protection. This study compared the antibody responses to pertussis toxin (PT) after aPV and infection. PT is an exotoxin that can elicit many biological activities and is included in all current aPVs. Binding strength, binding location, and the neutralizing activity of these antibodies were evaluated with newly developed immunoassays for patient and vaccination samples in Finnish, Danish, and Dutch populations.

Infection and aPV-derived antibodies recognized different epitopes of PT. The binding strength of antibodies was higher after aPVs compared to infection. Elevated concentrations of PT-neutralizing antibodies remained one year after aPV. These characteristics were influenced by the detoxification method of PT, amount of PT included in aPV, number of vaccination doses received, existing immunological memory of PT, and recency of the latest vaccination. The studied antibody characteristics may contribute as potential correlates of protection and thus aid the evaluation of the next generation of vaccines, vaccination programs, and diagnostics.

KEYWORDS: Bordetella pertussis, pertussis, pertussis toxin, vaccination, vaccine response, antibody, infection

TURUN YLIOPISTO
Lääketieteellinen tiedekunta
Biolääketieteen laitos
Lääketieteellinen mikrobiologia ja immunologia
AAPO KNUUTILA: Hinkuyskätoksiinivasta-aineiden ominaisuudet rokotuksen ja infektion jälkeen
Väitöskirja, 138 s.
Kliininen tohtoriohjelma
Maaliskuu 2024

TIIVISTELMÄ

Hinkuyskä on hengitystieinfektio, jonka aiheuttaa *Bordetella pertussis* -bakteeri. Jo pitkään jatkuneista rokotusohjelmista huolimatta hinkuyskän ilmaantuvuus on kasvanut viime vuosikymmeninä. Syitä tähän ei täysin ymmärretä, mutta siihen voivat vaikuttaa parempi diagnostiikka, kiertävien *B. pertussis* -kantojen muuntuminen, rokotuksen aiheuttaman immuniteetin nopea heikkeneminen sekä taudinaiheuttajan oireeton leviäminen väestössä.

Vaihtoa kokosolurokotteista soluttomiin hinkuyskärokotteisiin arvellaan keskeiseksi tekijäksi nopeasti heikkenevään immuniteettiin. Soluttomien rokotteiden matala reaktogeenisyys saavutetaan käsittelemällä rokotekomponentteja kemiallisesti, mikä johtaa niiden proteiinirakenteiden muuttumiseen. Tämä voi edelleen vaikuttaa rokotuksen jälkeen muodostuvien vasta-aineiden erilaisiin ominaisuuksiin.Tällä hetkellä hinkuyskärokotteista saatavan suojan arvioimiseksi ei ole olemassa kansainvälisesti vakiintuneita immunologisia vastemuuttujia. Tässä tutkimuksessa verrattiin vasta-ainevasteita hinkuyskätoksiinin (PT, engl. pertussis toxin) soluttoman hinkuyskärokotteen ja infektion jälkeen. Vasta-aineiden sitomisvoimaa, sitoutumispaikkaa ja neutralisointiaktiivisuutta arvioitiin varta vasten kehitetyillä immunomäärityksillä rokote- ja potilasnäytteistä Suomen, Tanskan ja Hollannin väestössä.

Rokotteista ja infektiosta muodostuneet vasta-aineet tunnistivat erilaisia epitooppeja. Vasta-aineiden sitoutumisvoimakkuus oli korkeampi pian rokotuksen jälkeen infektioon verrattuna. PT:tä neutraloivien vasta-aineiden pitoisuudet olivat merkittävästi koholla vielä vuoden kuluttua rokotuksen jälkeen. Näihin tutkittuihin ominaisuuksiin vaikuttivat PT:n eri kemialliset käsittelymenetelmät, rokotuksessa käytetyn PT:n määrä, rokoteannosten määrä, immunologinen muisti PT:lle ja viimeisimmän rokotuksen ajankohta. Tämän tutkimuksen perusteella vasta-aineiden eri ominaisuuksia voitaisiin käyttää seuraavan sukupolven rokotteiden, rokotusohjelmien ja diagnostiikan kehittämisessä.

AVAINSANAT: Bordetella pertussis, hinkuyskä, hinkuyskätoksiini, rokote, rokotevaste, vasta-aine, infektio

Table of Contents

Abbreviations

ACT	Adenylate cyclase toxin
ADP	Adenosine diphosphate
AI	Avidity index
aPV	Acellular pertussis vaccine/vaccination
BERT	Booster against pertussis vaccine study
BSA	Bovine serum albumin
CDC	Centers for Disease Control and Prevention
CMI	Cell-mediated immunity
DT	Diphtheria toxin
DTaP	Diphtheria-tetanus acellular pertussis vaccine (primary)
dTap	Diphtheria-tetanus acellular pertussis vaccine (booster)
DTwP	Diphtheria-tetanus whole-cell pertussis vaccine
ECDC	European Centre for Disease Prevention and Control
ELISA	Enzyme-linked immunosorbent assay
FHA	Filamentous hemagglutinin
FIM	Fimbriae
GM(C)	Geometric mean (concentration)
GSK	GlaxoSmithKline
Hib	Haemophilus influenzae type b
Ig	Immunoglobulin
IL	Interleukin
IPV	Inactivated poliovirus
IU	International units
kDa	Kilodalton
mAb	Monoclonal antibody
MIA	Multiplex immunoassay
PCR	Polymerase chain reaction
PRN	Pertactin
PT	Pertussis toxin
PTd	Detoxified pertussis toxin
PTdg	Pertussis toxin detoxified with glutaraldehyde

PTg	Genetically detoxified pertussis toxin
PTNA	Pertussis toxin neutralizing antibody
PTx	Native pertussis toxin
S1-5	Subunits 1-5
SP	Sanofi Pasteur
Th	T-helper cells
TT	Tetanus toxin
WHO	World Health Organization
wPV	Whole-cell pertussis vaccine/vaccination

List of Original Publications

This dissertation is based on the following original publications, which are referred to in the text by their Roman numerals:

I Knuutila, A., Dalby, T., Barkoff, AM., Jørgensen, C., Fuursted, K., Mertsola, J., Markey, K., He, Q. Differences in epitope-specific antibodies to pertussis toxin after infection and acellular vaccinations. Clin Transl Immunology. 2020; 9: e1161.

II Knuutila, A., Versteegen, P., Barkoff, AM., van Gageldonk, P., Mertsola, J., Berbers, G., He, Q., and PERISCOPE Consortium. Pertussis Toxin Neutralizing Antibody Response After an Acellular Booster Vaccination in Dutch and Finnish Participants of Different Age Groups. Emerg Microbes Infect. 2022; 11:956–963.

III Knuutila, A., Dalby, T., Ahvenainen N., Barkoff, AM., Jørgensen, C., Fuursted, K., Mertsola, J., He, Q. Antibody avidity to pertussis toxin after acellular pertussis vaccination and infection. Emerg Microbes Infect. 2023; 12(1):e2174782.

1 Introduction

Pertussis, also known as whooping cough, is a highly contagious upper respiratory tract disease caused mainly by the bacterium *Bordetella pertussis*. Pertussis was one of the most common childhood diseases in children before vaccinations against the disease were introduced [1], [2]. Although vaccinations against pertussis have significantly reduced the incidence of the disease since the 1950s, pertussis is still endemic in many countries. In 2014, the disease burden was estimated to be 24 million cases worldwide, with most cases occurring in low-income countries. The same report estimated that 160,000 children under five years of age die annually from pertussis. [3]. In recent decades, a resurgence of pertussis has been noted in many countries with high vaccination coverage [4]–[8]. The reasons behind this resurgence are not entirely understood, but factors, such as improved diagnostic methods, better awareness and surveillance of the disease, adaptation of circulating *B. pertussis* strains, and waning of the vaccination-induced immunity together with asymptomatic transmission of the pathogen have been suggested.

The shift from whole-cell pertussis vaccines (wPV) to acellular pertussis vaccines (aPV) in the 1990s and the early 2000s has been speculated as one of the causes of quickly waning vaccination-induced immunity. In Finland, wPVs were used between 1952–2005. Currently, aPVs are given at three, five, and twelve months of age, and additional booster doses are offered at four and fourteen years of age to compensate for quickly waning antibody-mediated immunity [9]–[13]. Like Finland, most high-income countries have changed to aPVs since they are less reactogenic [8], [14]–[16]. Essentially, the wPVs consist of heat-inactivated *B. pertussis* strains, which contain the full spectrum of immunogenic antigens made by the bacterium. However, many of the proteins included caused strong and reactogenic side effects, such as swelling, fever, and agitation [8]. For aPVs, a selected few purified protein antigens are included, which are considered central for protection against the disease. In addition, reduced reactogenicity of these vaccines is achieved by chemically treating the vaccine components, which results in the alteration of protein structures [17]. The altered structures may affect the properties of antibodies generated after immunization to vaccine antigens.

Although immunoglobulin G (IgG) antibodies against aPV antigens have been shown to correlate in general with protection against pertussis [18]–[21] estimating what the exact relevant levels required for protection are, has been challenging. As a result, no internationally established correlate of protection exists for pertussis [19]. To find such correlates would be important to evaluate the reliability of vaccine production, to recognize the susceptibilities of individuals and populations, and to validate current and future vaccines [19], [22]. Therefore, an essential task remains to characterize in which ways the aPV-induced immunity is similar to and different from infection-induced immunity. This thesis aims to study the antibody responses in humans to aPVs and infection and uncover their functional properties to one of the most central antigens of *B. pertussis,* pertussis toxin (PT). We believe this knowledge will help understand the underlying factors behind this waning immunity and aid the development of the next generation of vaccines.

2 Review of the Literature

2.1 Pertussis

2.1.1 Clinical pertussis

The *Bordetella* genus, classified in the *Alcaligenaceae* family, consists of ten genetically close species. Although *B. pertussis* is generalized as the leading cause of pertussis, other species such as *B. parapertussis, B. holmesii,* and *B. bronchiseptica* can cause comparable but milder symptoms in humans [23]–[26]. Pertussis results from the combined effect of several virulence factors (Chapter 2.2.1). The bvgAS regulon of *B. pertussis* plays a central role in controlling the expression of these virulence factors and in the pathogenicity of the disease [27]. The most characteristic toxin for pertussis is PT, which is only produced by *B. pertussis.*

The pathogenesis mechanism of *B. pertussis* is based on the attachment of adhesins, including filamentous hemagglutinin (FHA) to the cilia of epithelial cells [28]. Once attached, *B. pertussis* replicates locally, fights against the immune defenses, and causes local damage to the respiratory tract. Disease severity depends on several factors, such as the patient's age, immune response strength, bacterial load and spread, and recency of vaccination. [8].

The bacteria may cause bronchitis, internal and external bleeding, pneumonia, and other severe symptoms in infants. In the worst case, symptoms develop into lung hypertension, heart failure, and death. [28]. The disease's incubation period generally lasts a few weeks [29]. The disease is transmitted by coughing or sneezing during the first weeks of infection [30]–[32]. In infants and children, the symptoms vary from asymptomatic to severe and continuous bouts of coughing, which last for several weeks or months.

The disease progresses typically in three stages: catarrhal, paroxysmal, and convalescent. During the catarrhal stage, rhinitis, mild cough, and flu-like symptoms appear for 1–3 weeks. As the disease progresses, uncontrollable paroxysmal coughing begins, often followed by heavy inhalation. This “whooping” is the namesake for the disease. Vomiting can also occur with the worst symptoms. [28]. Other characteristics during the paroxysmal stage in infants include leukocytosis and

lymphocytosis. The symptoms can last up to six weeks or more. Finally, recovery begins, and both the coughing intensity and repetition decrease. When convalescing, the symptoms may continue for 2–3 months. [33].

In general, the symptoms are milder for adults [34]–[36], whose disease is easily undiagnosed. As much as 46% of disease cases can be asymptomatic. Thus pertussis could be suspected in cases where coughing continues for longer than three weeks, even if there are no typical whooping coughs. [37]–[40]. Subclinical pertussis infection in adults is concerning, as these individuals transmit the disease to infants.

2.1.2 Diagnosis

Clinical suspicion of pertussis is essential for an early and effective diagnosis. However, *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, respiratory syncytial virus, and adenoviruses can cause similar symptoms as pertussis [23], [41], [42]. Prolonged cough can also be misdiagnosed as asthma [43]. Therefore, clinical suspicion of pertussis should be combined with laboratory confirmation to ensure a correct diagnosis and avoid the underdiagnosis of pertussis [44].

Several laboratory tests are used to diagnose *B. pertussis* infection. Culture is the primary and the most specific method to detect the bacterium, generally obtained from a nasopharyngeal swab or aspirate specimens. However, *B. pertussis* is fastidious and slow-growing, taking three to seven days on a culture plate. The sensitivity obtained from cultures varies widely in clinical practice, even between 20 and 83% [45]. The use of Regan-Lowe agar has improved the success of the separation, but the cultivation of the bacteria is still challenging and susceptible to contamination.

Other tests include nucleic acid-based methods, such as polymerase chain reaction (PCR) from nasopharyngeal specimens, and serological tests, generally from a blood sample, which measure antibody concentrations to *B. pertussis* antigens. The most important factor when choosing a diagnostic test is the timing of the disease onset. Cultures and PCR are usable methods in the acute phase of the disease, when the pathogen is present in the body in large amounts, while serology can only be used a few weeks after disease onset [46]. PCR has become more common, especially in the diagnostics of acute cases, due to its ability to additionally detect (dead) bacterial fragments [47]. Consequently, PCR is more sensitive when patients have already started antibiotic treatment [48]. The main target sequences of PCR in diagnostics are insertion sequence 481 and the promoter of the *ptx* gene [49]–[51].

Serological testing is mainly performed with enzyme-linked immunosorbent assays (ELISA) or multiplex immunoassays (MIA) [52]. Numerous ELISAs have been developed for *B. pertussis* to detect different antigen-specific antibodies [53]–

[56], which have been most helpful in epidemiological studies and clinical diagnosis of pertussis, particularly in adolescents and adults [55], [57], [58]. Antibodies to pertussis antigens are also found in nasopharyngeal samples indicative of recent infection [55], [57], [59]. It is generally recommended that only anti-PT IgG antibodies with quantitative cutoffs should be used for serological diagnostics because of PT's uniqueness to *B. pertussis*. The generally used cutoffs for positive diagnosis vary between 40 and 125 international units/mL (IU/mL), which varies between countries depending on the target population, vaccination programmes, and possible exposure information such as local outbreaks. [60].

Cutoffs are necessary since PT is included in the aPVs, and the response between vaccination and infection cannot be distinguished based on sole IgG. An elevated IgA or IgM antibody response may better indicate an early infection, as IgG antibody response develops more slowly [61]. However, children produce lower amounts of IgA in general [62]–[65]. In addition, detectable levels of antibodies from previous infections may interfere with diagnostics [66]. The increasing number of aPV boosting in adults and adolescents further complicates the serological diagnosis [58], [67], [68]. Overall, these lead to challenges in choosing the proper test, interpreting the results, and standardization of tests. [69].

2.1.3 Epidemiology and disease burden

Pertussis is a prevalent disease in both high- and low-income countries. According to an estimate in 1999, there were up to 48.5 million pertussis cases worldwide and 295,000 deaths from pertussis [70]. In 2008, the corresponding figures were estimated at 30 million cases and 195,000 deaths [71]. The most recent estimate in 2014 accounted for 24 million patients and 160,000 deaths in children less than five years of age [3]. However, estimating the number of cases worldwide is difficult, as many countries have limited monitoring infrastructure, such as devices or molecular diagnostic tests [72]. The WHO has also noted that standardized pertussis tests are not uniform, and the countries' practices differ significantly. There have also been challenges in assessing disease rates due to changes in diagnostic methods, vaccine schedules, vaccine components, and vaccine manufacturers [73].

The disease burden encompasses both direct and indirect costs associated with the disease. Direct costs encompass hospitalization, laboratory tests, and travel expenses, whereas indirect costs are related to lost working days [74]. In the USA, pertussis among adolescents alone was estimated to result in financial costs of 3.2 billion dollars over a decade. A solid economic rationale exists for pertussis vaccines as immunization would save up to 50% of this sum [75], [76].

Over the last two decades, pertussis incidence has increased in highly vaccinated populations [6], [77], [78]. The increased number of disease cases has been attributed

to factors such as asymptomatic transmission, quickly waning immunity after (aP) vaccination, the transition from wPVs to aPVs, *B. pertussis* evolution, improved diagnostic methods and lower thresholds for testing, and increased awareness by clinicians [6], [79]–[81]. Other possible reasons include changes in age-specific contact patterns [82] or changes in the transmissibility of the disease [83].

In Europe, the highest incidence has consistently been observed in children younger than one-year-old. Worrisomely, the incidence in adults and adolescents doubled from two to four cases per 100,00 population between 1998 and 2002 [7]. Despite the implementation of aPVs in vaccination programmes, high vaccination coverage (>90%), and increased amount of booster doses administered to all age groups, the disease continues to circulate in adults [84]. The disease incidence is typically high among children in populations with low vaccination coverage, whereas an increased prevalence of the disease is noted to shift towards adults within highly vaccinated (child) populations [85]. The bacteria is likely transmitted from the parents and other household members to their unvaccinated infants [2], [8], [86], [87]. This view has been further emphasized by baboon model studies, in which vaccinated baboons transmitted the disease although they remained asymptomatic [88]. Furthermore, those baboons that received wPVs were able to clear *B. pertussis* infection in two and a half weeks after nasal challenge, whereas aPV-primed baboons struggled for nearly six weeks.

Notably, an increase in pertussis cases has occurred in North America [89]–[92], Australia [93], [94], and Europe (Figure 1), including the Netherlands [95], Finland [96] (Figure 1, 2), and of late in Denmark [97]. For example in the USA, despite an apparent decrease in the reported cases after the introduction of the first wPVs, the trend has turned into a gradual increase in recent years (Figure 3). The past large

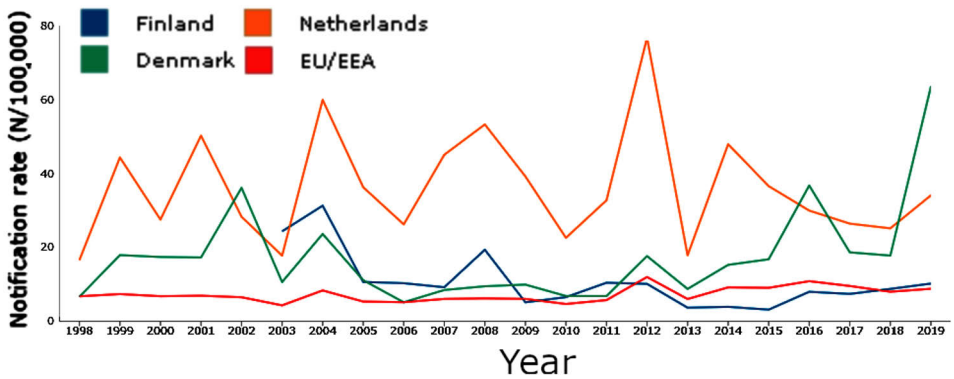

Figure 1. Laboratory confirmed pertussis notification rates in Europe and individual countries such as Finland, the Netherlands, and Denmark. The figure is reproduced from disease data from ECDC surveillance Atlas [97].

pertussis epidemics in the USA and Australia have demonstrated high incidence in adolescents after the transition from diphtheria-tetanus whole-cell pertussis vaccine (DTwP) to diphtheria-tetanus acellular pertussis vaccine (DTaP) [98], implying that the efficacy of aPVs is lower than that of traditional wPvs. Logically, this age group was the first to receive aPVs as a primary vaccine. The increased incidence and nationwide epidemics of pertussis have led to an increased amount of booster vaccination doses administered throughout life, as well as alternative strategies to immunize pregnant women in certain countries, such as the UK, to protect vulnerable infants [99].

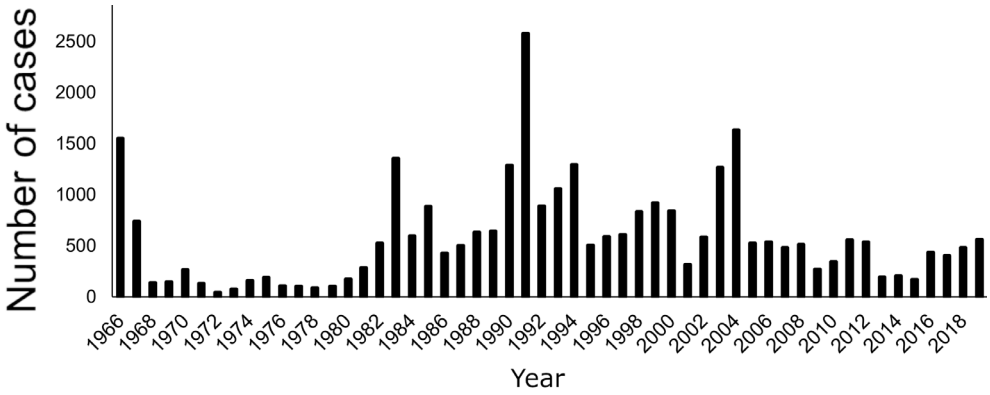

Figure 2. The reported number of pertussis cases in Finland from 1966 to 2019 (1966–1978 [100]; 1981–1994 (reports from Orion Oy); 1995–2019 [101]). Before 1995, both clinical and laboratory-confirmed cases were reported. Since 1995, only laboratory-confirmed patients have been notified to the National Infectious Disease Registry. wPVs were started in 1952, and the primary aPV program was started in 2005.

Figure 3. The number of pertussis cases in the USA between 1922 and 2019. Whole-cell vaccinations were started during the 1940s. aPVs were introduced in 1997. The figure is reproduced from CDC data [105].

When vaccination schedules are mofidied, pertussis epidemiology in the respective age group changes relatively quickly [102a], [102b]. For example in Sweden, the prevalence of pertussis decreased substantially in children once aPVs were introduced to the vaccination programme, after 17 years without vaccination in the country [103], [104]. A few years after the vaccine's introduction, infections among 2–4-year-olds decreased while the incidence increased in 7–8-year-olds, which would refer to quickly waning immunity.

2.2 *Bordetella pertussis*

Jules Bordet discovered *B. pertussis* in 1900 from a sputum sample. Later in 1904, Bordet and Gengou isolated *B. pertussis* after attaining the ideal growth medium, later named as Bordet-Gengou medium [106]. The bacterium was later named *B. pertussis* in honor of Bordet [107]. *B. pertussis* can be primarily separated from the other *Bordetella* species by its growth characteristics and with biochemical tests. It is a demanding species to grow, and catalase- and oxidase-positive [26], [108]. *B. pertussis* is a strictly aerobic, gram-negative, pleomorphic, mainly considered as a non-motile coccobacillus and β-proteobacteria [109]. It is solely a human pathogen, however, dogs, cats, and rabbits can have mild symptoms from direct *B. pertussis* challenge [110], [111]. Additionally, mice, rats, primates, and guinea pigs have been used as disease models for *B. pertussis* infection [112]–[115]. The disease spreads by coughing and from nasal drips since the bacterium can survive for short periods in droplets.

Whole-genome sequencing of the *Bordetella* genus has revealed that *B. pertussis* and *B. parapertussis* have evolved from *B. bronchiseptica* independently [116]–[119]. After the inclusion of aPVs, a recently noted trend in the evolution of *B. pertussis* has been the emergence of strains that lack specific virulence antigens. Several isolated strains have not produced PT, FHA, fimbria (FIM), or most commonly, pertactin (PRN) [120]–[125]. Although PT-negative strains are rare and not associated with more severe disease, it is concerning how *B. pertussis* can down-regulate aPV-related antigens.

2.2.1 Virulence factors

When *B. pertussis* adheres to the epithelium of the respiratory tract it produces various virulence factors for the pathogenicity of the bacteria, including toxins and adhesins. The production of most virulence factors is regulated by a two-component regulatory system called BvgA/S [126]. The environmental signals sensed by the BvgA/S system in vivo are not fully known [127], but include at least temperature, sulfate and nicotinic acid [27]. Toxins produced by *B. pertussis* include PT,

adenylate cyclase toxin (ACT), lipopolysaccharide (LPS), dermonecrotic toxin (DNT), and tracheal cytotoxin (TCT). Briefly, ACT has roles in manipulating the physiology of host cells, commonly neutrophils, as it interrupts cellular signaling pathways and ion homeostasis. LPS causes severe sepsis and septic shock and is essential to protect bacteria from innate immunity. TCT has a role in the evasion of the host defense, and DNT causes systemic effects. [31], [128].

2.2.1.1 Pertussis toxin

PT is exclusively produced by *B. pertussis*. *B. parapertussis* and *B. bronchiseptica* have the *ptx* gene, but it is not expressed due to several mutations in the gene promoter [129], [130]. PT consists of five subunits that compose a hexameric structure accounting for 105 kDa in size (Figure 4) [131]. The hexamer contains a catalytic subunit A [or subunit 1 (S1)] and a subunit B5, responsible for receptor-binding, consisting of S2, S3, two S4, and S5. Subunits 1 – 5, according to decreasing size, are produced by the genes *ptxA* to *ptxE* [132], [133]. Before exporting PT through the inner and outer membrane to outside the cell, it has to be assembled entirely in the periplasm [134]–[136]. The B5 subunit then transports the catalytic S1 into the host cells (Figure 5).

PT has multiple functions: it acts in cell receptor binding and as a catalytic adenosine diphosphate (ADP)-ribosyltransferase. The primary function of PT lies in the modulation of host immune responses [137]–[139], whereas its role in the adhesion of the bacteria to epithelial cells is considered minor [137], [140]. The receptor-binding capabilities of subunit B [141], [142] are independent of the toxin's enzymatic activity [143]. The genetic similarity of the carbohydrate-recognition domains of S2 and S3 has suggested that they can be substituted with each other to a certain extent [132], [133], [138], [143], [144]; if S2 is replaced by S3, ADP-ribosylation activity increases; if S3 is replaced by S2, PT production is enhanced [145].

PT can attach to a high variety of host cell types. The ligands are generally glycoproteins with sialic acid or glycolipids. However, glycosylation is not necessary for attachment. [138], [146]–[148]. The C-terminal binding sites on S2 and S3 mediate PT binding to sialylated compounds. In contrast, the N-terminal sites mediate attachment to nonsialylated N-linked glycans [144]. Polar and charged groups of Tyr102, Ser104, and Arg125 are responsible for binding to sialic acid receptors. Overall, amino acid residues 180–219 appear to confer high affinity binding to guanine nucleotide proteins (G proteins). [149]. The N-terminal regions of S2 and S3 are also included in receptor binding: The N-terminal of S2 reconstitutes adherence to ciliated cells, whereas S3 reconstitutes binding to macrophages [138]. On the other hand, the C-terminal domains of S2 and S3 have

similar fold structures to other carbohydrate-binding proteins [150]. The wide variety of target proteins and cells for PT reflects well the wide variety of biological activities of the toxin [148].

Figure 4. The three-dimensional structure of pertussis toxin. PT is composed of a hexameric AB5 structure. Subunit A (or S1) (red) on top withholds the catalytic activity of the molecule, and the pentameric base of the molecule, the B5 subunits (S2 (cyan), S3 (purple), two S4 (yellow), and S5 (green)) act as a mediator for adherence and transportation to target cells. S2 and S3 each form a dimer with S4. Generated by PyMOL (version 2.3.1, Schrödinger, LLC).

Subunit 1 becomes active after dissociating from the B subunit into the host cell's cytosol (Figure 5) [151]. PT is an ADP-ribosylating toxin like cholera and diphtheria toxin [131], [141]. In the cytoplasmic membrane, S1 catalyzes the transfer of ADP-ribose from nicotinamide adenine dinucleotide (NAD) to the α-subunit of G proteins, inactivating them in the process [141], [148], [152]. The amino acids surrounding the NAD binding cavity are Arg-9, Trp-26, His-35, Cys-41, and Glu-129. More specifically: Trp26 [153], [154], Arg-9 [155], and Cys-41 [156] are involved in NAD binding, whereas His-35 [157], [158] and Glu-129 [159] have been identified as catalytic residues. The genetic substitution of Arg-9 and Glu-129 with other amino acids (Chapter 2.3.1.4) has been enough to abolish the enzymatic activity of S1.

The inactivation of G-proteins causes the accumulation of cyclic adenosine monophosphate in the cells. This in return leads to dysregulation of immune responses. These effects include histamine sensitization, enhancement of insulin

secretion, and various both suppressive and stimulatory immunological outcomes: leucocytosis, lymphocytosis, and delayed recruitment of neutrophils to the respiratory tract [8], [161]–[169]. Additionally, the B oligomer interferes with the signal transduction response between macrophages and T cells [170]. It blocks chemokine receptors, such as CCR5 and CXCR4, expressed on immune cells, which affect T-cell migration [171]. PT helps to establish the colonization of the bacteria as it targets antigen-presenting macrophages [172].

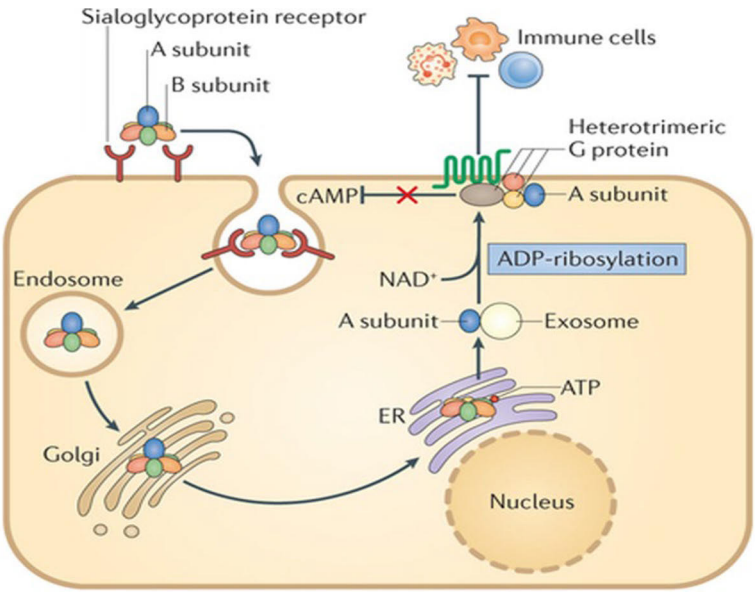

Figure 5. The activation pathway of PT. As B-subunit attaches to glycoprotein receptors, PT is administered to the cell via endosomes and is further transferred to the endoplasmic reticulum (ER). The A-subunit gets cleaved from the B-subunit and starts to ADP-ribosylate G-proteins. The accumulation of cyclic adenosine monophosphate (cAMP) in the cell disrupts various inflammatory signaling pathways. Reproduced from [160].

PT also promotes the maturation of dendritic cells (DC) through activation of myeloid differentiation primary response protein 88 (MyD88)-dependent and independent signaling pathways [173]. For future consideration, it is worthwhile to note that an enzymatically inactive form of PT preferentially triggers only the MyD88-independent pathway [174]. Both toll-like receptor 4 (TLR4) and TLR2 signaling pathways of macrophages and DCs are activated by PT [174], [175,], [176]. PT also enhances Th1 and Th2 type $CD4^+$ T cells [177], [178] and promotes Th17 differentiation [179], [180]. Additionally, PT induces the polyclonal activation and effector functions of $CD8^+$ T cells [181].

There is some controversy about whether the colonization of *B. pertussis* is not significantly affected if PT is not present (in mice) [182], [183]. Essentially, there

remains a debate about whether pertussis is a toxin-mediated disease or not [184]. Despite PT's inevitable role as a central molecule in the pathogenicity of *B. pertussis*, its role is not fully understood, aside from its association with leukocytosis, a general clinical feature of pertussis. Especially, whether PT is the cause of coughing remains elusive. [127], [185].

2.2.1.2 Adhesins

B. pertussis has several virulent surface structures. These include FHA [186], pilus, PRN [187], FIM [188], and several other metabolic proteins that are needed for adherence and colonization of *B. pertussis* [189]. FHA has immunomodulatory and immunosuppressive effects. Its several binding motifs indicate its important role in adhesion, bacterial colonization [190], and biofilm formation [191], [192]. PRN is a 69 kDa autotransporter protein that contributes to the adherence of *B. pertussis* to the ciliated respiratory epithelium. Additionally, PRN is central to resisting neutrophil-mediated clearance [193]–[195]. FIMs are filamentous adhesion molecules bound at the outer membrane of *B. pertussis*, which has three serotypes: FIM2, FIM3, and FIM2,3 [188], [196]. FIMs aid the adherence and spreading of *B. pertussis* in the respiratory tract [197], [198]. *B. pertussis* also produces other adhesins such as BrkA [199], tracheal colonization factor [200], and Vag8 [201].

2.3 Immunity after pertussis vaccination or infection

2.3.1 Vaccination against pertussis

Before vaccines, pertussis was one of the most common cause of infant mortality [2]. The vaccine development against *B. pertussis* started soon after its successful isolation. The first experimental whole-cell vaccine was designed in 1933 and was successfully used to immunize against pertussis [202]. Starting from 1940 several countries began pertussis vaccinations. wPVs drastically reduced the incidence and mortality from pertussis (Figures 2 and 3). wPVs are still used in many countries, but most often as combinatory vaccines with tetanus and diphtheria toxoids (TT and DT, respectively) [8]. Additional subunit components such as *Haemophilus influenzae* type b (Hib), hepatitis B, and inactivated poliovirus (IPV) have also been included in some aPVs [203].

2.3.1.1 Vaccines in Finland(, the Netherlands, and Denmark)

In Finland, vaccinations started in 1952. The vaccine contained a suspension of inactivated *B. pertussis* in combination with DT and TT (Orion Oy, Finland). Infants were vaccinated with three primary doses at three, four, and five months of age. In 1957, the vaccine was extended to be administered as boosters at four and six years of age. From the 1970s onward, a fourth primary dose was included at two years of age. [204], [205].

In 2003, the first aPV booster was included in the national vaccination program for six-year-old children. It contained purified PT, FHA, and PRN (dtap, Boostrix, GlaxoSmithKline Biologicals (GSK), Belgium) (Table 1) [80]. Two years later, DTwPs were replaced from the primary vaccination schedule with the DTaP-IPV vaccine (Tetravac, Sanofi Pasteur (SP), France), including only PT and FHA. As a side note, the different spellings of DTaP and dtap are related to the amount of antigen included in the vaccine. Smaller letters indicate a reduced quantity of antigens, which is more common for booster vaccines. In this work, the term aPV is generally used to mean either of these vaccines, in combination with DT and TT.

Since then, the vaccine schedule has undergone many changes: The primary vaccine schedule was changed to three, five, and twelve months, and a booster (Boostrix, GSK) was added at four years of age. In 2009, a vaccine with PRN replaced the primary vaccine (Infanrix, GSK), but it was changed back to a two-component (Pentavac, SP) vaccine without PRN in 2019. Current booster doses are given at four years, 14–15 years, and for persons attending military service. Since 2018, a booster dose has been recommended at the age of 25 years. [206]. Detailed compositions and schedules from the 1950s in Finland and the Netherlands have been covered in the supplementary Figure 1 (in original publication II) and in the study by Versteegen et al. [207].

In the Netherlands, fewer changes have been made regarding the timings of the vaccines since the 1950s. However, multiple different aPV compositions have been used [207], [208] [II]. In short, wPVs were used until 2001, and aPV boosters are only given at four years of age after four doses of the primary series at two, three, four, and eleven months (Table 1).

In Denmark, wPVs were replaced in 2004, and a monocomponent aPV containing only hydrogen peroxide-detoxified PT in addition to DT and TT was used from 1997 until 2019 (DiTeKiPol or DiTeKiPol/Act-Hib, previously Statens Serum Institut, now AJ Vaccines). Primary vaccines are given at three, five, and twelve months of age, and children receive a booster at five years of age (Table 1). The vaccine was found to protect children against severe disease in an efficacy trial conducted in Sweden. [209]–[211].

Table 1. Pertussis vaccination programmes in Denmark, Finland, and the Netherlands at the time of the study.

Country	Acellular pertussis vaccine since	Primary vaccination schedule	Booster vaccination schedule	Pertussis Antigens
Denmark	2004	3, 5, 12 months, DTaP-IPV/Hib	5 years, dTap-IPV	Solely pertussis toxin (PT) (40 µg primary, 20 µg booster)
Finland	2005	3, 5, 12 months, DTaP-IPV-Hib	4 years, dTaP-IPV; dtap: 14–15 years; military(18–30 years)	PT (25 µg in primary, 8µg in booster), pertactin, filamentous hemagglutinin
The Netherlands	2005	2,3, 4, 11 months, DTaP-IPV-Hib-HepB+Pneu*	4 years, dTaP-IPV	PT (8 µg in booster), pertactin, filamentous hemagglutinin, fimbriae 2/3

*DTap including Hemophilus influenza (Hib), hepatitis B (HepB), and Pneumococci (Pneu) components

2.3.1.2 Whole-cell pertussis vaccines

wPVs are produced from heat-killed bacteria, and thus they contain the full spectrum of proteins made by *B. pertussis*. However, the wPVs' reproducibility is not simple as *B. pertussis* is fastidious to cultivate, and the manufacturing methods and strains are often different between companies. The included bacterial strains were naturally those circulating in the population at that time [212]. The vaccines undergo several quality tests, such as potency and toxicity testing (in mice), opacity testing, and sterility control. wPVs often use aluminum salt as an adjuvant, which increases the vaccine response by stimulating the immune system. Thiomersal is commonly used as a preservative. [213], [214].

The impact of wPVs on pertussis incidence was observed rapidly. Over just twenty years, the number of pertussis cases in the USA decreased from more than 150,000 to less than 10,000 cases per year (Figure 3). However, the wPVs often resulted in reactogenic side effects [215]. Local reactions such as fever, redness, swelling, and agitation were common and more frequent with later vaccine doses. Thereafter wPVs are not recommended for adolescents or adults. [8]. The most detrimental, but very rare reactions included neurological disorders and deaths.

The public pressure towards a growing anxiety to the adverse reactions led to a suspension of wPVs in Sweden between 1979–1996 [104], and in Japan, vaccine coverage declined to 10% after two vaccine-related deaths [216]. Today, wPVs are used in certain countries, such as Poland, and most low-income countries due to the

significantly lower costs in comparison to aPVs [98], [217], [218]. Although current aPVs may be more expensive for now, combination dtap-vaccines with even more antigens and target diseases (such as hexavalent Hexyon® [203]) may represent better economic value when the costs of extra injections, handling, storage, and complexity of manufacturing processes of individual vaccines are taken into account [219], [220].

2.3.1.3 Acellular pertussis vaccines

The development of aPVs started in the 1970s as it became technically possible to extract and purify the desired antigens as subunit components of the vaccine [8]. Japan was the first to replace DTwP with DTaP in 1981 [216]. In the 1990s, multiple randomized and double-blinded efficacy trials evaluated the immunogenicity, efficacy, and safety of aPVs used for primary and booster immunizations [221]–[224]. The data obtained supported the safety and efficacy of aPVs. Essentially, DTaPs were significantly less reactogenic and thereafter more tolerated than DTwPs. Consequently, aPVs are safer to administer to adolescents and adults. [14], [15]. Currently, available aPVs contain up to five antigens: PT, PRN, FHA, FIM2, and FIM3 in several combinations and formulations of either PT and FHA, PT, FHA and PRN, sole PT, or all five antigens. Aluminum is generally included as an adjuvant.

2.3.1.4 Detoxication methods of pertussis toxin

To avoid adverse reactions to the aPVs, native PT (referred to as PTx) has to be detoxified while maintaining its antigenicity/immunogenicity. Detoxification can be achieved chemically [17], [225] or genetically [226]. Formaldehyde was used to detoxify PT and FHA in the first developed aPV [168], [216]. Today, the most widely used chemical treatments include formaldehyde, glutaraldehyde, and hydrogen peroxide (Table 2).

The simple proof that the immunogenicity is preserved in detoxified PT (referred to as PTd) is that antibodies, which protect well against severe disease symptoms, are found against PTx after aP vaccination (Chapter 2.3.2) [227]. However, some individuals have low anti-PTx IgG concentrations after a single dose of aPV [207]. It is hard to outline whether this is due to individual genetic factors or detoxification methods and loss of immunogenicity. Higher serum antibody titers to *B. pertussis* antigens can be noted upon infection if S1 is inactivated beforehand, implicating it has immunosuppressive properties [161]. The resurgence of the disease (Chapter 2.1.3), together with quickly waning immunity (Chapter 2.3.2), further highlights the importance of finding out whether they are linked with detoxification procedures and their impact on the immunogenicity of PT.

The generalized effects of the different detoxification methods on the structure of PT are detailed in Table 2. Inactivation of PT by chemical treatment has been achieved either by using formaldehyde [228], [229], glutaraldehyde [165], [230], trinitrobenzene sulfonic acid [231], hydrogen peroxide [232] or a combination of formaldehyde and glutaraldehyde [233], [234]. Generally, nonlinear and conformation-dependent epitopes are more sensitive to detoxification treatments [17]. Most PT-neutralizing epitopes were noted to be conformational. Likewise, potentially protective epitopes in the intact PT are not present in individual recombinant PT subunits. [235].

Table 2. Methods used and their effect on detoxifying pertussis toxin.

Detoxification method	Amino acids affected	Subunits affected	Reference
Formaldehyde	Arg, Asn, Cys, Gln, His, Lys, Tyr, Trp	S1-S5	[17], [236]–[238]
Glutaraldehyde	Lys, trisialylated tri-antennary N-glycans	S2-S5	[17], [236], [239]–[241]
Trinitrobenzene-sulfonic acid	ε-amino groups	Not studied	[231]
Hydrogen peroxide	Asp, Cys, His, Met, Trp, Tyr	S1-S5	[17], [242]
Genetic modifications	Arg9 to Lys, Glu129 to Gly	S1	[148],[226],[243]

Formaldehyde treatment effectively inactivates the ADP-ribosyltransferase activity, whereas glutaraldehyde is potentially weaker. In contrast, glutaraldehyde has a more potent and less reversible denaturing effect on the carbohydrate-binding activity of PTx compared to formaldehyde. [236]. Glutaraldehyde is known to induce more cross-linking of proteins with more lysine residues [240], [241], [244]. The observations above are understandable, as there is little lysine in S1, whereas S2, S3, S4, and S5 each contain several lysine residues. Additional detoxification procedures are often required to compensate for glutaraldehyde's weak effect on S1's enzymatic activity. Fewer PT epitopes are affected by hydrogen peroxide treatment than glutaraldehyde and formaldehyde, nevertheless, the S1 activity is generally well-neutralized. [17].

Inactivation of PT by chemical treatments is often complex, difficult to control, and can result in varying degrees of cross-linkages and aggregation [17], [237], [245]. The exact sites and extent of modification are challenging to characterize. Additionally, different detoxification conditions: *e.g.,* temperature, buffers, or reaction times, may contribute to the specific inactivation degree and duration of the biological activities of PT [237]. Additionally, the possibility of reversion of toxicity

or incomplete detoxification of PT following chemical treatment cannot be neglected [17], [246].

Alternatively, the enzymatic activity of S1 may be inactivated directly with the genetic alteration of amino acids (referred to as PTg). In principle, extensive conformational changes in the structure of PT are avoided, and the genetic treatment always results in similar changes in the molecule. After the successful sequencing of the *ptx* gene [132], [133] and the characterization of the relationship between the structure and function of PT [247], several PTg mutants were engineered for vaccine development [226], [248]–[250]. A well-established PTg analog contains two changes in the S1 (Arg9 to Lys; and Glu129 to Gly), which individually remove the enzymatic activity of PT (Table 2). Consequently, it has been included in new-generation pertussis vaccines. [148], [246], [251]. Additionally, a *B. pertussis* strain producing this analog has been developed as a live attenuated nasal pertussis vaccine candidate which was safe and effective in preclinical models [252]-[255]. The stabilization of PTg at the time was done using low doses of formaldehyde [250]. Even treatment with as low as 0.052% formaldehyde was shown to hinder the binding of certain S1-targeting monoclonal antibodies (mAbs) [246].

2.3.2 Humoral immunity post-vaccination

It is first essential to recognize that antibodies against PT alone are often enough to protect against severe pertussis [20], [256]. Clinical trials in the 1990s indicated that a level of IgG antibodies against aPV antigens correlate in general with protection. More specifically, antibody concentrations above a threshold of 5 IU/mL anti-PT IgG have been shown to correlate with protection [18]–[21]. Additionally, the administration of PT-specific mAbs to baboons prevented pertussis symptoms when challenged with *B. pertussis* [257]. Thus, PT is an integral part of aPVs. [31]. Nevertheless, some children with low anti-PT IgG concentrations are still protected [258] due to components of cell-mediated immunity and immunological memory (Chapter 2.3.4). Therefore, estimating the relevant levels of vaccination-induced antibody responses that correlate with protection (against the disease or infection) is difficult. Today, no internationally established correlate of protection exists for pertussis (toxin), unlike, e.g., for DT and TT-specific antibodies [19]. More likely, the truth consists of multiple parameters to consider instead of a single correlate, such as anti-PT IgG [259]. To find such correlates of vaccine-induced immunity is of high interest from multiple viewpoints: to evaluate the consistency of vaccine production; to acknowledge the susceptibilities of individuals and populations; to validate already licensed prior-generation vaccines; and to the licensure of combination vaccines [19], [22].

Both aPVs and wPVs induce high IgG concentrations in general. However, IgG concentrations are higher after aPVs [221], [222], [227], [260]. Generally, the quantity of antibody response can be related to the amount of the attached antigens in the vaccine [98], [261]. Naturally, as wPV consists of the entire bacteria, antibodies are produced to a broader number of antigens. Intriguingly, only aPV stimulates IgE response [262], [263]. The presence of IgE is typically associated with a Th2 response in allergies. After wPVs, IgE is most likely downregulated by LPS, which suppresses Th2 responses and elevates a Th1 response instead [264]. Vaccinations do not usually induce IgA production, especially in children or adolescents [98], [207]. In a recent study, a rise in IgA was observed among older adults (higher than 60 years) after aPV [207].

The induced antibodies, especially to PT, decrease very sharply after vaccination. However, there is a high individual variance between the degradation kinetics [265]–[270]. Retrospective studies have estimated that wPV-induced protection against the disease lasts longer than with aPVs [98], [271]–[273]. The estimated duration was four to 12 years after wPV and six years after aPV. Although wPVs induce lower concentrations of antibodies (to aPV-specific antigens), the immunity declines more slowly [274], [275]. The different combinations of primary and booster vaccines as well as previous natural infections influence the duration of protection [98]. Nevertheless, the odds of acquiring a pertussis infection rise as the duration from the latest vaccine increases [103], [271], [274], [276], [277]. This connection is considered as quickly waning immunity.

Despite high antibody concentrations after vaccination, the shortcomings of the current aPVs have been well illustrated in baboon studies. aPVs protect baboons against pertussis disease but cannot protect against infection by *B. pertussis* and further transmission of the organism [88].

2.3.3 Humoral immunity post-infection

B. pertussis infection induces IgM, IgA, and IgG responses to the whole bacteria. In contrast, vaccination induces IgG and IgM antibodies only to vaccine-specific antigens. Infection with *B. pertussis* induces secretion of nasal and mucosal IgG and IgA, which is a major difference to vaccination-induced immunity. [57], [278], [279]. Antibodies neutralize bacterial toxins, inhibit bacterial binding to the mucosal tract, enable phagocytosis by macrophages and neutrophils, and induce cellular immune functions [280], [281].

Induced IgG antibodies are similarly crucial for protection against the disease. The antibody-mediated protection after infection is not lifelong [18], [20], and the decay of infection-induced antibodies seems faster than those after aPV [265]. Of note, the magnitudes of the anti-PT IgG responses after infection vary widely

between and within (300-fold) the studies [265], [282]. In addition to individual differences in immune responses, the degradation kinetics of antibodies may be influenced by various factors, such as previous vaccinations, infections, and disease severity [282], [283]. Despite decreasing antibodies, protection against future infections is maintained longer by T-cell and B-cell memory [11], [12].

2.3.4 Innate and cell-mediated immunity

Although PT, among other virulence factors, delays the recruitment of neutrophils and specifically targets the early capabilities of macrophages, eventually macrophages, together with neutrophils, start clearing the bacteria (and its bacterial components) and together with DCs act as central antigen-presenting cells. Once TLR4-mediated recognition has occurred, the bacteria are phagocyted by macrophages. The elimination of *B. pertussis* is further enabled by interferon-γ and proinflammatory cytokines. [284]. However, some bacteria evade intracellular killing and replicate intracellularly [285]. *B. pertussis* can avoid (alternative pathway) complement recognition by producing proteins such as BrkA and Vag8. [286], [287].

Cell-mediated immunity (CMI) is activated after innate recognition. DCs are critical in antigen-presenting and cytokine production, and in activating the adaptive immune system and CMI. CMI is intermediated by CD4+ T cells, which include Th1, Th2, Th17, regulatory T cells and follicular helper cells (Tfh) [139], [288], and CD8+ cytotoxic cells. In brief, Th1 cells activate phagocytic cells, whereas Th2 cells produce interleukins that initiate B cells to produce antibodies [289]. Th17 cells contribute to the clearance of a primary infection of *B. pertussis* in the respiratory tract. [290], [291]. Recently, Tfh cells, which are crucial for the development of memory B-cells and affinity maturation, were shown to be induced only after wPV, and not by aPV in mice [292]. An increase of CD8+ cells was shown to be stimulated by all aPV antigens [181], [293] as well as after infection specifically by FHA [294]. However, studies on CD8+ T-cell responses are scarce.

There is a distinct CMI response pattern depending on which vaccine is used. As wPV includes the whole bacteria, hypothetically, the numerous surface antigens of *B. pertussis* activate a similar response to infection. After infection and wPV, IL-1, IL-6, IL-12, and IL-23 are induced by macrophages and DCs, which stimulate the differentiation of Th1 and Th17 cells [295]. Th1 and Th17 responses are associated with a high level of interferon-γ, and IL-17 production, which, respectively, mediate the build-up of opsonizing antibodies and further activation of macrophages and neutrophils [296]–[298].

aPVs, on the other hand, stimulate IL-10-producing macrophages, leading to a Th2 dominant response with elevated IL-4, IL-5, and IL-13 concentrations [280],

[296], [299], [300]. FHA, in particular, stimulates the secretion of IL-10 but inhibits IL-12 secretion, which transits naïve T-cells to the Th2 direction [301]. However, in older children and adolescents, both Th1 and Th2 cells are detected [9], [293], [302], which may be due to previous infections. The distinct T cell memory patterns were noted still at four years after an aPV or wPV [303]. The difference in Th responses may result in poorer long-term protection with aPVs compared to wPVs [103], [189], [284], [290].

PT induces also a Th17 response in an IL-6-dependent manner [179]. Mice defective in IL-17A had a more persistent *B. pertussis* infection after aPVs than wild-type mice. aPV-mediated bacterial clearance appears to depend on the recruitment of Th17 cells to the lungs after the challenge. [290]. Mucosal IL-17, IL-6, and IL-23 were found in the nasopharynx of *B. pertussis*-infected baboons [291]. Together, these findings highlight the role of IL-17A as a central goal for protective immunity attained from vaccinations.

2.4 Pertussis toxin-specific antibodies

There are several laboratory techniques used to measure antibodies against PT. When considering the quantity of anti-PT IgG, the most common methods are ELISAs or multiplex immunoassays [52]. Also, lateral flow point-of-care assays [304] or immunoblots [305] can be used. However, they are less sensitive. Essentially, it is suggested to use purified PTx as an antigen. Tests can measure anti-PT IgG from serum, whole blood, or oral fluid [304], [306], [307].

Problematically, laboratory assays measuring only anti-PT IgG cannot separate recent infection from vaccination. Thus, much research has been committed to finding alternatives for diagnostic purposes. These include other (non-vaccine) antigens, such as ACT [308], [309], anti-PT IgA, IgM [309], [310], and other qualitative aspects such as anti-PT IgG antibody subclasses, or fucosylation status [311]. Simultaneous measurement of IgA, IgG, and IgM may prove helpful due to the isotype composition heterogeneity at different stages after the onset of the disease. [312].

2.4.1 Characterization of antibodies

Numerous qualitative characteristics have been evaluated for PT-specific antibodies after infection, vaccination, and for mAb clones. These include structural analysis, functional tests, and biochemical properties, such as binding strength (Figure 6). As PT evokes various biological activities, antibodies' effectiveness in preventing these events may be evaluated. Features such as ADP-ribosyltransferase activity, leukocytosis promotion, islet activation, hemagglutination, and protection upon

challenge have been studied extensively in vitro and mouse models. Essentially, mAbs targeting different subunits of PT have alternating binding affinities and excel in preventing different functions of PT. [313]–[316].

Generally, the IgG1 subclass is elevated after both vaccination and infection. However, IgG3 antibodies are often present after infection, whereas aPV promotes IgG4 antibodies. [312], [317]–[320]. Like infection, whole-cell vaccines increase IgG3 antibodies [319]. IgG2 and IgG4 are also found after infection [312], [320]. Additionally, the presence of IgG4 seems age-dependent. The frequency of occurrence of IgG4 antibodies was highest in children at the age of 6 years and decreased with age. This phenomenon likely has nothing to do with infection but is tied to being more recently vaccinated with aPVs [321]. Intriguingly, IgG4 antibodies decrease faster than other subclasses. IgG4 antibodies can be linked to a Th2-type response from repeated aPVs, whereas IgG2 and IgG3 subclasses are more upregulated by Th1 cells. [322], [323].

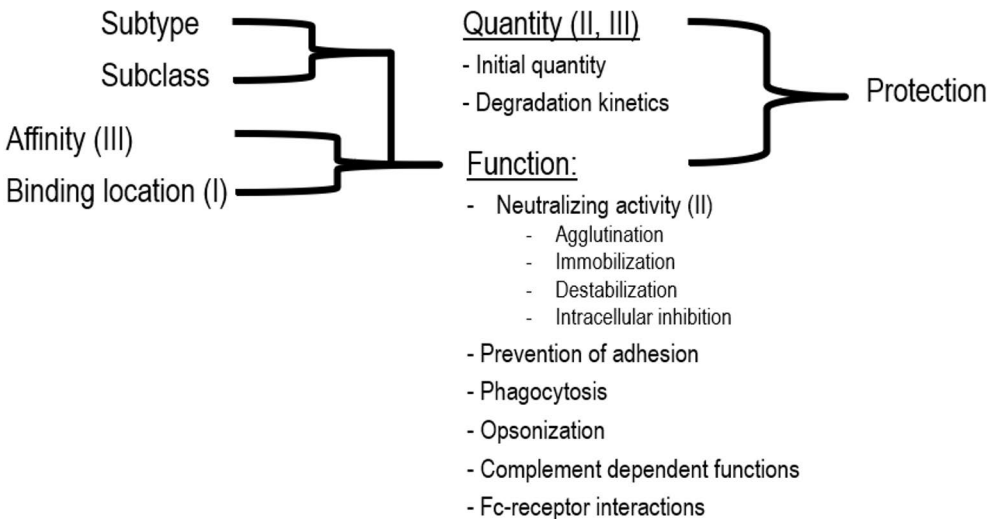

Figure 6. Characteristics influencing antibody-mediated protection. Antibodies have various functions to prevent the activity and function of pathogens and foreign antigens. In return, antibodies' effectiveness and proposed mechanism of function may be reflected by certain factors. The aspects studied in this thesis are outlined as I-III.

The neutralization capability of vaccine and infection-induced antibodies is commonly evaluated for toxin-mediated bacterial diseases to study protectivity against the pathogen of interest [324], [325]. For anti-PT IgG this is most commonly studied via the clustering effect and form changes of Chinese hamster ovary (CHO) epithelial cells [326]–[328]. Less commonly, a method based on peripheral blood mononuclear cells has been used [329]. The CHO cell assay was initially used to

study whether PT is properly detoxified in the vaccines [330]–[333]. The assay was widely used to evaluate vaccine and infection responses [261] and to show the protective properties of mAbs targeted to different epitopes of PT subunits [313], [314]. In short, the general conclusion is that immunization with aPV, wPV, and infection induce PT-neutralizing antibodies. However, of late, the assay has been used less after several demonstrations that anti-PT IgG ELISAs give a reasonable estimate of the quantity of neutralizing antibodies [261]. Nevertheless, the assay may prove useful as high antibody concentrations do not always guarantee high neutralization capacity [334]–[337] [II].

The affinity of antibodies may correlate with long-term immunity against diseases based on the constant development of antibodies through avidity maturation [338]–[341]. Avidity describes the general affinity resulting from multivalent antibody binding with multivalent epitopes on the antigen. As an antigen-driven selection process within the germinal centers [342]–[345], somatic hypermutations lead to the progressive selection of higher-affinity antibodies [346]–[348]. Avidity is generally measured by ELISAs, in which the proportion of strongly and weakly binding antibodies to PT is estimated by treating the antibodies with chaotropic solutions, such as urea, ammonium thiocyanate, or diethylamine, which break down weak antigen–antibody bonds. [261]. Thus far, it has been demonstrated that avidity towards PT increases after vaccination and remains elevated up to one year [349]–[352], which is affected by previous aPVs or wPVs [353], and that it declines over time [354]. With infection, avidity increases between the acute and the convalescent phase, and the attained avidity remains high up to three years after recovery [351], [355]. Studies regarding avidity are somewhat limited, and respective research settings have rarely been replicated [261].

One of the functions of antibodies is to facilitate the uptake of bacteria via Fc-γ-receptor-mediated opsonophagocytosis by neutrophils and macrophages that have a role in clearing *B*. *pertussis* during infection [356]. PT- and FHA-specific antibodies were shown to be the second-best predictors for opsonophagocytosis after anti-PRN antibodies, although anti-PT antibodies might not be mechanistically related to the process. [355]. Likewise, no evidence has been found to highlight a role for anti-PT antibodies in potential complement-mediated bactericidal activity after aPV or natural infection [357].

Antibodies targeting specific epitopes on the S1 and B-oligomer of PT may induce protective immunity [358]. The target subunit for antibody binding may be deduced by immunoblotting [316]. mAbs against specific epitopes have been further utilized for epitope mapping of human immune responses towards PT with competitive ELISAs. In comparison to infection, aPVs seem to induce fewer antibodies toward specific epitopes 1B7 in S1 and 11E6 in S23 [359]. In the study by Sutherland et al., aPV-derived antibodies were found to preferentially bind to PTd

over PTx. The 1B7 epitope is the most studied so far, and its location on PT has been defined in great detail [359]–[362]. 1B7-targeting mAb is a candidate therapeutic that neutralizes PTx, prevents leukocytosis (in mice), and treats established disease (in baboons) [257], [363], [364]. Furthermore, after aPVs, antibodies from genetically distinct plasmablasts were found to 1B7 but not to the S23-targeting epitopes [365]. However, extensive epitope mapping of the human antibody response has not been performed for PT. It remains elusive whether aPVs skew the immune response to non-protective epitopes. Likewise, wPV-induced epitope-specific antibody responses have not been studied. [366]. Presumably, epitopes remaining in PTg should resemble closely to those of PTx [226], [359].

3 Aims of the Study

This thesis aimed to examine the different functional and quantitative aspects regarding the antibody response in humans after exposure to *Bordetella pertussis* bacteria's unique and likely the most central antigen, pertussis toxin, via vaccination or infection.

More specifically, the aims were:

I To compare whether vaccination- and infection-induced antibodies recognize different epitopes of PT because of the chemical detoxification procedures of the vaccines.

II To analyze the aPV-induced antibodies' capability to neutralize PTx in participants with different vaccination backgrounds and ages.

III To investigate the binding strength of vaccination and infection-induced antibodies to PTx.

IV To examine the possible association between the antibodies' functional characteristics.

4 Materials and Methods

A more detailed description of the materials and methods can be found in the original publications (I-III)

4.1 Study subjects

The samples used in the studies are summarized in Table 3. The vaccination programs of the countries and general compositions of the vaccines included in the studies are presented in Table 1.

4.1.1 Booster vaccination in different age groups (II, III)

The Booster against pertussis vaccine ("BERT") study was performed in 2017 in Turku, Finland, and at the Spaarne Academy, Hoofddorp, the Netherlands [207]. Overall, 258 participants from different age groups from the two countries were immunized with a single dose of the Tdap3-IPV vaccine (Boostrix™, GSK, Wavre, Belgium), and the collected serum samples were measured for anti-PT IgG, IgA, avidity, and neutralizing antibodies. The vaccination history of these study subjects is presented in more detail in original publication II. Of note, the adolescent cohort included participants primed either with aPVs or wPVs in their infancy. Serum samples were collected at days 0, 28, and 365 post-vaccination.

4.1.2 Infant vaccination cohorts (I, III)

To study PT-specific epitopes induced after a primary series of aPVs, 50 Finnish infants were selected from a prospective study cohort called the Steps to Children's Healthy Development and Wellbeing conducted in Turku, Finland [367]. They received three primary doses of two- or three-component aPVs (Tetravac, SP, Lyon, France, or Infanrix, GSK, Rixensart, Belgium) at 3, 5, and 12 months of age. Serum samples were collected at the age of 13 months.

Another cohort of twenty Danish toddlers, aged 13–25 months, who received their third primary dose of aPV (Statens Serum Institut, Copenhagen, Denmark) at 12 months, was measured for avidity (III).

4.1.3 Child vaccination cohorts (I, III)

Danish children aged 5–7 years were tested for avidity and epitopes of PT antibodies. They were vaccinated at the age of five years with the Danish monocomponent booster vaccine (Statens Serum Institut, Copenhagen, Denmark). Notably, this vaccine included high amounts of only PT, detoxified by hydrogen peroxide. An additional cohort measured for epitopes included Finnish children, ages 4–5, who had received a booster dose of a two-component aP (Tetravac, SP) at the age of four years. The samples were collected from routine diagnostic samples unrelated to respiratory infections during 2014–2017. Another cohort of Finnish adolescents was tested for epitopes, who received a booster dose of a three-component dTaP vaccine (Boostrix, GSK) at the age of 11–13 in 1997, and their serum sample was collected one month after vaccination. They had received four doses of wPVs in earlier childhood [224]. In Boostrix and Infanrix, PT is detoxified using a combination of glutaraldehyde and formaldehyde; Tetravac is detoxified using glutaraldehyde and stabilized with formaldehyde.

4.1.4 Pertussis patients (I, III)

Danish and Finnish patients were tested for avidity and epitopes of PT antibodies. The Danish serologically confirmed patients were adolescents vaccinated in childhood with the Danish aP (at three, five, and twelve months). The diagnosis of these patients was based on anti-PTx IgG ELISA [368]. The diagnosis of the Finnish patients was based on IgA and IgM antibody levels measured by ELISA using sonicated *B. pertussis* as a coating antigen [369]. In addition, the patients selected for the epitope study (I) were defined to have at least 50 IU/mL of anti-PT IgG and at least 25 IU/mL for the measurement of avidity (III).

4.2 Ethics

Before the inclusion into the study, all subject data, except for age, gender, and pertussis vaccination history, were anonymized. The Finnish sera of patients and 4- to 5-year-old children had been sent for diagnostic purposes of pertussis and Lyme borreliosis, respectively, to the diagnostic laboratory at the Department of Medical Microbiology and Immunology, at the University of Turku, Finland. The use of these clinical samples was approved by the Ethics Committee of the Hospital District of Southwest Finland by the chief of the operative group of Turku University Hospital (Decision 14/17 MBG). Denmark's regional research ethics committee did not require discrete approval to use Danish sera for this study. The clinical trials and permissions regarding the use of serum from Finnish infants and 11–13-year-old children were described earlier in their respective investigations [224], [367].

Table 3. Study subject characteristics.

Group	Year of sample collection	Study	N	Age, years (median, range)	Female /Male	Time since last vaccination (range)	Anti-PT IgG (IU/ml, median)
Danish infants, primary aPV	2011–2016	III	20	1.5 (1–2)	7/13	15–413 days	47
Danish children, booster aPV	2009–2017	III I	34 22	5.7 (5–7) 5.0 (5–6)	18/16 10/12	10–677 days 10–580 days	134 113
Danish patients	2011–2017	III I	39 21	13.5 (10–19) 13.0 (11–17)	21/18 11/10	5–13 years 5–16 years	257 149
Finnish patients	2015–2016	III I	95 51	13.5 (3–67) 14.3 (3–70)	13/13* 27/24	not available not available	202 118
Finnish children, aPv	2014–2017	I	30	4.3 (4–5)	17/13	2–52 weeks	104
Finnish adolescents, aPv	1997	I	50	11.6 (11–12)	24/26	1 month	94
Finnish and Dutch children, aPv	2017–2019	II III**	73 29	8.6 (7–11)	36/37	3–7 years	***
Finnish and Dutch adolescents, aPv	2017–2019	II III**	85 29	13.6 (11–16)	49/36	6–10 years	***
Finnish and Dutch young adults, aPv	2017–2019	II	95	29.4 (21–35)	31/19	not available	***
Finnish and Dutch older adults, aPv	2017–2019	II	51	64.5 (60–70)	35/15	not available	***

*Data available for only 26 individuals

** Only Finnish samples

*** Detailed antibody concentrations at each timepoint and age group in Versteegen et al. 2021 [207]

The BERT clinical study (Chapter 4.1.1) was registered at the European Clinical Trials register under the study number: 2016-003678-42 and approved by the medical research ethics committee of Turku University Hospital (ETMK Dnro: 129/1800/2017) and by the Medical Research Ethics Committees United (MEC-U, NL60807.100.17-R17.039) in the Netherlands. Parents or legal guardians of minor participants provided written informed consent. The study was conducted in compliance with the principles of the Declaration of Helsinki (1996).

4.3 Anti-PTx IgG antibody measurements (I, II, III)

Concentrations of PTx-specific IgG (and IgA, II) antibodies were measured from all study samples either with ELISA (I, III) or MIA (II). The anti-PT IgG and IgA results using MIA had been measured and published before [207]. For ELISA measurements, 96-well plates (Immulon, 735-0462, VWR, Finland) were coated with 100 ng of PTx (GSK) in 100 µl of PBS (pH 7.4) overnight at room temperature. After washing the plates thrice with 200 µls of 0.9% NaCl-0.05% Tween-washing buffer (Sigma P-1379, St. Louis, USA), 100 µl of four-fold serial dilutions (1:60–1:3840) of serum samples, and two-fold serial dilutions of WHO anti-PT IgG control sample (06/142, National Institute for Biological Standards and Control (NIBSC), Potters Bar, UK) were incubated in the wells for two hours at 37 °C. Anti-PT IgG negative and positive controls from in-house serum sample pools were included with each test plate. After three washes, 100 µl of 1:2000 diluted goat anti-human IgG alkaline-phosphatase conjugate (0751-1002, KPL Inc., Maryland, USA) was incubated for two hours at 37 °C. Last, after three washes, 100 µL of p-Nitrophenylphosphatase substrate (cat no S0942, Sigma, Helsinki, Finland) in diethanolamine-$MgCl_2$-buffer (Reagena, cat no 170057, Toivala, Finland) was incubated and covered from light for six minutes, and the reaction was stopped with 100 µl of 3 M NaOH. Absorbance was measured at 405 nm with Multiskan EX (Thermo Scientific Vantaa, Finland) or Victor X4 devices (PerkinElmer, Turku, Finland).

4.4 Antibody avidity to PTx (III)

The binding strength of serum antibodies to PTx was determined by two ELISAs, similarly to anti-PT IgG-ELISA, with few modifications: 96-well plates (Greiner Microlon, art. No. 655061, Frickenhausen, Germany) were coated with 200 ng of PTx (GSK) in 100 µL of PBS. After the wells were washed once with washing buffer, the plates were blocked with 1% BSA-PBS (cat no 810033; MP Biomedicals, Solon, Ohio) for one hour at 37 °C. Next, after three washes, the difference between the two distinct ELISAs included diluting the serum samples either by a constant 1:50 dilution or to match a fixed concentration of 0.025 IU per well of anti-PT IgG

between the samples in 100 µL of 1% BSA-PBS. Samples were incubated in duplicate wells for two hours at 37 °C. The constant dilution was performed only for subjects with at least 50 IU/mL anti-PT IgG antibodies. Likewise, only samples with more than 25 IU/mL anti-PT IgG were included in the fixed dilution approach. Blank samples (1% BSA-PBS and PBS) and anti-PT IgG negative and positive controls from in-house serum sample pools were included with each plate and were used to adjust for inter-assay variation. After washing, half of the wells were treated with 100 µl of either constant or a dilution series of antibody–antigen binding disturbing detergents (2–8 M urea, 30 mM diethylamine (DEA), or 1 M ammonium thiocyanate, NH_4SCN), and the other half with PBS for 15 minutes. The concentrations of these detergents were selected based on the previous literature [261]. Wells were then washed once, and the addition of conjugate and substrates, and measurement of the absorbance would proceed similarly as described in chapter 4.3. The avidity index (AI) was counted from the background (PBS and BSA-PBS wells) reduced signals as absorbance (detergent well) / absorbance (PBS well). AI-values over 100% were converted to 100.0% for data analysis.

4.5 Binding location of PT-specific antibodies (I)

A total of 27 murine mAbs (NIBSC) which targeted the five subunits of PT (10 to S1, 2 to S2, 5 to S23, 6 to S3, 3 to S4 and 1 to S5), were first screened against PTx with a small set of sera (N = 11, anti-PTx IgG range 0–387 IU/mL) from Finnish patients. Based on the amount of binding to PTx, and the biological protection in mice, five mAbs 1B7 (S1), 1D7 (S1), 10D6 (S1), 11E6 (S23), and 7E10 (S3) were selected for further testing with the study samples. Blocking of the mAbs 1B7, 1D7, 10D6, and 7E10 was also studied with glutaraldehyde detoxified PT (PTdg) as the capture antigen. These mAbs are later referred to as the corresponding epitopes.

The blocking of the epitope-specific mAbs' binding to PTx and PTdg by serum antibodies was determined by a competitive ("blocking") ELISA: 96-well plates (Art. No. 655061; Greiner Microlon, Frickenhausen, Germany) were coated with 250 ng of PTx (GSK) or PTdg (SP) in 100 µL of PBS (pH 7.4). The wells were washed once with PBS and blocked with 150 µL of 1% BSA-PBS for one hour at 37 °C. After three washes with 0.9% NaCl-0.05% Tween-wash buffer, serum samples were diluted 1:20 in 100 µL of 1% BSA-PBS and incubated for two h at 37 °C. Only samples with at least 50 IU/mL anti-PT IgG but not more than 270 IU/mL were included to ensure an even distribution of antibody concentrations across the study cohorts. All samples were tested in duplicate wells, including a blank sample (1% BSA-PBS) and anti-PTx IgG-negative and IgG-positive controls. After washing the plates, 100 µL of individual mAbs were diluted in PBS according to NIBSC-recommended dilutions and incubated for one h at 37°C on the well plates. Goat anti-mouse IgG conjugate (AP124; Merck,

Espoo, Finland) was then diluted 1:2000 in 100 µL of BSA-PBS and incubated for one h at 37°C. Last, 100 µL of p-nitrophenylphosphatase substrate (Sigma) in diethanolamine-$MgCl_2$-buffer (Reagena) was incubated and covered from light until the blank sample reached an absorbance value of ~ 1.5. Absorbance was measured at 405 nm with the Multiskan EX device. The reduction/blocking of the maximum signal of mAb binding caused by the similar epitope-like antibodies from study sera, referred to as 'specific inhibition', was counted from background reduced signals as 1 - absorbance (sample)/absorbance (blank). All values of specific inhibition equal to 0% or less were converted to 0.1% for data analysis.

4.6 Antibodies' capability to neutralize PTx (II)

PT-neutralizing antibodies (PTNA) were determined based on their ability to prevent the clustering of CHO cells caused by PT. Fifty µl of 3.36 ng/mL PTx (GSK) was incubated for three hours with 50 µls of a two-step dilution series of serum (1:8 to 1:4096) in Dulbecco's modified Eagle medium (Corning, USA, or Labnet, Finland) with 10% fetal bovine serum (Corning, USA, or Hyclone, Canada). Next, 5,000 or 10,000 CHO cells were mixed with PT and sera in 96-well plates (CLS3596, Corning USA). Each 96-well plate included controls: 1) only PT and cells, 2) only testing sera and cells, and 3) only cells. The wells were evaluated visually after 24 hours by microscopy, Incucyte, or IncucyteZoom (Essen Bioscience, Michigan, USA) instruments. The neutralizing titer was reported as the reciprocal value of the serum dilution in the last well without clusters of CHO cells. Samples with titers below the assay cutoff "1:8" were arbitrarily attributed to "1:4" to allow for statistical analyses. The PTNA titer results were further analyzed in a model in which the proportion of neutralizing titer was divided by anti-PT IgG concentration [370]. Exploratory proportion values of <0.5 and >2.0 were chosen to describe low and high neutralization ratios, respectively.

4.7 Statistics

Data were analyzed with IBM SPSS statistics software (various versions from 25 to 28.0) for Windows (IBM Corp., Armonk, NY, USA). Two-sided p-values less than 0.05 were considered statistically significant. The correlation between functional antibody responses and anti-PTx IgG IU/mL was calculated with the Pearson or Spearman correlation coefficients, based on the Shapiro-Wilk test. The differences in means between groups were tested either with analysis of variance (ANOVA) (III), Mann–Whitney U (I, II), or Kruskal-Wallis tests (I, II) for more than two groups with Bonferroni corrections. Wilcoxon signed-rank test was used to compare median titers between the before and post-booster time points (II).

5 Results

5.1 Antibody avidity

The binding strength of antibodies to PTx after aPV and infection was evaluated with antibody-binding interfering detergents in two distinct ELISAs.

5.1.1 Avidity correlation with overall antibody concentration

Study samples were first measured with a constant 1:50 serum dilution with 30 mM DEA as a detergent. The Danish patients had higher avidity than children who received aPVs (Figure 7). However, the Finnish patients and vaccine recipients had similar avidity. Avidity correlated strongly with anti-PT IgG concentration (Pearson R = 0.801 and 0.804 in Danish and Finnish samples). If the anti-PT IgGs were compared, there was a similar significant difference between Danish patients and vaccinees but no difference between the Finnish cohorts (III).

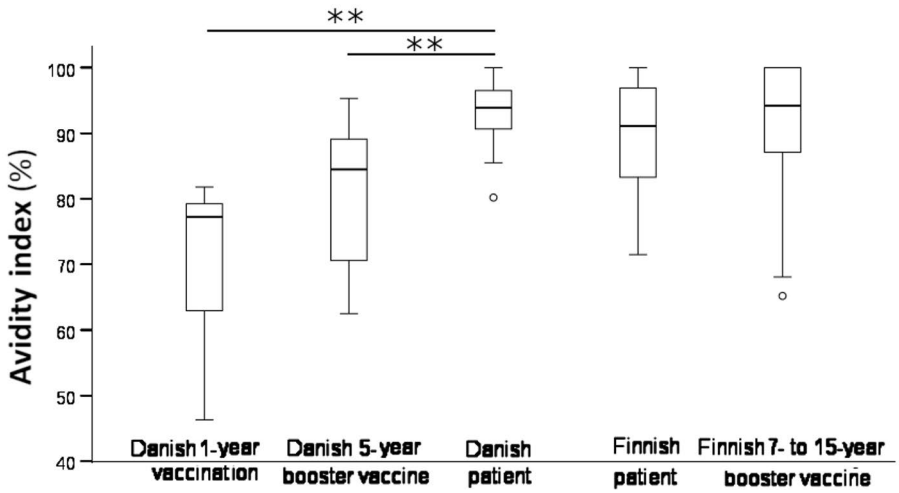

Figure 7. Avidity of anti-PT IgG antibodies using 30 mM DEA with a constant 1:50 serum dilution. Only samples with above 50 IU/mL anti-PT IgG were included in this analysis. The number of samples in each group in respective order = 10, 25, 38, 42, and 42. $^{**}p < 0.01$. The box plots present the median, quartile range, and 1.5 times the quartile range of avidity. O = values exceeding 1.5 times the interquartile range. Modified from (III).

Consequently, the differences between anti-PT IgG concentrations led to contradictive observations regarding avidity between the study populations. Alternatively, the DEA concentration could be too mild, resulting in the incapability to separate different avidity levels between high-concentration samples. The presumption was that the PT capture surface becomes saturated with only "high-affinity" antibodies, and the detergent cannot interfere with their binding.

5.1.2 Comparison between fixed and constant dilution methods for avidity measurement

To confirm whether anti-PT IgG concentration was the cause of different avidities, the Danish samples were diluted in three distinct ways: either by a constant 1:50 dilution or fixed (estimated) concentrations of 0.2 or 0.1 IU per well for all samples. If the samples were diluted to have matching anti-PT IgGs, there was no longer a difference in avidity between the groups (Figure 8a). A strength of this analysis was that it included a patient sample (matching of 100 IU/mL) which was diluted similarly in the constant 1:50 dilution and the 0.2 IU per well assays, and it could be thus ensured there was no or minimal assay-to-assay and plate-to-plate variation. A similar assay was performed with a subset of Finnish samples, with the same result: avidity was similar between the groups (Figure 8b). These experiments also demonstrated that avidity increased when the amount of antibodies doubled (from 0.1 to 0.2 IU per well). A question arises whether a threshold amount of antibodies exists, after which the avidity (of an individual sample) would not change.

5.1.3 Comparison between detergents and concentration ranges

First, the antibodies' hypothetical lowest concentration limit where avidity would remain unchanged was determined. With both urea and DEA, the anti-PT IgG concentration-dependent change of avidity in individual samples was noted to slow down after 0.013 IU per well (Figure 9). For 1M NH_4SCN, no threshold limit was found with the performed dilution series, hypothetically indicating a lower limit of even less than 0.005 IU per well needed for avidity testing. However, from a technical standpoint, diluting to 0.013 IU was found unpractical since several samples at this point were found to be negative by the PBS-control well of the assay. Based on this factor, the dilution to 0.025 IU per well was chosen as the concentration for the following assays. Of note, this would still be four-fold less than what was used in the earlier test (Figure 8).

Since urea is non-toxic and demonstrated a similar quantity of interference to the binding strength of antibodies as DEA, it was selected for the following assays.

However, selecting a single detergent concentration might result in a lack or too strong interference in the binding. A range of detergent concentrations was chosen for the following assays to capture the full spectrum of the avidity response [371].

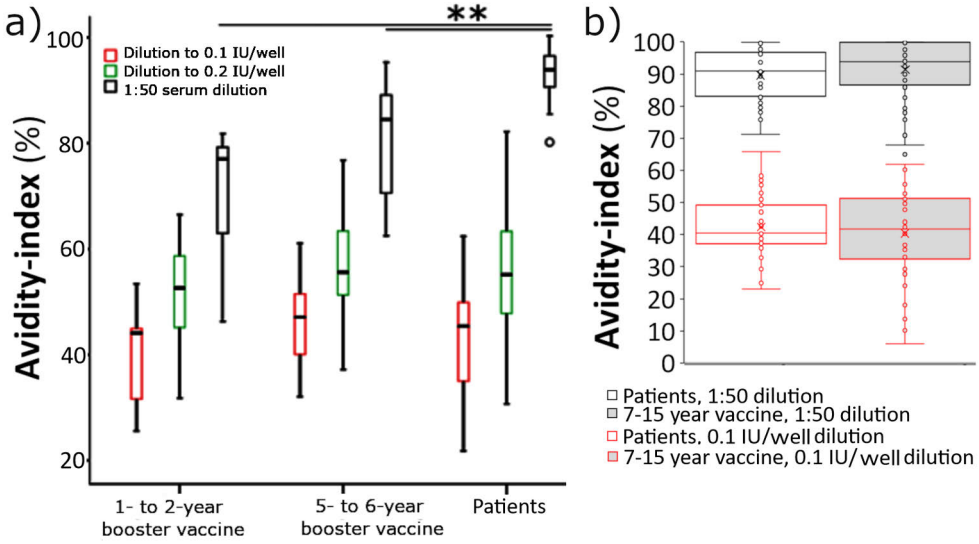

Figure 8. **a)** Danish samples were diluted by a constant 1:50 dilution ratio (Black), or to have an equal amount of antibodies, corresponding to 0.1 (Red) or 0.2 IU anti-PT IgG (Green) per well. Only samples with >100 IU/mL were included in this analysis. **b)** Finnish samples were diluted in two ways: by a constant 1:50 dilution ratio (Black) or corresponding to 0.1 IU anti-PT IgG per well (Red). Samples with >50 IU/mL were included in this analysis. ***p* < 0.001.

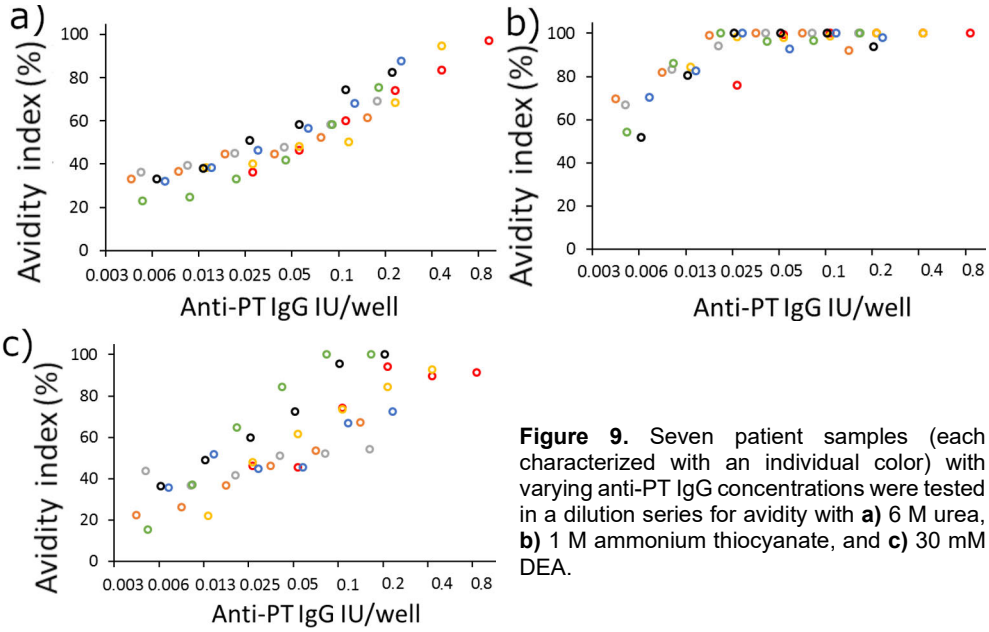

Figure 9. Seven patient samples (each characterized with an individual color) with varying anti-PT IgG concentrations were tested in a dilution series for avidity with **a)** 6 M urea, **b)** 1 M ammonium thiocyanate, and **c)** 30 mM DEA.

5.1.4 Avidity of antibodies after infection and vaccination

The Danish samples were tested within a range of 0–10 M urea concentrations, and the Finnish samples were tested with 0 and 8 M urea. Only the more potent urea concentrations used revealed that aPV induced higher avidities than patients in both countries ($p < 0.01$) (Figure 10, III). However, 10 M urea was too high a concentration, resulting in very low AI-values and no differences between the groups (data not shown).

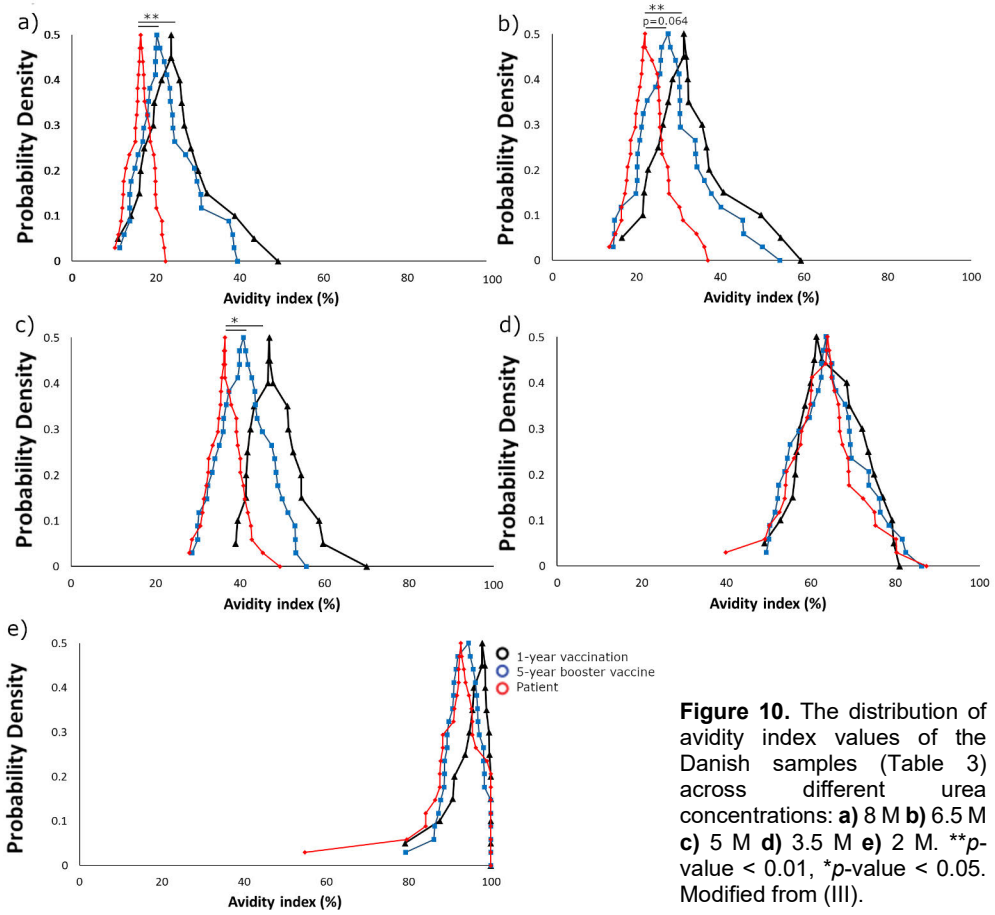

Figure 10. The distribution of avidity index values of the Danish samples (Table 3) across different urea concentrations: **a)** 8 M **b)** 6.5 M **c)** 5 M **d)** 3.5 M **e)** 2 M. ***p*-value < 0.01, **p*-value < 0.05. Modified from (III).

An additional analytical benefit of the developed fixed antibody dilution method was that the data was normally distributed within all studied groups (Shapiro-Wilk $p > 0.05$). Second, there was no correlation between avidity and anti-PT IgG concentration (Pearson R = -0.157). If the absorbance values between the PBS control wells were compared (which reflect the overall amount of bound antibodies

to PTx), there were no differences between the study groups, indicating that the dilutions to 0.025 IU per well had been performed precisely.

5.1.5 Effect of vaccination history on antibody avidity

Antibody avidity was significantly higher in patients, who had been more recently vaccinated, within a wide range of urea concentrations ($p < 0.05$) (Figure 11). Conversely, no difference in avidity was found with the 1:50 constant dilution method ($p = 0.827$) since high anti-PT IgG concentration in this cohort led to nearly a stagnant >90% avidity response in these participants (Figure 11f). Nor was there a difference with initial anti-PT IgG concentrations ($p = 0.710$) (Figure 11g).

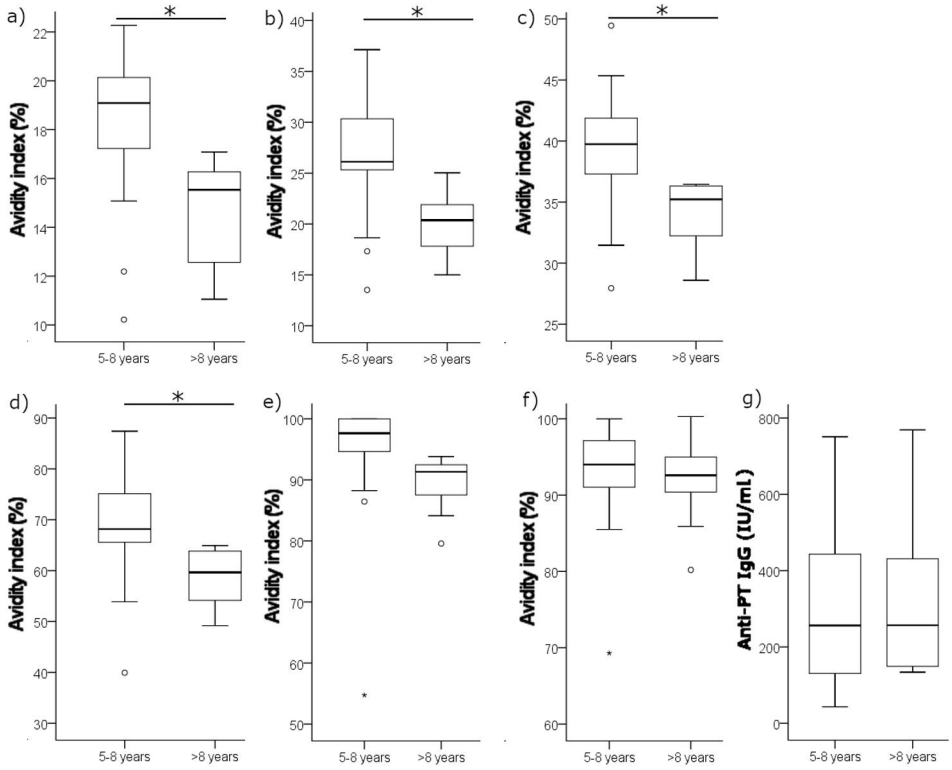

Figure 11. Avidity index of Danish patients vaccinated 5–8 years or 8–13 years before infection. Avidity is presented after treatment of the samples with either **a)** 8 M, **b)** 6.5 M, **c)** 5 M, **d)** 3.5 M, or **e)** 2 M urea using the fixed dilution method, and after **f)** 6.5 M urea using the constant dilution method. There was no difference between the initial anti-PT IgG concentrations **(g)**. * p < 0.05.

A similar effect of vaccination recency could be observed for vaccination-induced antibodies: Danish toddlers who received their third primary dose (six months after their second dose, sampling within a year) had the highest avidity (Figure 10, GM for 8 M urea = 23.7%), following Danish children who received their booster dose four years after their primary series (21.3%), and last, Finnish children who had 4–10 years since their latest vaccination (19.3%). However, differences in country background and vaccine composition (and resulting epitopes, Chapter 5.2) may considerably affect avidity. Thus, country comparisons should be considered with caution. A negative correlation was found within the Danish vaccination samples between the time since the latest vaccination and avidity across all urea concentrations (for 8 M urea Spearman R = -0.336, $p < 0.05$).

5.2 PT-specific epitopes after infection and vaccination

The binding locations of vaccination and infection-induced antibodies to PTx and PTd were evaluated with a competitive ("blocking") ELISA, utilizing epitope-specific mAb binding sites.

5.2.1 Selection of epitopes

Initially, 27 PT-specific mAbs targeting all five PT subunits were screened for overall binding to PT and tested with several patient sera. Generally, low binding of mAbs (Figure 12) and low specific inhibition from the patient samples were observed in S4 and S5 targeting epitopes (data not shown). For the following experiments, mAbs targeting the 1B7-epitope (S1), 1D7 (S1), 10D6 (S1), 11E6 (S23), and 7E10 (S3) were selected based on the following criteria: the strength of binding to PTx (Figure 12), correlation to anti-PT IgG in individual and pooled patient samples (Figure 13), the variety of subunits, the capability for biological protection in a mouse model, and other in vivo characteristics, such as affinity. For a detailed description of characteristics of mAbs see supplementary table 1 (in original publication I) and references [315], [316].

The developed ELISA was confirmed to be quantitative and demonstrated a clear correlation in clinically relevant antibody concentrations (Figure 13). The positive sample pool was a mixture of 16 serologically confirmed pertussis samples, with individual samples having at least 280 IU/mL anti-PT IgG, and resembles an estimation of what to expect for epitope binding in an infected population. The different epitope-binding mAbs appear to have different binding kinetics and affinities to PT [316] (Figure 13).

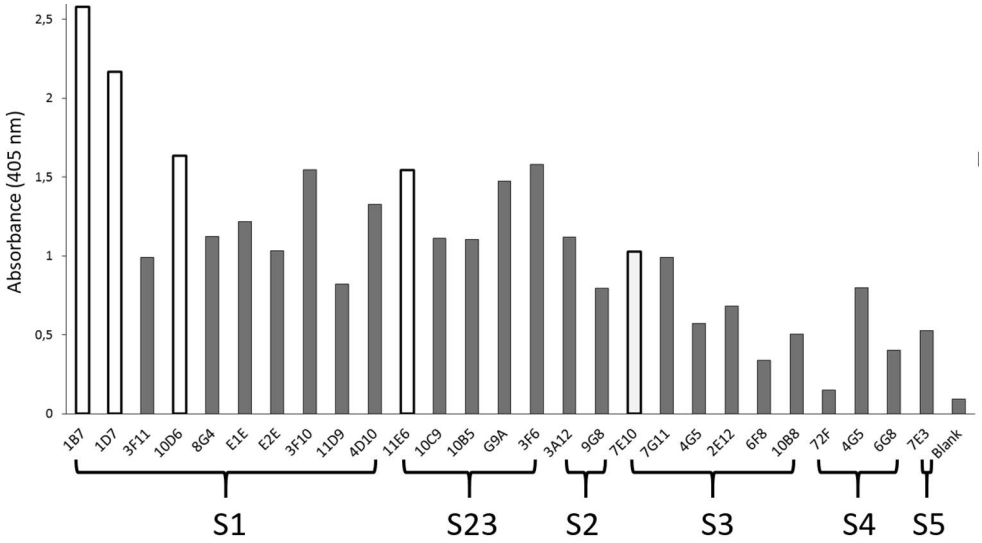

Figure 12. Twenty-seven mAbs were initially screened for the quantity of binding against PTx after 20 minutes of substrate incubation. mAbs 1B7, 1D7, 10D6, 11E6, and 7E10 (white columns) were selected for future testing. Reproduced from (I).

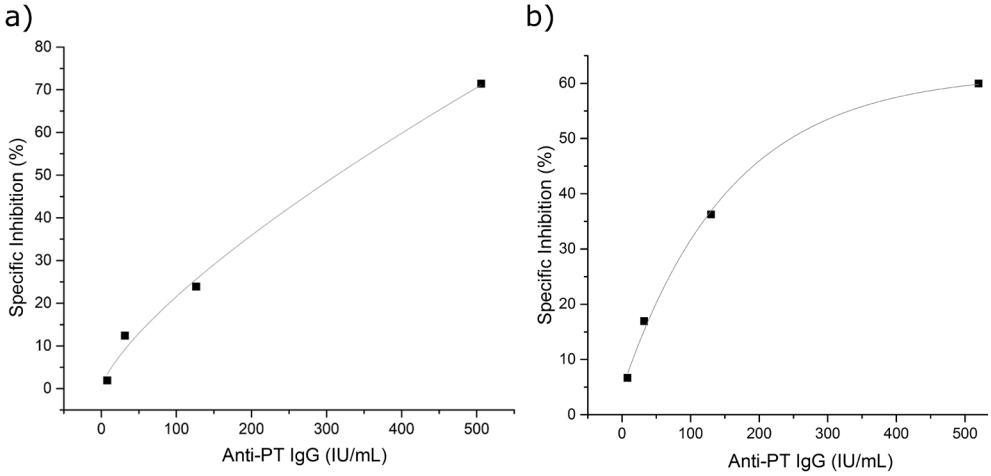

Figure 13. Specific inhibition of a four-fold dilution series of the in-house anti-PT IgG positive sample pool with epitopes 1B7 **(a)** and 7E10 **(b)**.

5.2.2 Differences in infection and vaccination epitopes

In the Finnish samples, there were significantly more 1D7, 11E6, and 7E10 binding antibodies after infection than in all vaccination groups combined ($p < 0.001$) (Figure 14). Infants after three primary vaccination doses had more 1D7 and 10D6 antibodies

than the aPV booster groups ($p < 0.05$). Like the Finnish groups, Danish patients had more 7E10 and 1D7 but also 1B7 compared to vaccination (I).

It was noted that specific inhibition by 7E10-targeting antibodies after infection correlated with overall anti-PTx IgG concentration [Pearson's R = 0.35, 0.079–0.617 95% confidence interval, $p = 0.012$], whereas vaccination-induced antibodies did not (Pearson's R = 0.05). Intriguingly, a ROC analysis revealed that the 7E10-epitope alone could demonstrate some potential for a diagnostic biomarker in recently vaccinated populations as a 5% specific inhibition threshold had a sensitivity of

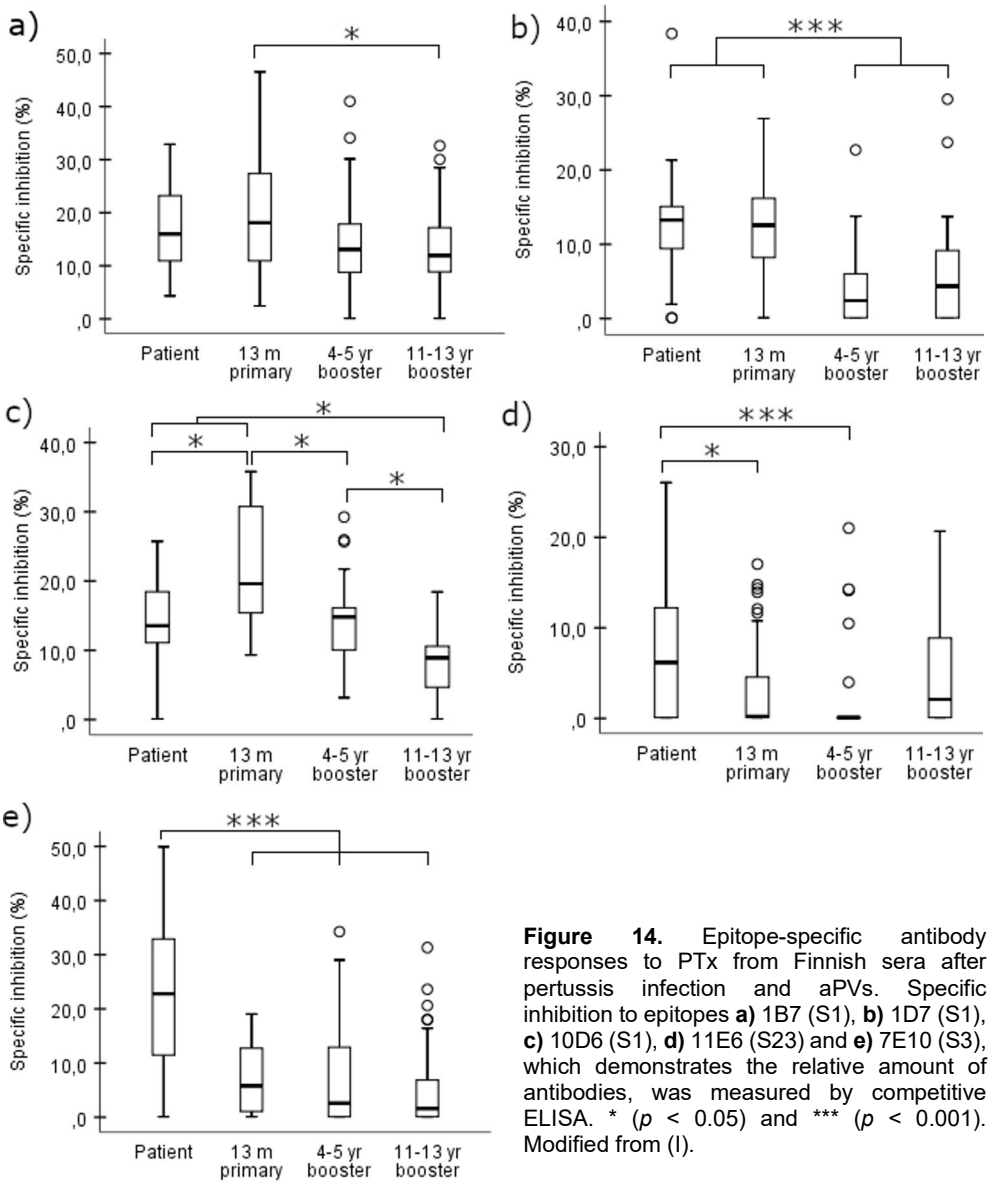

Figure 14. Epitope-specific antibody responses to PTx from Finnish sera after pertussis infection and aPVs. Specific inhibition to epitopes **a)** 1B7 (S1), **b)** 1D7 (S1), **c)** 10D6 (S1), **d)** 11E6 (S23) and **e)** 7E10 (S3), which demonstrates the relative amount of antibodies, was measured by competitive ELISA. * ($p < 0.05$) and *** ($p < 0.001$). Modified from (I).

96.4% and a specificity of 63.4% (all study samples included). Moreover, when additional sera from vaccinees with very high concentrations of anti-PTx IgG (> 300 IU/mL) were tested, only a portion (5/12) of the samples had measurable 7E10 antibodies (I). Additional S3 epitopes 2E12, 6F8, and 10B8 were studied to confirm whether other S3 epitopes are similarly affected as 7E10; however, no such difference as in 7E10 was observed (Figure 15).

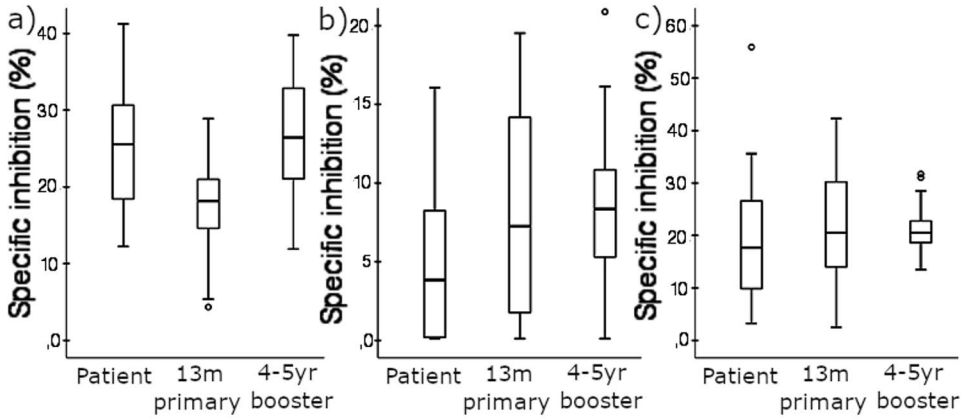

Figure 15. Epitope-specific antibody responses to PTx from Finnish sera after pertussis infection and aPVs were evaluated in additional S3 epitopes **a)** 2E12, **b)** 6F8, and **c)** 10B8. Number of samples was 20 in each group.

5.2.3 PT epitopes affected by glutaraldehyde detoxification

Epitope-specific antibodies to glutaraldehyde-detoxified PT were tested for epitopes 1B7, 1D7, 10D6, and 7E10 with the Danish samples and the Finnish 4- to 5-year-old booster group. The correlation between the PTdg and PTx 7E10-responses was non-existent in all study groups (Table 4), whereas 1B7 and 10D6 appeared to be more preserved in PTdg. Further, a correlation of 1D7-specific antibody responses between PTdg and PTx was found only among the Danish vaccine group.

The overall correlation between epitope-specific responses of 1B7 and 1D7 within PTdg increased compared with PTx from 0.62 (Figure 16a, PTx) to 0.98 (Figure 16b, PTdg) in the vaccine groups. However, within patients, the correlation between 1B7 and 1D7 decreased slightly, from 0.55 (PTx) to 0.44 (PTdg), primarily because of two strongly deviating outliers. If these were removed, the trend would be similar to vaccinations. The correlation of 10D6 response to 1B7 and 1D7 increased similarly in the Finnish vaccination group from an average of 0.63 to 0.92.

Table 4. Pearson correlation of epitope responses between PTdg and PTx capture surface. Modified from (I).

	Danish patients	Danish 5–6-year booster	Finnish 4–5-year booster
1B7	0.705*	0.526*	0.443*
1D7	0.036	0.639*	-0.011
10D6	-	-	0.534*
7E10	-0.004	0.241	0.154

* Correlation is significant $p < 0.05$

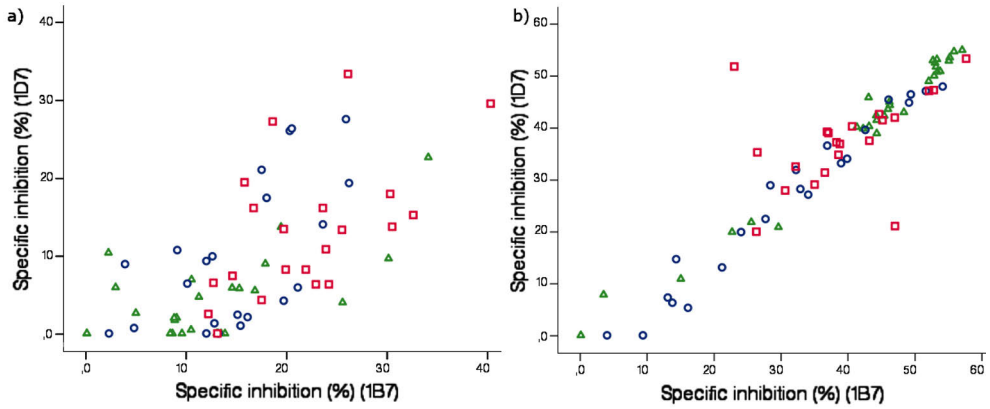

Figure 16. Correlation of epitope-specific inhibition between epitopes 1B7 and 1D7 of native **(a)** and glutaraldehyde-detoxified PT **(b)**. O = booster vaccination in 5- to 6-year-old Danes (N = 22); □ = Danish patients (N = 21); Δ = booster vaccination in 4- to 5-year-old Finns (N = 26). Reproduced from (I).

5.3 PT-neutralizing antibodies after vaccination

Vaccination-induced antibodies' capability to neutralize PTx was evaluated as the antibodies' ability to prevent PTx-mediated CHO cell clustering. PTNAs were measured from samples before vaccination, one month, and one year post-vaccination in different age groups.

5.3.1 Effect of existing antibodies on PTNA response

The PTNA titers correlated well with overall anti-PT IgG concentrations across the study time points (Figure 17). The greatest factor influencing the PTNA response's magnitude was the concentration of anti-PT IgG before vaccination (Figure 18): Those subjects with higher than 10 IU/mL anti-PT IgG before boosting had 2.7 and 3.4 times more PTNAs at one month and one year, respectively. This effect was

observed across all age groups in both countries (II). A similar observation was made with solely anti-PT IgG concentrations.

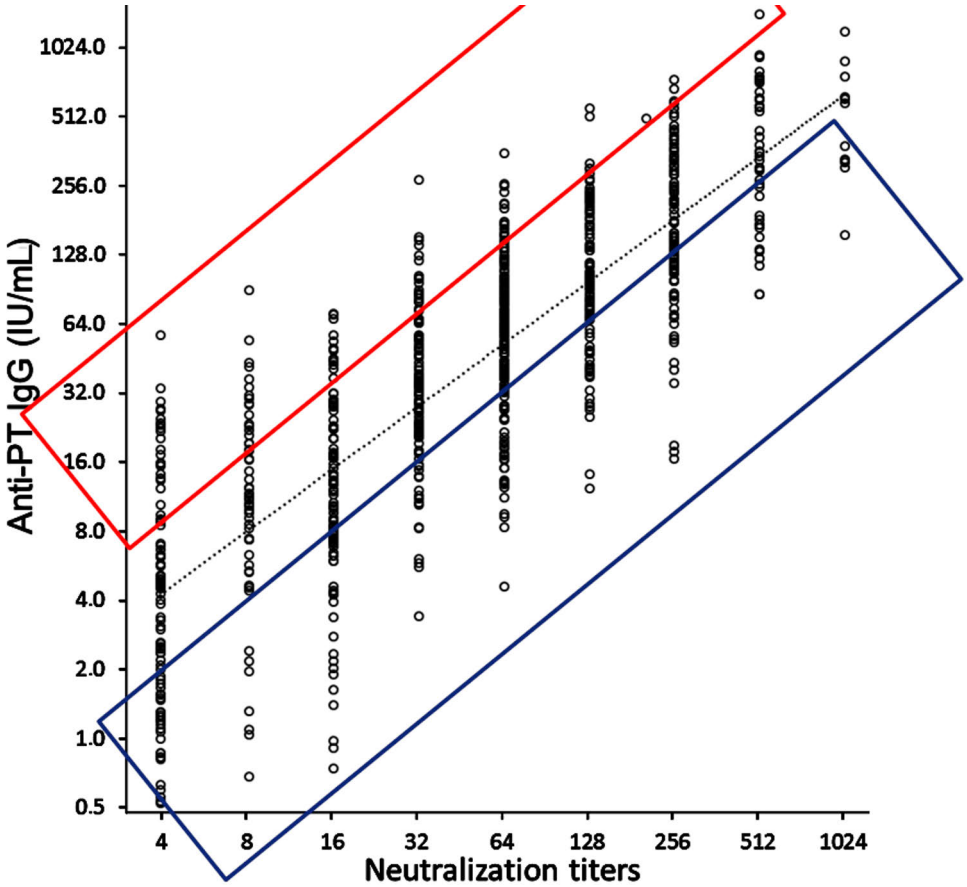

Figure 17. The correlation between overall anti-PT IgG concentration and PT-neutralizing antibody titers was high (Pearson R = 0.829). All samples: before, one month, and one year after vaccination are presented (N = 769). The red box highlights samples with a low PTNA/IgG ratio of < 0.5 (N = 114), and the blue box highlights samples with a high PTNA/IgG ratio of > 2 (N = 206). Modified from (II).

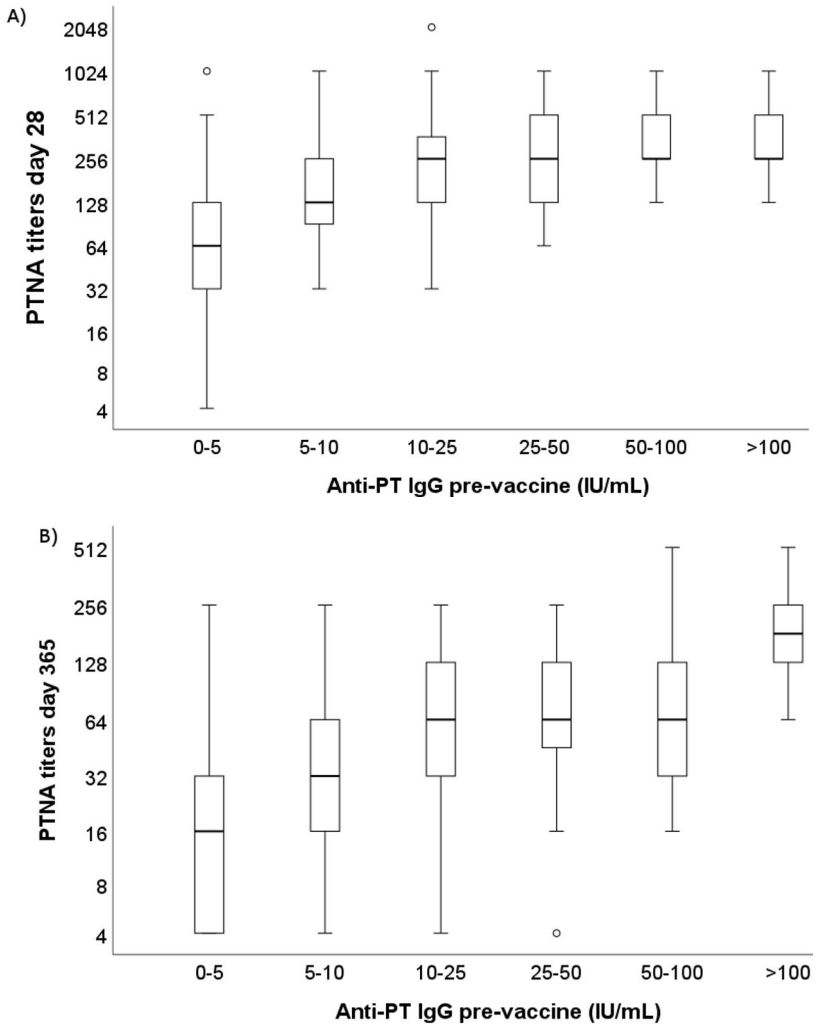

Figure 18. PTNA titers at one month **A)** and one year **B)** post-vaccination based on anti-PT IgG concentrations before the vaccination (x-axis). The number of samples in each range in respective order: 70, 45, 66, 44, 21, and 10.

5.3.2 Effect of age and vaccination background on PTNAs

No direct correlation could be seen between PTNAs and age. Young adults in both countries had significantly lower PTNAs post-vaccination than other age groups ($p < 0.05$) (II) (Table 5). However, this could be associated with low PTNA titers before vaccination; 78% of young adults had less than 10 IU/mL anti-PT IgG before vaccination (other age groups, on average, 37%). Only older adults demonstrated a correlation between IgA and PTNAs (Pearson R = 0.733 and 0.648 at one month and year, respectively.)

The adolescents in the BERT study were recruited based on their birth year, which was tied to the shifts in the national vaccination programs from wPVs to aPVs in both countries. A significant difference between these vaccination backgrounds was noted only in Finland at one year (Figure 19). This finding may however be influenced by anti-PT IgG concentrations before vaccination.

Table 5. GM values of anti-PT IgG (IU/mL), PTNA titers, and PTNA to IgG ratio before, a month, and a year after vaccination. Modified from (II).

Cohort	Country	PTNAs			anti-PT IgG			PTNAs per anti-PT IgG		
		0M	1M	1Y	0M	1M	1Y	0M	1M	1Y
Children	FI	18	198	47	16	199	39	1.13	1.00	1.22
	NL	20	210	22	12	147	30	1.72	1.43	0.96
Adolescents (aPV)	FI	12	154	36	10	187	43	1.11	0.82	0.82
	NL	28	203	51	17	140	41	1.61	1.47	1.29
Adolescents (wPV)	FI	19	181	69	16	219	68	1.16	0.83	1.02
	NL	43	272	49	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	23	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

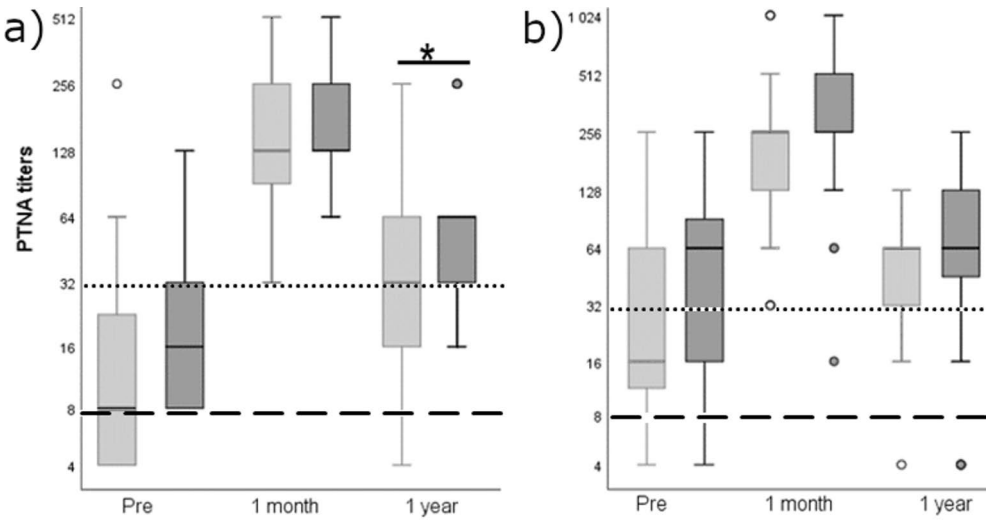

Figure 19. PTNA titer responses of Finnish **(a)** and Dutch **(b)** adolescents before vaccination, one month, and one year after vaccination based on aPV (light grey, N = 19 FI, N = 25 NL) or wPV (grey, N = 18 FI, N = 23 NL) priming background. Titer values of eight and below were considered undetectable PTNA titers (dashed line), and titers equal to and above the value of 32 as protective titers (dotted line). *$p < 0.05$.

To account for the high correlation between PTNAs and IgG and the effect of existing antibodies before vaccination, a relative model, which divided the PTNA titers by anti-PT IgG concentration, was applied to evaluate whether individuals with the same amount of anti-PT IgG antibodies neutralize PTx equally well (Figure 17, Table 5). The ratio model showed no statistically significant differences between age groups or priming backgrounds one year after vaccination in either study country (II). Interestingly, the proportion of individuals with low PTNA/IgG ratio increased post-vaccination in both countries (II). The ratios remained at the same level from one month to one year after vaccination (Wilcoxon $p = 0.84$).

5.4 The relationship between neutralization, avidity, and epitopes

Many of the samples tested for avidity were also tested for epitopes or PTNAs. Although in the BERT study, a positive correlation was noted between the initial and end outcome of avidity (Pearson R = 0.69, unpublished data) and between the initial and end outcome of PTNA titers (Pearson R = 0.48), there was no correlation between avidity and PTNAs, suggesting that strong antibody binding is not necessarily a requisite or a predictor for efficient neutralization. In this study, no overlapping data is available to evaluate the relationship between PTNAs and epitopes.

When the Danish avidity data with constant dilution ratio (Chapter 5.1.1) were compared with the epitope data, epitopes 1B7 and 1D7 correlated positively with avidity among vaccinees (Spearman R = 0.49 and 0.61, respectively) but negatively among patients (R = -0.57 and -0.72). There was no correlation between avidity and epitope 7E10. However, subjects who had high (>14% specific inhibition) 7E10 antibodies also had significantly higher avidity ($p = 0.041$, T-test). With the fixed concentration dilution approach (Chapter 5.1.4), avidity correlated negatively with epitopes 1B7 and 1D7 within patients (Spearman R = -0.52 and -0.54 respectively), but no correlation was found between vaccination responses (R = -0.23 and 0.05). Once again, there was no correlation to 7E10.

In general, epitopes measured against PTdg did not correlate with avidity after vaccination ($R < \pm 0.09$). However, only in Danish patients, 1B7-specific epitopes in PTdg were negatively correlated with avidity (R = -0.61), whereas the correlation to 1D7 was notably lower (R = -0.30) than between avidity and 1D7 in PTx, further indicating that 1D7 may be influenced by glutaraldehyde detoxification.

6 Discussion

6.1 Vaccination and infection-induced functional antibodies

The re-emergence of pertussis throughout the aPV era has given a reason to speculate that these vaccines have not successfully protected against infection or prevented further transmission of *B. pertussis*. The shift from wPV- to aPV-induced immunity in humans indicated a higher possibility of contracting the disease after aPVs [98]. The second piece of evidence is through convincing findings in various baboon model studies. Essentially, compared to infection-mediated immunity, aPV-induced immunity is inferior in preventing infection upon rechallenge. What is worse, the immunity by aPVs seems to further complicate the disease, pro-longing infection, thus making others more susceptible to the disease. [88]. Immunological data indicates that antibodies protect against disease, but Th1 and Th17-mediated immune responses are needed for bacterial clearance and long-lasting protection [88], [290], [372].

The observations in the baboon model can be tightly linked to differences in the induced mucosal immunity, in which aPV is obviously at a disadvantage. But how are the differences in immunity between aPV and wPV explainable since both are administered intramuscularly? Critical factors in the observed differences include the more expansive antigen repertoire of wPVs and differently stimulated and guided immune memory. However, in a recent report, *B. pertussis* was noted to be highly circulating in the entire Finnish population during the 1960–1970s when wPVs were used [373]. Currently, the overall situation is more complex to analyze, with individuals having a variety of mixed wPV and aPV doses and infections throughout their lives [98].

It is justified to ask – does waning immunity have anything to do with PT? And more particularly, do the chemical treatments of PT and destruction of central epitopes have a role in waning immunity? Based on the baboon model, PT antibodies alone can prevent severe disease [88], [257]. Second, in humans, a certain threshold of anti-PT antibody concentration decreases the likelihood of the disease [18]–[21], [374], [375]. Third, it is evident that individuals have vastly different quantities (and quality) of the antibody response following vaccination, but this is also the case with

infection. Fourth, there is clear scientific evidence (and the overall purpose of detoxification) that the different amino acids and (conformational) PT epitopes are affected by chemical treatments.

Following these remarks, it is evident that aPV-mediated immunity towards PT is sufficient to protect against the disease. However, the properties of antibodies may be altered due to detoxification methods. Essentially, the following should be initialized for the discussion of this thesis. Optimally, vaccination against *B. pertussis* should aim to prevent infection, further transmission of the pathogen, and severe disease and symptoms. The studies included in this thesis have the potential only to influence the later aim. In the following discussion, terms such as "protection" or "immunity," will mean protection against the disease. As specified earlier, no established correlate of protection exists for pertussis (infection or disease). Thus, the premise is whether some characteristic of anti-PT IgG antibodies would be suitable to determine (persistent) protection against the disease.

In this thesis, aPV- and infection-induced protection were studied by following different characteristics of anti-PT IgG antibodies: quantity, avidity, neutralization capability, and epitope specificity. These were analyzed with retrospective (I, III) and longitudinal vaccination studies (II), with the latter having the stricter benefit of comparing the initial response up to one year. In contrast, the sampling time varied in retrospective studies after aPV (or infection) and lacked information of existing antibody concentrations. A strength of the included studies is that they cover many age groups: infants, children, adolescents, and adults, thus giving a comprehensive look at the implications of the findings in the earlier and later stages of life. The general findings and comparison between infection and vaccination have been summarized in Table 6. The following sections (6.1.1-6.1.3) aim to recite the discussions in original publications I-III.

6.1.1 Avidity

At first, it was noted that initial antibody and detergent concentrations significantly affect the notified avidity index. Notably, the AI seems to saturate at high antibody concentration, also observed by others [349], [376]. Presumably, antibodies from individual samples should result in similar avidities regardless of how much antibody is used in a test, as the proportion of strong–weak antibodies is always the same. Contradictory interpretations were made where vaccination-induced antibodies were, at the same time, inferior, equal, or better in avidity compared to infection, depending on which samples or immunoassay was used. Therefore, an approach in which the samples were diluted to a fixed concentration of anti-PT IgG [376]–[379] was developed for this purpose. The approach was combined with various detergent concentrations to catch the full spectrum of antibody avidities [371].

Following these principles, vaccinated children were observed to have higher antibody avidity to PTx than patients. It could be logical as children have multiple vaccine doses and thus repeated encounters with PT, and the vaccines contain a higher amount of PT than what is derived from infection [259], [371], [380]. Yet, most patients should have received pertussis vaccinations in their childhood, and therefore yet another re-exposure to PT should lead to a strong avidity if any immunological memory remains.

The timing from an individual's latest vaccination was observed as a central attribute to describe the binding strength of antibodies: avidity was the highest in Danish babies (latest aPV within six months), the second highest within 5-year-olds (four years since primary vaccines), then 8–15-year-olds (4–10 years since latest booster), and finally patients with 5–13 years since booster vaccines. A similar decreasing age trend was noted between children of 1–2 years and 3–6 years in a study observing healthy individuals by Fumimoto et al. [370]. However, the finding by Fumimoto is likely influenced by overall anti-PT IgG concentrations, which were five times higher in 1–2-year-olds. Notably, these findings might contradict the general concept that antibody maturation, which develops with age, is not uniformly reflected in the notified strength of antibody avidity [343], [347], [381], [382].

Likewise, among the Danish patients, the highest avidities were noted among the younger and more recently vaccinated subjects. Astonishingly, the estimated period of 5–8 years from the latest vaccination for having a higher level of avidity upon infection is in line with what has been estimated as the period of protective immunity after aPV [98], [271]–[273]. It seems that, in the end, the avidity of antibodies persists at a higher level longer after infection [355]. Whereas after vaccination, the high avidity noted after one month may be short-lived and decrease quickly after one year (unpublished avidity data of BERT).

The variety of data analysis models and assay designs in the study of anti-PT IgG avidity makes it particularly challenging to make reliable comparisons to earlier studies [261]. At this moment, comparisons may be made strictly between the general conclusions of the studies, not absolute values. Additionally, the detailed proof in this study regarding the influence of antibody concentration on AI-values gives reason to revisit the findings of previous studies. Mainly, whether results are affected by sample selection or whether the assays can separate avidity in samples with very high concentrations of anti-PT IgG should be considered. These issues will be revisited in more detail in Chapter 6.3.

6.1.2 Epitopes

The epitope-specific anti-PT antibodies were compared between patients and children who had received either primary or booster doses of aPVs. Despite the study

groups having near equal anti-PT IgG concentrations, single doses of aPVs induced a notably weaker epitope response than infection. Three doses of aPVs, on the other hand, seem to enforce the antibody maturation more strongly, particularly towards epitopes 1D7 and 10D6. Nevertheless, the aPVs used in Finland induced considerable antibody titers to the protective epitope 1B7. Hydrogen peroxide-treated vaccines generated a higher response towards 1D7 than glutaraldehyde- and formaldehyde-treated vaccines based on quantity (I) and correlation between PTx and PTdg-induced epitopes (Table 4). The interpretation would be that hydrogen peroxide treatment preserves the 1D7-resembling epitope as opposed to that of glutaraldehyde.

Antibodies to S2 and S3 were noted mainly in patients, which reassuringly correlated with overall anti-PT IgG. Overall, out of the other tested 11 mAbs targeting S2 and S3, there was either no correlation with anti-PT IgG, or in the few more thoroughly tested S3 epitopes, no significant difference between vaccination and infection. These would suggest that 11E6 and 7E10 are distinct from any other S2/3 epitopes. Deletions or substitutions of amino acids such as Tyr102, Tyr103, or Asn105 in S2 affected toxin binding to haptoglobin or fetuin. In contrast, deletions or substitutions of Tyr102, Tyr103, or Lys105 in S3 affect binding to surface receptors on CHO cells or sialic acid. [149], [248]. These amino acids are affected, particularly by formaldehyde treatment (Table 2). Although there is no certainty detoxification would alter these specific amino acids, changes such as these to a conformational epitope would be reasonable explanations for why epitopes 11E6 and 7E10 are only found from PTx and which would have consequences to antibody function. Further studies are certainly needed to find links between antibody function and amino acid or conformational changes in PT structure.

Aside from 1B7 and 11E6, unfortunately, the exact information on which binding sites they bind on the PT surface is not available [360], [362]. Without this information, it is challenging to speculate about the connection between chemical treatments, their effects on epitope-specific residues and conformations, and further antibody function (Chapter 2.3.1.4). Nevertheless, chemical treatments affect certain epitopes more than others. It is also important to remember the “epitope-like” viewpoint of the generated data. E.g., what is indeed measured by the 1D7-targeting mAb if the region has been altered or a conformation change has occurred? What is the role of the antibodies with cross-reactive epitopes? A daunting task lies ahead to fully confirm the implications of these findings, as although antibodies would be found competitive towards a certain epitope, there is no certainty of their protective capabilities towards PTx. E.g., individual plasma blasts could be separated after infection and vaccination (including PTd or PTg), sequenced, and from unique human-induced antibodies to test their specificity towards epitopes in combination with other functional in vitro tests [365].

In the study by Sutherland et al. [359], significantly more 1B7 and 11E6 antibodies were noted in patients than in recently vaccinated adults. Why such a difference in 1B7 was not observed in the Finnish cohort could be due to differences in the age of the study subjects or previous infections. On the other hand, a difference was observed in the case of Danish children, adding speculation about whether this difference is emphasized due to the different chemical treatments. The interpretation would be that glutaraldehyde treatment affects the 1B7-resembling epitope less than hydrogen peroxide does.

Table 6. Overall conclusions regarding the comparison between infection- and vaccination-induced antibodies to PT.

Feature	Vaccination	Infection
Anti-PT IgG (IU/mL)	- High variance between individuals - $IgG_{1,4}$, IgE	- High variance between individuals - IgM, IgA, $IgG_{1,2,3}$
Epitopes (S1)	- Present: 1B7, 10D6 - Affected: (glutar-/formaldehyde) 1D7 - 1D7 less affected in hydrogen peroxide	- Present: 1B7, 1D7, 10D6
Epitopes (S2-3)	- Absent: 11E6, 7E10	- Present: 11E6, 7E10
PTNA	- High variance between individuals: high PTNAs but low IgG, and vice versa - 71% have protective levels (one year) - A decreasing trend of PTNA/IgG ratio - Influenced by existing antibodies	- High variance between individuals: high PTNAs but low IgG and vice versa - Similar PTNA/IgG ratio with aPV - Influenced by vaccination history
Avidity	- Influenced by vaccination history - Stronger than infection (within a year)	- Influenced by vaccination history

The following can be concluded: both glutaraldehyde+formaldehyde and hydrogen peroxide treatment of PT manifest in drastically less epitope specificity towards S2/3 and seem to skew the response towards other epitopes (than 1B7) in S1. Especially 1D7 in S1 appears more affected in PTdg than after hydrogen peroxide treatment. However, this remains to be confirmed by measuring epitopes to hydrogen peroxide-treated PT. It appears that multiple doses of aPVs or a greater quantity of PT in the vaccine may compensate for these lacks to some extent. The presence of impaired B-cell epitope structures may raise the amount of toxoid needed to achieve a protective antibody response [17].

6.1.3 Neutralization

The functionality of aPV-induced antibodies to neutralize PT was evaluated in different age groups in Finland and the Netherlands. An increased amount of PTNAs

were found in all age groups at one month and one-year post-vaccination, which were generally well-correlated with overall anti-PT IgG concentrations, as previous studies would indicate [261]. Additionally, it was noted that young adults had relatively weaker IgG and PTNA responses. Why? Interestingly, it was possible to pinpoint the best vaccine response among those study participants with evidence of more recent vaccination or infection, as measured by having higher than 5 or 10 anti-PT IgG IU/mL before the study. A reasonable explanation, since pertussis is the most common in younger age groups [383], more common in the Netherlands [77], [207], [208], [384], and children have had the shortest time since their latest vaccination. These groups were also the ones with the highest responses. On the other hand, older adults presumably have encountered pertussis throughout their lives. They are exposed through interacting with their grandchildren and thus could have a better immunological memory than 20–35-year-olds who have yet to build a family.

A follow-up model was considered to answer whether it is feasible to suggest that young adults produce weaker PTNAs than the other age groups. The simple model divided the PTNA titer by overall anti-PT IgG concentration. In this manner, it can be evaluated whether individuals would fall off categorically more often to either producing many antibodies and having relatively weak PTNAs, or the contrary (Figure 17). A more thorough look into this matter felt necessary since it is often cited that a high anti-PT IgG concentration does not guarantee good PTNAs, also demonstrated in this study (II). Indeed, according to this model, young adults had an equally high proportion of PTNAs per anti-PT IgG compared to other age groups one-year post-booster across both study countries. Vaccination seems to induce different quantities of antibodies in different age groups, but they have relatively similar neutralizing activity in vitro.

Nevertheless, up to 16-fold differences between individuals in PTNA titers (32-fold differences in PTNA/IgG) existed within the cohorts. On a similar note, by only looking at PTNA titers, it can be concluded that the aPV-induced antibodies' ability to neutralize PT is worse at one year than at one month. Following the proportion model instead, it can be concluded the PTNA/IgG ratios remain the same from one month to one year after vaccination, shifting the interpretation that just the quantity of IgG decreases over time. The ability of a single unit of antibodies to neutralize PT remains the same. The long-term development remains to be evaluated in further studies.

Although primary vaccination with aPV induces significantly higher PTNA titers than wPVs [221], [227], [385]–[388], in the BERT cohort there was no direct evidence of priming background having a significant influence on adolescents' booster response. Although the PTNA titers in the wPV group were higher one month and one year after vaccination in Finland, the titers were also higher before

immunization. Knowing what we do, higher pre-titers were an excellent predictor for a higher response after vaccination.

Two alternatives can be thought of. First, the wPV-primed adolescents had longer persisting antibodies and immunological memory (8–10 years since their latest vaccine) than aPV primed (6–8 years). Although antibodies after wPV decline slower [274], [275], they are of less quantity than after aPV. Nevertheless, their latest booster was an aPV, and as indicated by the BERT study [207], and other studies [265], that response declines rapidly.

The second alternative would be that wPV-primed children have been more exposed to pertussis. This idea seems contradictory to previous literature [98]. Ultimately, it is challenging to evaluate whether infections during the last 6–10 years influence the readout. Since 35% of the adolescents in the study had PTNAs ≥ 64 (near equivalent of 50 IU/mL) before the study, there is good reason to suggest that many have been infected with *B. pertussis* within a few years [60]. On the other hand, based on a study by Carlsson et al. 94% of children had detectable PTNAs four years after primary vaccines [389]. In contrast, in the BERT adolescent cohort, 89% had detectable PTNAs before the study, highlighting that antibody-mediated protection has partially waned in this population. Country-to-country variation in the background of participants regarding infections and vaccinations may have a significant effect on the readout. Another such example was the Dutch children and adolescents who had declining PTNA per anti-PT IgG ratios after vaccination, whereas Finnish children and adolescents had an increasing or constant ratio between one month and one year post-booster. This could be a reflection of how differently primed and existing memory of individuals further influences future vaccination responses.

The magnitude of the PTNA response was strongly linked with existing indications of memory, such as anti-PT IgG or PTNA concentration. This finding may have immense implications for interpreting previous studies. Signs of this were reported earlier with an aPV booster in Finnish adolescents [224], and also recently with a booster administered to pregnant women [390]. It was now possbile to establish exact quantitative limits to this phenomenon. Should one always address the background levels of antibodies? How much interpretation can be addressed to the existing memory? Based on the presented data (Figure 18), having a higher level of existing anti-PT IgG could result in a 2–8-fold higher PTNA response, regardless of age group. This phenomenon was not observed in the PTNA per anti-PT IgG ratio. Therefore, future studies should present and analyze data more explicitly regarding pre-antibody concentrations, and not just for PT, as similar effects were recently demonstrated also for other DTaP antigens [390].

Following the thesis title, it is fair to ask – what about infection and PTNAs? Although no patients were included in this study, the recent study by Zhang et al.

was performed with the same methodology, equipment, and laboratory as the Finnish PTNA readout of this study [337]. Based on a personal estimate, the ratio of PTNAs to anti-PT IgG in that study was slightly above 1.0 in young pertussis patients who had received at least three vaccine doses. In this light, aPV booster produced sufficiently high proportions of PTNAs in all age groups. Similar to vaccination, not all pertussis patients develop high PTNAs and anti-PT IgG concentrations [336], [337], [391], [392]. Similar to vaccination, a lack of immunization (= existing memory) negatively affected PTNAs in infection [228], [278], [337]. Additionally, some patients have significant increases in IgA or IgM without a noticeable rise in PTNAs. Understandably, as in serology in general, sampling timing dramatically affects how much PTNAs can be found [392].

As stated before, a correlate of protection against pertussis remains to be established. The best (and the only) evaluation of protective PTNA titers was the studies by Granström et al. demonstrating that no symptoms appeared in individuals with higher than 32 PTNA titers [374], [375]. With this in mind, in the BERT study, 29% of study participants already had less than these titers after one year post-vaccination (Figures 18 and 19). It should be kept in mind that the studies performed by Granström et al. were conducted during a time in which the pertussis vaccinations were stopped in Sweden. In addition, the purity of PT and sensitivity of the assays may differ between the studies. More extended follow-up studies are lacking in the field, with vaccination [389], [393] and infection [375] being followed at best up to two to four years.

In general, the results are difficult to compare between PTNA studies since the CHO cell assays are challenging to standardize. It is sometimes challenging to interpret the morphological changes of the cells and score the results objectively or consistently between individuals. Even when performed at the same lab, higher PTNAs were found after sixteen months compared to seven months after vaccination [388]. Essentially, the country-to-country comparisons between the presented Finnish and Dutch data may be hindered as different CHO cell lines, analysis equipment, and personnel were involved in the laboratory analysis of the samples.

Additionally, there exist numerous ways to report significant changes in PTNAs, from changes in titers, different thresholds for fold increases of PTNAs [392], PTNA/IgG [354], or arbitrarily defined proportions of samples with less than a single titer [374]. Combined with the influence of existing PTNA titers before vaccination, fold increase comparison may be even less reliable to compare with. To compensate for these challenges, an alternative, highly sensitive, and animal-free methodology could be used to evaluate PT activity [394].

6.1.4 The relationship between functional properties

The connection between antibody characteristics to PT and protection from disease in humans has not been studied extensively. It seems evident that the current aPV formulations significantly affect the full capability of the epitope specificity of the formed antibodies and likely their effectiveness. At the same time, aPVs induce a high quantity of anti-PT IgG, which have, in general, robust affinity (one month post-vaccine), highly varying PTNA/IgG ratios regardless of age, and shifting epitope specificities, depending on detoxification method and the number of doses. It is uncertain whether a low PTNA/anti-PT IgG proportion reflects the quality or function of antibodies through epitope-specificity or affinity of the induced antibodies. Alternatively, sera with low PTNA/anti-PT IgG may contain many other antibody classes that excel in other protective functions.

In this study, no correlation was observed between avidity and PTNAs, probably because the CHO cell assay was concentration-dependent, and the avidity assay had fixed concentrations (Chapter 6.3). Thus, after a fashion, their data speak slightly different languages. However, these two characteristics seem unrelated since no correlation was observed either between avidity or PTNA/IgG-ratio (close to a concentration-independent model). This can indeed be the case, as demonstrated by studies involving mAbs to PT; individual mAb clones have highly varying affinities and biological properties [313]–[315], not to mention what the situation is when evaluating heterogeneous antibody responses in humans. It would seem that strong antibody affinity is not necessarily a prerequisite or a predictor for efficient neutralization.

Comparison of single epitope-like antibodies with the other characteristics is not very feasible since the other functional tests cover all epitopes of all antibodies to PT, not just single antibodies and epitopes. Sutherland et al. did find a correlation (Pearson r = 0.45) between human-induced 1B7-like antibodies and PTNAs, including vaccination and infection samples [359]. However, this analysis has the same underlying issues. Additionally, since both PTNAs and epitopes are correlated with anti-PT IgG, it remains elusive whether all correlation values indicate the same thing: anti-PT IgG concentration.

This study observed a negative correlation between avidity and epitopes in patients, but not vaccination to S1 epitopes 1B7 and 1D7. One could thereafter speculate, whether high and low-affinity antibodies are indeed directed toward different epitopes and have different functions. And particularly, if other than S1 epitopes have higher affinities, what are their functions? Similarly, as mentioned above, the assays generate different types of data regarding anti-PT IgG dependency, and avidity evaluates all epitopes. Nevertheless, this observation should be confirmed in a concentration-independent assay setup with many more epitopes to

confirm, particularly whether high-affinity antibodies are directed to other S1 epitopes instead of 1B7 or 1D7 in infected patients (Chapter 6.3).

6.1.5 Considerations for possible diagnostic biomarkers

For the most part, this thesis has discussed the comparison of antibodies between vaccination and infection to be potential drivers for biomarkers for protection and vaccination development. However, there is an evident need for serological diagnostic assays to separate infection and vaccination, particularly in highly vaccinated populations as both induce anti-PT IgG, the only recommended and specific antigen for pertussis diagnostics. Neutralizing antibodies seem a poor candidate for this because of the high correlation to anti-PT IgG. Avidity, on the other hand, demonstrated differences between infection and recent vaccination. First, it should be presumed that anti-PT IgG concentrations over 100 IU/mL would be used as a cutoff for diagnostics [60]. Using 8 M urea avidity data from the Danish samples with such anti-PT IgG concentrations, a ROC analysis yielded an area under the curve value of 0.836, and an AI threshold limit of 25.3% resulted in a sensitivity and specificity of 90% and 75%, respectively.

Regarding epitopes, a clear candidate would include either 11E6 in S23 or 7E10 in S3 since they were explicitly induced by infection. On further inspection, 11E6 had poor diagnostic performance. On the other hand, 7E10, with a diagnostic threshold for specific inhibition of 5%, demonstrated a sensitivity and specificity of 94.4% and 63.4%.

Both avidity and epitopes indicate some potential diagnostic use in a recently (aP) vaccinated population. Regarding the avidity and epitope analyses, it can be questioned how likely it is for a subject with only one month after the vaccination to have an infection. Thus, the diagnostic performance can be better or worse for samples one year after vaccination. Likewise, there is limited information about the sampling timing since the beginning of symptoms in the included patient samples. Still, these samples would likely match the typical timing to seek diagnostic testing.

6.2 Indications of waning immunity

Multiple aspects throughout the studies pinpointed factors of waning antibody-mediated immunity after aPV. These were generally attributed to too long intervals from the latest vaccination. On the contrary, in the BERT cohort, the mucosal antibody [59], B-cell [395], IgG, and PTNA responses (II) were higher in participants with evidence of prior infection. A recent study involving pregnant women noted a similar positive effect on IgG, PTNA, and B-cell responses following vaccination [390]. These results demonstrate that preserving long-term memory is

central to the immune response's success after immunization or infection to PT. In Table 7, the general observations influencing vaccine and infection responses and their implication for waning immunity from the previous literature and this study have been outlined. The message seems clear: more frequent boosting is required to upkeep aPV-induced immunity, or new types of vaccines are needed to generate long-term protection.

As a brief overview, the more specific notions were the following. With epitopes, it was noted that infants receiving three consecutive doses have an increased response to multiple epitopes, including particularly S1-specific antibodies. Children who had a longer time from their latest vaccines and received only a single dose had fewer of these epitope-specific antibodies.

Table 7. Overall conclusions regarding factors influencing vaccination and infection responses, as well as waning immunity.

Feature	Indications of quickly waning immunity	Factors influencing vaccination or infection responses
Anti-PT IgG (IU/mL)	- Low antibody concentrations already one year after vaccination - Low antibody concentrations before vaccination in many age groups - Poor vaccination responses follow in individuals with low pre-vaccine anti-PT IgG	+ Previous infections + Recency of vaccination (age) + >10 IU/mL pre-vaccination a predictor for a stronger response + Number of vaccine doses received + Quantity of PT in the vaccine
Epitopes	- Absence of epitope-specific antibodies on S2 and S3 after vaccination - Many individuals have no response at all to protective epitopes 1B7 or 11E6	+ Number of vaccine doses received + Quantity of PT in the vaccine - Detoxification methods
PTNA or PTNA/IgG	- Low protective levels one year post-vaccine - Low titers before vaccination in general - Many individuals with an indication of being infected within a few years pre-study	+ Influenced by previous infections and recency of vaccination + >10 IU/mL pre-vaccination a predictor for a stronger response
Avidity	- >8 years since the latest vaccination leads to a weaker avidity response upon infection	+ Recency of vaccination + Amount of PT encountered

It was noted that the shorter the period from the last aPV, the better the avidity responses at the subsequent PT-antigen exposure, whether via vaccination or infection. Although age, in general, affects the overall capability of antibody maturation [343], [347], [381], [382], results obtained in this study instead highlight the significance of multiple vaccination doses and the recency of vaccination (or reoccurring exposure in general) to avidity, epitopes, and neutralizing antibodies in children, adolescents, and adults (Table 3) [396]–[398].

In general, with anti-PT IgG or PTNAs, the absolute increase after aPV in these respective levels was related to pre-existing anti-PT IgG concentrations. The induced PTNA titers were significantly higher in participants with a pre-booster concentration of at least 10 IU/mL anti-PT IgG antibodies. Regardless of age, individuals with less than 10 IU/mL PT antibodies would have required more frequent boosting or a second booster dose to reactivate immunological memory to attain higher antibody levels (for a longer time). Maintaining respectable antibody titers may be emphasized in the future because the PTx used in the vaccines is isolated from ptxP1 strains, whereas recently isolated strains carry the ptxP3 allele. The ptxP3 strains produce higher amounts of PTx and were demonstrated to be more virulent in a mouse infection model. [399], [400]. The increase of PTx production may thus be reflected in the waning of aPV-induced immunity.

No substantial evidence could be attributed to differences between adolescents' aPV or wPV priming backgrounds regarding waning immunity: both cohorts had statistically similar antibody concentrations before and after the aPV booster. However, wPV-primed adolescents appeared to have higher GMC before the study, and as has been now often stated, this was a predictor for a good response. This could be noted at a month post-vaccine. It is a bit contradictory to the hypothesis that individuals with wPVs would encounter more pertussis. At least based on the quickly decreasing anti-PT IgG kinetics demonstrated in the BERT study and by others [265], this high pre-study antibody concentrations (Table 5, Chapter 6.1.2) would be unlikely to be found after 6–10 years without naturally encountering *B. pertussis*. The research setting should preferably be repeated with a larger cohort.

Long-lasting memory from previous exposure to *B. pertussis* or vaccination may present a challenge for evaluating vaccine responses and studying related background factors. Generally, this may influence how reliably we can compare different age groups or cross-country data with different relative seroprevalences of the disease. To minimize these background effects, a feasible approach for studying antibody characteristics could derive from concentration-independent assays.

6.3 Concentration-independent assays

Multiple studies have shown that both avidity [261], [350], [370], [401] and PTNAs correlate with anti-PT IgG [261]. However, a correlation can sometimes be challenging to find due to a meager quantity of antibodies or a low difference in antibody concentrations [349], [351], [354]. It was thoroughly demonstrated with the avidity study (III) what issues the analyses face if functional assays do not consider overall anti-PT IgG concentration. To list a few, these were: study population-dependent conclusions, high intra-population variation, and saturation of readout. These were solved with careful planning and optimization for experimental

conditions, which led to normally distributed population data without signal saturation. The different epitopes (Figure 13) and PTNAs (III, Chapter 5.3) were also well-correlated with anti-PT IgG and are thus under the influence of similar factors: differently selected subpopulations could yield different results, depending on what anti-PT IgG concentrations are found in the population of interest. This may create uncertainty about what the assays measure: individual properties or another, more complicated way to measure anti-PT antibody concentrations.

Following these conclusions, also in the epitope study, the slight differences in the GMCs of the study groups may influence the results. If the patient and vaccination cohorts were pooled from Denmark and Finland, the patients had significantly higher anti-PT IgG concentrations ($p < 0.01$). Nevertheless, even with a concentration bias, it seems evident that some epitopes are affected by chemical treatments more than others. In the future, either fixed concentration dilutions or analysis based on dilution series of sera and estimation of 50% IC values [359] could be used. However, the later type of assay has identical issues to avidity: the cost and workload of the assays increase with excessive dilution series exponentially the more epitopes one wishes to study.

What was attempted with the PTNA/IgG ratio model was, after a fashion, to consider the influence of IgG concentration of study populations retrospectively. A concern in the model was how low anti-PT IgG concentrations (< 2 IU/mL) could extrapolate the ratio values, particularly in samples before vaccination (N = 38). Indeed, the MIA assay can quantify as low as 0.2 IU/mL [52]. However, excluding these samples did not significantly affect the outcome of GM values of individual study groups. Another issue with the PTNA assay overall is the readout's less sensitive (visual) interpretation. There is always a natural risk of a two-fold difference in reporting between individual wells. Preferably, PTNAs would be evaluated with a titration series of PT in a fixed antibody amount instead of titration of antibodies. This method would have a similar challenge of the sensitivity of two-fold dilutions series of PT and in the reliability of direct dilutions of sera. Alternatively, a simplified assay design could include testing of threshold levels to demonstrate semi-quantification: are there enough PTNAs (and protection) or not? Presumably, if a protective level of PTNAs could be demonstrated, a standardized test would only need to include two dilutions around the protective threshold [e.g., 1:16 and 1:32 dilution, if 32 titers (1:32 dilution) is selected as the protective cutoff.]

There is a clear indication for future studies to present and analyze data more explicitly regarding the subjects' pre-antibody levels to visualize the effect of subject background on reported vaccination responses. These further developments may aid in studying and recognizing those individuals with high antibody concentration and low neutralization capacity/avidity/epitopes or vice versa. This would be a further tool to evaluate who are in reality protected against the disease.

6.4 Limitations of the study

In addition to apparent challenges regarding assay methodology and sampling (Chapter 6.3), numerous factors surrounding the study subjects affect the interpretation of the obtained results and comparisons. At times, particularly for subgroup analyses, the number of subjects in the study is limited. This limitation was partly artificial since threshold values were included beforehand for samples to have in the tests (> 50 IU/mL and > 25 IU/mL anti-PT IgG for studies I and III).

No patients from Finland or other European countries were included to analyze PTNAs, a population that would have had matching age, pertussis seroprevalence, and childhood vaccinations. It is uncertain whether study subjects have had pertussis infections in the past, and the presence of anti-PT IgG (and partly IgA) was used as the only predictor to evaluate the past. Culture or PCR combined with clinical confirmation of pertussis would be a preferable background to define infection. As demonstrated in this work, a lack of knowledge regarding previous infection may significantly impact the interpretation of subsequent immune responses.

In general, the sampling times of vaccinated subjects were well-defined in the study. In contrast, serologically diagnosed patients' sampling timing may vary from weeks to possibly even years after the infection. Unfortunately, no exact data was available regarding the vaccination history of the Finnish patients. This study estimated responses shortly after vaccination up to a year. Long(er) follow-up studies would be needed to confirm any noted implications for waning immunity.

Although the research setting for evaluating vaccination background in the BERT study was thought to be simple, the vaccination background in the adolescent age group appeared quite divergent, making the results hard to interpret. Additionally, the subjects had received different numbers of doses and slightly different schedules between the two countries. Ultimately, this may have a minor effect since, in previous studies, no differences were noticed in anti-PT IgG or PTNAs between children who received four or three doses of vaccines in childhood [334], [389]. Nevertheless, these aspects may, in return, influence the likelihood of being infected by pertussis.

In this study, other functional antibodies e.g., serum bactericidal antibodies or mucosal antibodies were not determined. The aPVs also induce antibodies to FHA, PRN, or FIM 2/3, and the possible relationships of these antibodies were not cross-analyzed with PT, or their avidity, neutralizing capacity, and epitopes. Existing antibodies to these vaccine antigens may also influence the magnitude of response towards PT [402].

6.5 Considerations for future vaccines

Licensure of vaccines typically involves a rigorous and multi-step process to ensure their safety and efficacy. Particularly, the manufacturing processes for combinatory dtap vaccines are one of the most complex ones. Since the lack of an effective correlate of protection for pertussis, two different, and rather laborious, animal assays are routinely used to measure and predict their expected efficacy against pertussis. [219]. Antibody responses are generally used to assess the ability of many vaccines to protect against the target disease. For pertussis vaccines, as discussed in Chapter 6.1, this is currently sufficient to demonstrate protection against the disease, but not against infection and to prevent the spread of pertussis. Are either epitopes, avidity, or neutralization alternative hallmarks and biomarkers of successful vaccination? Preserving critical epitopes appears central to protection, as demonstrated in the baboon models [364]. Instead of solely relying on 1B7, which still is a reasonable starting point, identifying alternative epitope-targeting mAbs could be considered worthwhile. Alternatively, as it is routinely checked that PT is properly inactivated in the vaccines [328], affected epitopes could be used to qualify for successful vaccination, either their existence (e.g., 1B7) or their absence, such as for an epitope covering the genetically detoxified vaccines amino acids Arg9 and Glu129 [250]. To practically implement such analysis, certainly, high throughput assays are needed. In this case, peptide pools consisting of PT subunits could be thought of to define critical epitopes, although they lack cross-subunit conformational structures of typical PT epitopes.

Due to the inaccurate nature of defining epitopes in the whole PT in the competitive assay format, there is a need to develop individual PT subunit-specific assays, to reduce the impact of cross-reacting antibodies. This approach would also be beneficial to evaluate the antibodies' neutralization capability or affinity to individual subunits. Although Sutherland et al. demonstrated that PTg withholds more similar epitopes to PTx, it remains of interest to characterize what PTg-induced epitope-specific antibody responses look like [359].

Important epitopes already characterized in S1 and S2/3 within human infections and in mouse and baboon models suggest different inactivation strategies to block PT's receptor binding and catalytic pathways [257]. They represent potential as serological correlates for next-generation vaccine development or even as biomarkers for separating infection- and vaccination-induced anti-PT antibodies. Extensive epitope mapping of the human antibody response is still lacking [366]. Further identified epitopes could be necessary to fully grasp epitope-specific antibodies' potential for vaccine evaluation and develop therapeutic antibodies against pertussis.

Regarding avidity, a clear interest exists for future vaccines to demonstrate and focus on developing long-preserving affinity after vaccination. These studies are

lacking in the field. The experience in this study covered only the short-term response at one month; however, up to 5 or 10 years would be central to evaluating long-term protection. Nevertheless, this study successfully demonstrated what potentially happens after such a period upon the next booster vaccination or infection. As demonstrated, there is currently a clear positive direction for the development of avidity if vaccines are administered more often. Preferably, the interval of booster vaccines should match the needs of tetanus and diphtheria (every ten years), as instead, avidity data presented here suggests having pertussis boosters every 5–8 years is beneficial. Although the assay is currently laborious to perform, once a sufficient detergent concentration is defined to be measured in a set population, only a single detergent concentration could be used, which would make the method easier to perform. As a general notion, this thesis presents many considerations of suggested assays, analysis, and technical formats for possible vaccine evaluation (Chapter 6.3). As such, this study presents clinically relevant cutoffs to define high avidity antibodies concerning infection, which could be used to validate vaccination responses in the future.

No research has thus far convincingly demonstrated any difference in PTNAs that would not be visible with just plain anti-PT IgG at a population level. The role of further analysis models, such as those suggested by Fumimoto et al. [370] and this study, may be essential to revisiting old and newly conducted research. E.g., in the recent study where the novel nasal pertussis vaccine was evaluated for PTNAs, these analytical viewpoints were not considered [255]. Although the authors pinpoint that the PTNA levels induced by the vaccine are equal with aPVs, the vital notion that this is achieved with fewer overall anti-PT IgGs is left out. Similarly, PTg was shown to have a higher PTNA seroconversion than PTdg vaccines at one month [330], [403], although being formulated at a smaller dose of PT [404].

Nevertheless, there remains an essential narrative for evaluating PTNA capability as a small proportion of patients and vaccine recipients do not induce neutralizing antibodies. In this regard, a straightforward vaccination “failure” could provide a reason for a subsequent additional vaccine dose. Additionally, this study demonstrated that general booster timing recommendations might not be ideal for everyone: to achieve the highest and long-preserving avidity, anti-IgG or PTNA concentrations, some memory towards PT should remain. It could be considered whether an individual would need a second dose to compensate for lacking memory already one month after the initial dose. It can be concluded that PTNAs are laborious to conduct for vaccine evaluation at a population level and provide limited information in addition to anti-PT IgG; however, at an individual level, it may pose significant implications on the success of vaccination.

Altogether, the findings presented in this thesis stress the importance of determining functional antibody characteristics after pertussis vaccinations. Larger

studies are needed to prove whether or not a connection exists between the different antibody characteristics. Additionally, sufficient, justified, and long-lasting thresholds for these characteristics reflecting protection against the disease, are needed to utilize them for future vaccination development. In future work, epitope mapping, immune profiling, utilization of epitope databanks, and whole-genome sequencing of antibody-producing B-cells will likely be instrumental in determining whether protection against PT requires antibodies binding discrete epitopes versus a more diverse antibody response [361], [405]. In addition, the constant evolution of *B. pertussis* may lead to crucial changes in PT epitopes [406]–[409] and therefore hinder vaccination-induced protection. For now, before the arrival of the next generation of vaccines, the many implications found in this study regarding waning immunity stress that the currently used aPVs should be administered more frequently to guarantee immunity and protection against the disease.

7 Summary and Conclusions

Since implementing pertussis vaccination programs worldwide, the overall prevalence of *B. pertussis* has continued to decrease. Despite these actions, the bacterium remains circulating in the population, and the latest shift from wPVs to aPVs has not significantly improved the situation. Worrisomely, the scientific evidence seems to prove that aPV-induced protection is insufficient to prevent the transmission of the bacteria. Further studies are needed to identify the current and future shortcomings of vaccines. Second, it is pivotal to estimate what level of protection would be relevant to be achieved from an immunization, with studies observing infection-related immunity. These actions will give direction for the future development of vaccines.

Although cell-mediated protection against the disease is central to consider regarding these factors, in the end, antibodies against *B. pertussis* antigens are the product of T-cell to B-cell-mediated pathogen protection and have a significant role in protecting and preventing severe symptoms. The quantity of anti-PT antibodies alone does not reflect long-lasting immunity, and functional antibody assays should be performed to evaluate immune responses. However, a clear understanding of a proper functional response is lacking. Functional characteristics of pertussis toxin binding antibodies, such as binding strength, binding location, and function, may be an essential piece of the puzzle to find what could be improved for the next generation of vaccines to attain effective and long-lasting protection against pertussis. The findings of this thesis underline the following:

I Antibody-recognized epitopes of PT differ depending on whether an individual has encountered PTd through acellular vaccination or PTx in its native confirmation through an infection—notably, vaccination induced few antibodies targeting certain S2/3 or S3 epitopes. The quantities of antibody-recognized epitopes were influenced by the detoxification method of PT, the amount of PT used in a vaccine, and the number of consecutive vaccination doses received.

II aPV induced PTNAs in children, adolescents, young, and older adults one month after vaccination, which remained at least two-fold higher one year

post-booster than before the booster. The most substantial increase in PTNAs was observed in participants with higher anti-PT IgG concentrations before boosting. This suggests that the existing memory of PT is critical for an efficient PTNA response.

III A fixed serum dilution method together with a wide detergent concentration range was reliable for evaluating antibody avidity between samples with highly varying anti-PT IgG antibody concentrations. Avidity after a recent vaccination was significantly higher than after a recent infection. The recency of the latest vaccination boosting was found to affect avidity positively on the subsequent PT-antigen exposure with either vaccination or infection.

IV The apparent lack of strong associations between avidity, neutralizing capability, and epitopes indicates that these are individual characteristics and should be studied separately. It remains essential to further develop assay and analysis formats to improve the interpretation of the different antibody characteristics.

Acknowledgements

This work was carried out at the Institute of Biomedicine, Research Centre for Infections and Immunity, University of Turku, and the Doctoral Programme in Clinical Research (DPCR) in 2018–2023. I express my deep gratitude to the grand pertussis trio of UTU, whose interest in supporting our research group and the overall study of pertussis over the last decades, particularly in Finland and Europe, is unparalleled. First, my humble gratitude to my thesis supervisor Prof. Qiushui He, with whom I am equally often happy to agree and disagree on matters. Nevertheless, our discussions have always led to the best results. Thank you for the opportunity and goodwill to have me in the group since 2016. Second, Prof. Jussi Mertsola, thank you for always having a high interest in all my research efforts and for your support and advice. I am grateful to both of you for the opportunities your life-long dedication has provided me (and others). The extent has been extraordinary: from large clinical projects, international collaboration, trips, training, and rapid new projects. I am grateful for all these valuable, challenging, and fun experiences.

Third, my sincere thanks to Alex Barkoff for sharing the everyday challenges of science and making the Periscope study a manageable experience. Thank you for always sharing your time, no matter your hurry. My warm thanks to all pertussis group members: Johanna Teräsjärvi, Elina Tenhu, Kaisu Kaistinen, Tuula Rantasalo, Lauri Ivaska, Raakel Luoto, Päivi Haaranen, and Liisa Lund for your selfless work for the day-to-day contributions of our lab and Periscope. I want to thank the many students who have joined and put their open-minded efforts into this work: Thanks, Niina Ahvenainen, Carita Rautanen, and Joonatan Mäkelä. It has been a joy to supervise your progress and do research together.

Without the help of the laboratory staff at Mikro and later at Medisiina D, it would be impractical to do anything in the lab. Thank you all for your daily efforts in keeping our tight work environment up and running. Not only in the lab, but you are also the cornerstone of maintaining the meaningful, supportive, and encouraging atmosphere of which we all can feel a part. I am thankful for all the fellow Ph.D. candidates and fellow researchers with whom it has always been easy to share the joys and nuances of studies, research, and the endless university bureaucracy. Likewise, I am grateful to all my colleagues at the Department of Biotechnology

with whom I have had the pleasure of sharing the last few years in teaching and research while writing this thesis. Particularly, I thank Teppo Salminen for initially introducing and bridging together a long collaboration between point-of-care diagnostics and pertussis. Overall, there are so many of you to thank in person that it would fill half of this page. Thus, to address the matter briefly:

Thank you.

I want to acknowledge my co-authors and collaborators abroad: Tine Dalby, Charlotte Sværke Jørgensen, Kurt Fuursted, Kevin Markey, Pauline Versteegen, Pieter van Gageldonk, and Guy Berbers, with whom I have not only had the privilege to share expertise and learn from but also share many meetings in person, casual discussions, lunches, and good drinks! I want to thank the pre-examiners of this thesis, Minna Hankaniemi and Hanna Jarva for their good advice and thoughtful orientation toward this work.

This work was mainly 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 program; the European Federation of Pharmaceutical Industries and Associations; and the Bill & Melinda Gates Foundation.

Many foundations and grants financially supported my work throughout the years. The sheer abundance of support has always been a source of admiration for me: Thank you, Päivi and Sakari Sohlberg Foundation, The Finnish Cultural Foundation Varsinais-Suomi Regional Fund, Tampere Tuberculosis Foundation, Alfred Kordelin Foundation, Juhani Aho Foundation, Väinö and Laina Kivi Foundation, Turku University Foundation, University of Turku, The DPCR and PGE doctoral programmes, and the Special Governmental Fund for University Hospital (EVO) for making this research possible.

I am ever grateful to my parents for always supporting my efforts. Having a little brother to always rely on is a safe comfort I can acknowledge daily. I express my humble gratitude to my wife, Elisa. Your diligence for your work, our family, and yourself are my greatest inspiration. Our family brings the greatest joy in my life.

Lieto, 12.1.2024

Aapo Knuutila

References

[1] World Health Organization, 2015. Pertussis vaccines: WHO position paper. Weekly Epidemiol Record 2015;90(35):443–460.

[2] Halperin, S.A., 2007. The control of pertussis--2007 and beyond. N Engl J Med 356, 110–113.

[3] Yeung, K.H.T., Duclos, P., Nelson, E.A.S., Hutubessy, R.C.W., 2017. An update of the global burden of pertussis in children younger than 5 years: a modelling study. Lancet Infect Dis 17, 974–980.

[4] Elomaa, A., He, Q., Minh, N.N., Mertsola, J., 2009. Pertussis before and after the introduction of acellular pertussis vaccines in Finland. Vaccine 27, 5443–5449.

[5] Spokes, P.J., Gilmour, R.E., 2010. NSW Annual Vaccine-Preventable Disease Report, 2009. N S W Public Health Bull 21, 197–209.

[6] Mooi, F.R., Van Der Maas, N. a. T., De Melker, H.E., 2014. Pertussis resurgence: waning immunity and pathogen adaptation - two sides of the same coin. Epidemiol Infect 142, 685–694

[7] Celentano, L.P., Massari, M., Paramatti, D., Salmaso, S., Tozzi, A.E., EUVAC-NET Group, 2005. Resurgence of pertussis in Europe. Pediatr Infect Dis J 24, 761–765.

[8] Mattoo, S., Cherry, J.D., 2005. Molecular pathogenesis, epidemiology, and clinical manifestations of respiratory infections due to Bordetella pertussis and other Bordetella subspecies. Clin Microbiol Rev 18, 326–382.

[9] Ausiello, C.M., Lande, R., Urbani, F., la Sala, A., Stefanelli, P., Salmaso, S., Mastrantonio, P., Cassone, A., 1999. Cell-mediated immune responses in four-year-old children after primary immunization with acellular pertussis vaccines. Infect Immun 67, 4064–4071.

[10] Hallander, H.O., Andersson, M., Gustafsson, L., Ljungman, M., Netterlid, E., 2009. Seroprevalence of pertussis antitoxin (anti-PT) in Sweden before and 10 years after the introduction of a universal childhood pertussis vaccination program. APMIS 117, 912–922.

[11] Hendrikx, L. H., Oztürk, K., de Rond, L.G.H., Veenhoven, R.H., Sanders, E.A.M., Berbers, G.A.M., Buisman, A.-M., 2011. Identifying long-term memory B-cells in vaccinated children despite waning antibody levels specific for Bordetella pertussis proteins. Vaccine 29, 1431–1437.

[12] Mahon, B.P., Brady, M.T., Mills, K.H., 2000. Protection against Bordetella pertussis in mice in the absence of detectable circulating antibody: implications for long-term immunity in children. J Infect Dis 181, 2087–2091.

[13] Edelman, K., He, Q., Makinen, J., Sahlberg, A., Haanpera, M., Schuerman, L., Wolter, J., Mertsola, J. 2007. Immunity to pertussis 5 years after booster immunization during adolescence. Clin Infect Dis 44, 1271-7

[14] Jacquet, J.M., Bégué, P., Grimprel, E., Reinert, P., Sandbu, S., Silfverdal, S.A., Faldella, G., Nolan, T., Lambert, S., Richmond, P., Marshall, H., Roberton, D., Schuerman, L., 2006. Safety

and immunogenicity of a combined DTPa-IPV vaccine administered as a booster from 4 years of age: a review. Vaccine 24, 2440–2448.

[15] Zepp, F., Heininger, U., Mertsola, J., Bernatowska, E., Guiso, N., Roord, J., Tozzi, A.E., Van Damme, P., 2011. Rationale for pertussis booster vaccination throughout life in Europe. Lancet Infect.Dis. 11, 557–570.

[16] Zhang, L., Prietsch, S.O.M., Axelsson, I., Halperin, S.A., 2014. Acellular vaccines for preventing whooping cough in children. Cochrane Database Syst Rev 2014, CD001478.

[17] Ibsen, P.H., 1996. The effect of formaldehyde, hydrogen peroxide and genetic detoxification of pertussis toxin on epitope recognition by murine monoclonal antibodies. Vaccine 14, 359–368.

[18] Cherry, J.D., Gornbein, J., Heininger, U., Stehr, K., 1998. A search for serologic correlates of immunity to Bordetella pertussis cough illnesses. Vaccine 16, 1901–1906.

[19] Plotkin, S.A., 2010. Correlates of protection induced by vaccination. Clinical and vaccine immunology : CVI 17, 1055–1065.

[20] Storsaeter, J., Hallander, H.O., Gustafsson, L., Olin, P., 1998. Levels of anti-pertussis antibodies related to protection after household exposure to Bordetella pertussis. Vaccine 16, 1907–1916.

[21] Taranger, J., Trollfors, B., Lagergård, T., Sundh, V., Bryla, D.A., Schneerson, R., Robbins, J.B., 2000. Correlation between pertussis toxin IgG antibodies in postvaccination sera and subsequent protection against pertussis. J Infect Dis 181, 1010–1013.

[22] PERISCOPE Consortium, 2019. PERISCOPE: road towards effective control of pertussis. Lancet Infect.Dis.

[23] Hallander, H.O., 1999. Microbiological and serological diagnosis of pertussis. Clin.Infect.Dis. 28 Suppl 2, 99.

[24] He, Q., Viljanen, M.K., Arvilommi, H., Aittanen, B., Mertsola, J., 1998. Whooping cough caused by Bordetella pertussis and Bordetella parapertussis in an immunized population. JAMA 280, 635–637.

[25] Hoppe, J.E., 1999. Update on respiratory infection caused by Bordetella parapertussis. Pediatr Infect Dis J 18, 375–381.

[26] Leber, A.L., 2014. Pertussis: relevant species and diagnostic update. Clin.Lab.Med. 34, 237–255.

[27] Mekalanos, J.J., 1992. Environmental signals controlling expression of virulence determinants in bacteria. J Bacteriol 174, 1–7.

[28] Paddock, C.D., Sanden, G.N., Cherry, J.D., Gal, A.A., Langston, C., Tatti, K.M., Wu, K.-H., Goldsmith, C.S., Greer, P.W., Montague, J.L., Eliason, M.T., Holman, R.C., Guarner, J., Shieh, W.-J., Zaki, S.R., 2008. Pathology and pathogenesis of fatal Bordetella pertussis infection in infants. Clin Infect Dis 47, 328–338.

[29] Heininger, U., Cherry, J.D., Stehr, K., Schmitt-Grohé, S., Uberall, M., Laussucq, S., Eckhardt, T., Meyer, M., Gornbein, J., 1998. Comparative Efficacy of the Lederle/Takeda acellular pertussis component DTP (DTaP) vaccine and Lederle whole-cell component DTP vaccine in German children after household exposure. Pertussis Vaccine Study Group. Pediatrics 102, 546–553.

[30] Crowcroft, N.S., Pebody, R.G., 2006. Recent developments in pertussis. Lancet 367, 1926–1936

[31] Kerr, J.R., Matthews, R.C., 2000. Bordetella pertussis infection: pathogenesis, diagnosis, management, and the role of protective immunity. Eur J Clin Microbiol Infect Dis 19, 77–88.

[32] Heininger, U., 2001. Recent progress in clinical and basic pertussis research. Eur J Pediatr 160, 203–213

[33] Tiwari, T., Murphy, T.V., Moran, J., National Immunization Program, CDC, 2005. Recommended antimicrobial agents for the treatment and postexposure prophylaxis of pertussis: 2005 CDC Guidelines. MMWR Recomm Rep 54, 1–16.

[34] Gregory, D.S., 2006. Pertussis: a disease affecting all ages. Am Fam Physician 74, 420–426.

[35] Hewlett, E.L., Edwards, K.M., 2005. Clinical practice. Pertussis--not just for kids. N Engl J Med 352, 1215–1222.

[36] von König, C.H.W., Halperin, S., Riffelmann, M., Guiso, N., 2002. Pertussis of adults and infants. Lancet Infect Dis 2, 744–750.

[37] Cornia, P.B., Hersh, A.L., Lipsky, B.A., Newman, T.B., Gonzales, R., 2010. Does this coughing adolescent or adult patient have pertussis? JAMA 304, 890–896.

[38] Mertsola, J., Kuronen, T., Turunen, A., Viljanen, M.K., Ruuskanen, O., 1984. Diagnosis of pertussis. J Infect 8, 149–156.

[39] Paisley, R.D., Blaylock, J., Hartzell, J.D., 2012. Whooping cough in adults: an update on a reemerging infection. Am J Med 125, 141–143.

[40] Schellekens, J., von König, C.-H.W., Gardner, P., 2005. Pertussis sources of infection and routes of transmission in the vaccination era. Pediatr Infect Dis J 24, S19-24.

[41] Korppi, M., Hiltunen, J., 2007. Pertussis is common in nonvaccinated infants hospitalized for respiratory syncytial virus infection. Pediatr Infect Dis J 26, 316–318.

[42] Wheeler, J.G., Simmons, A.L., 2005. Pertussis update. Pediatr Infect Dis J 24, 829–830.

[43] Weinberger, M., Abu-Hasan, M., 2007. Pseudo-asthma: when cough, wheezing, and dyspnea are not asthma. Pediatrics 120, 855–864.

[44] Greenberg, D., Bamberger, E., Ben-Shimol, S., Gershtein, R., Golan, D., Srugo, I., 2007. Pertussis is under diagnosed in infants hospitalized with lower respiratory tract infection in the pediatric intensive care unit. Med Sci Monit 13, CR475-480.

[45] Lewis, F.A., Gust, I.D., Bennett, N.M., 1973. On the aetiology of whooping cough. J Hyg (Lond) 71, 139–144.

[46] CDC, 2023a. https://www.cdc.gov/pertussis/clinical/diagnostic-testing/specimen-collection-diagnosis.htmL, accessed 18.8.2023

[47] Loeffelholz, M.J., Thompson, C.J., Long, K.S., Gilchrist, M.J., 1999. Comparison of PCR, culture, and direct fluorescent-antibody testing for detection of Bordetella pertussis. J Clin Microbiol 37, 2872–2876.

[48] Edelman, K., Nikkari, S., Ruuskanen, O., He, Q., Viljanen, M., Mertsola, J., 1996. Detection of Bordetella pertussis by polymerase chain reaction and culture in the nasopharynx of erythromycin-treated infants with pertussis. Pediatr Infect Dis J 15, 54–57.

[49] Fry, N.K., Tzivra, O., Li, Y.T., McNiff, A., Doshi, N., Maple, P.A.C., Crowcroft, N.S., Miller, E., George, R.C., Harrison, T.G., 2004. Laboratory diagnosis of pertussis infections: the role of PCR and serology. J Med Microbiol 53, 519–525.

[50] Muyldermans, G., Soetens, O., Antoine, M., Bruisten, S., Vincart, B., Doucet-Populaire, F., Fry, N.K., Olcén, P., Scheftel, J.M., Senterre, J.M., van der Zee, A., Riffelmann, M., Piérard, D., Lauwers, S., 2005. External quality assessment for molecular detection of Bordetella pertussis in European laboratories. J Clin Microbiol 43, 30–35.

[51] Riffelmann, M., Wirsing von König, C.H., Caro, V., Guiso, N., Pertussis PCR Consesus Group, 2005. Nucleic Acid amplification tests for diagnosis of Bordetella infections. J Clin Microbiol 43, 4925–4929.

[52] van Gageldonk, P.G., van Schaijk, F.G., van der Klis, F.R., Berbers, G.A., 2008. Development and validation of a multiplex immunoassay for the simultaneous determination of serum antibodies to Bordetella pertussis, diphtheria and tetanus. J.Immunol.Methods 335, 79–89.

[53] He Q., Barkoff, A.M., Mertsola, J., Glismann, S., Bacci, S., European Bordetella expert group, & European surveillance network for vaccine-preventable diseases, 2012. High heterogeneity in methods used for the laboratory confirmation of pertussis diagnosis among European countries, 2010: integration of epidemiological and laboratory surveillance must include standardisation of methodologies and quality assurance. Eur Communicable Dis Bull 17, 20239–0.

[54] Håkansson, S., Sundin, C.G., Granström, M., Gästrin, B., 1984. Diagnosis of whooping cough--a comparison of culture, immunofluorescence and serology with ELISA. Scand J Infect Dis 16, 281–284.

[55] Friedman, R.L., 1988. Pertussis: the disease and new diagnostic methods. Clin Microbiol Rev 1, 365–376.

[56] Reizenstein, E., Hallander, H.O., Blackwelder, W.C., Kuhn, I., Ljungman, M., Mollby, R., 1995. Comparison of five calculation modes for antibody ELISA procedures using pertussis serology as a model. J.Immunol.Methods 183, 279–290.

[57] Goodman, Y.E., Wort, A.J., Jackson, F.L., 1981. Enzyme-linked immunosorbent assay for detection of pertussis immunoglobulin A in nasopharyngeal secretions as an indicator of recent infection. J.Clin.Microbiol. 13, 286–292.

[58] Cherry, J.D., Grimprel, E., Guiso, N., Heininger, U., Mertsola, J., 2005. Defining pertussis epidemiology: clinical, microbiologic and serologic perspectives. Pediatr Infect Dis J 24, S25-34.

[59] van Schuppen, E., Fröberg, J., Venkatasubramanian, P.B., Versteegen, P., de Graaf, H., Holubová, J., Gillard, J., van Gageldonk, P.G.M., Joosten, I., de Groot, R., Šebo, P., Berbers, G.A.M., Read, R.C., Huynen, M.A., de Jonge, M.I., Diavatopoulos, D.A., 2022. Prior exposure to B. pertussis shapes the mucosal antibody response to acellular pertussis booster vaccination. Nat Commun 13, 7429.

[60] Guiso, N., Berbers, G., Fry, N.K., He, Q., Riffelmann, M., Wirsing von Konig, C.H., EU Pertstrain group, 2011. What to do and what not to do in serological diagnosis of pertussis: recommendations from EU reference laboratories. Eur.J.Clin.Microbiol.Infect.Dis. 30, 307–312.

[61] Burstyn, D.G., Baraff, L.J., Peppler, M.S., Leake, R.D., St Geme, J., Manclark, C.R., 1983. Serological response to filamentous hemagglutinin and lymphocytosis-promoting toxin of Bordetella pertussis. Infect Immun 41, 1150–1156.

[62] Chen, Z., Liu, X., Zhang, Y., Peng, X., Zhang, N., Chen, N., Li, Y., He, Q., 2023. Evaluation of serum anti-pertussis toxin IgA antibodies for the diagnosis of Bordetella pertussis infection in young children. Journal of Infection and Public Health 16, 1167–1173.

[63] Fumimoto, R., Otsuka, N., Kamiya, H., Sunagawa, T., Tanaka-Taya, K., Kamachi, K., Shibayama, K., 2019a. Seroprevalence of IgA and IgM antibodies to Bordetella pertussis in healthy Japanese donors: Assessment for the serological diagnosis of pertussis. PLOS ONE 14, e0219255.

[64] Hendrikx, L. H., Öztürk, K., Rond, L.G.H. de, Greeff, S.C. de, Sanders, E.A.M., Berbers, G.A.M., Buisman, A.-M., 2011. Serum IgA Responses against Pertussis Proteins in Infected and Dutch wP or aP Vaccinated Children: An Additional Role in Pertussis Diagnostics. PLOS ONE 6, e27681.

[65] Nagel, J., Poot-Scholtens, E.J., 1983. Serum IgA antibody to Bordetella pertussis as an indicator of infection. J.Med.Microbiol. 16, 417–426.

[66] Pebody, R.G., Gay, N.J., Giammanco, A., Baron, S., Schellekens, J., Tischer, A., Olander, R.M., Andrews, N.J., Edmunds, W.J., Lecoeur, H., Levy-Bruhl, D., Maple, P.A., de Melker, H., Nardone, A., Rota, M.C., Salmaso, S., Conyn-van Spaendonck, M.A., Swidsinski, S., Miller, E., 2005. The seroepidemiology of Bordetella pertussis infection in Western Europe. Epidemiol.Infect. 133, 159–171.

[67] Locht C., 2007. Bordetella, Molecular Microbiology. Norfolk, UK, Horizon Bioscience

[68] Loeffelholz M.J., 2003. Bordetella. Manual of Clinical Microbiology. Murray P. Washington, DC, USA, ASM Press. 8: 780-788

[69] Fierz, W., 2004. Basic problems of serological laboratory diagnosis. Methods Mol Med 94, 393–427.

[70] Crowcroft, N.S., Stein, C., Duclos, P., Birmingham, M., 2003. How best to estimate the global burden of pertussis? The Lancet.Infectious diseases 3, 413–418

[71] Black, R.E., Cousens, S., Johnson, H.L., Lawn, J.E., Rudan, I., Bassani, D.G., Jha, P., Campbell, H., Walker, C.F., Cibulskis, R., Eisele, T., Liu, L., Mathers, C., Child Health Epidemiology Reference Group of WHO and UNICEF, 2010. Global, regional, and national causes of child mortality in 2008: a systematic analysis. Lancet 375, 1969–1987.

[72] van der Zee, A., Schellekens, J.F.P., Mooi, F.R., 2015. Laboratory Diagnosis of Pertussis. Clin Microbiol Rev 28, 1005–1026.

[73] World Health Organization, 2014. SAGE Pertussis Working Group, background paper.

[74] Caro, J.J., Getsios, D., Payne, K., Annemans, L., Neumann, P.J., Trindade, E., 2005. Economic burden of pertussis and the impact of immunization. Pediatr Infect Dis J 24, S48-54.

[75] Ehreth, J., 2003. The global value of vaccination. Vaccine 21, 596–600.

[76] Purdy, K.W., Hay, J.W., Botteman, M.F., Ward, J.I., 2004. Evaluation of strategies for use of acellular pertussis vaccine in adolescents and adults: a cost-benefit analysis. Clin Infect Dis 39, 20–28.

[77] Barkoff, A.M., Grondahl-Yli-Hannuksela, K., He, Q., 2015. Seroprevalence studies of pertussis: what have we learned from different immunized populations. Pathog.Dis. 73, 10.1093/femspd/ftv050. Epub 2015 Jul 24.

[78] de Greeff, S.C., de Melker, H.E., van Gageldonk, P.G., Schellekens, J.F., van der Klis, F.R., Mollema, L., Mooi, F.R., Berbers, G.A., 2010. Seroprevalence of pertussis in The Netherlands: evidence for increased circulation of Bordetella pertussis. PLoS One 5, e14183.

[79] Cherry, J.D., 2012. Epidemic pertussis in 2012--the resurgence of a vaccine-preventable disease. N Engl J Med 367, 785–787.

[80] He, Q., Mertsola, J., 2008. Factors contributing to pertussis resurgence. Future Microbiol. 3, 329–339.

[81] Schmidtke, A.J., Boney, K.O., Martin, S.W., Skoff, T.H., Tondella, M.L., Tatti, K.M., 2012. Population diversity among Bordetella pertussis isolates, United States, 1935-2009. Emerg Infect Dis 18, 1248–1255.

[82] Rohani, P., Zhong, X., King, A.A., 2010. Contact network structure explains the changing epidemiology of pertussis. Science 330, 982–985.

[83] Aguas, R., Gonçalves, G., Gomes, M.G.M., 2006. Pertussis: increasing disease as a consequence of reducing transmission. Lancet Infect Dis 6, 112–117.

[84] Berbers, G., van Gageldonk, P., Kassteele, JV., Wiedermann, U., Desombere, I., Dalby, T., Toubiana, J., Tsiodras, S., Ferencz, IP., Mullan, K., Griskevicius, A., Kolupajeva, T., Vestrheim, DF., Palminha, P., Popovici, O., Wehlin, L., Kastrin, T., Maďarová, L., Campbell,

H., Ködmön, C., Bacci, S., Barkoff, AM., He, Q.; Serosurveillance Study Team, 2021. Circulation of pertussis and poor protection against diphtheria among middle-aged adults in 18 European countries. Nat Commun 12, 2871

[85] Pavlopoulou, I.D., Syriopoulou, V., Daikos, G.L., Fourlani, H., Tzivaras, A., Petychakis, D., Theodoridou, M., 2007. Pertussis seroprevalence in different age groups in Greece. Scand J Infect Dis 39, 14–18.

[86] Vitek, C.R., Pascual, F.B., Baughman, A.L., Murphy, T.V., 2003. Increase in deaths from pertussis among young infants in the United States in the 1990s. Pediatr Infect Dis J 22, 628–634.

[87] Wendelboe, A.M., Njamkepo, E., Bourillon, A., Floret, D.D., Gaudelus, J., Gerber, M., Grimprel, E., Greenberg, D., Halperin, S., Liese, J., Muñoz-Rivas, F., Teyssou, R., Guiso, N., Van Rie, A., Infant Pertussis Study Group, 2007. Transmission of Bordetella pertussis to young infants. Pediatr Infect Dis J 26, 293–299.

[88] Warfel, J.M., Zimmerman, L.I., Merkel, T.J., 2014. 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. 111, 787–792.

[89] Ntezayabo, B., De Serres, G., Duval, B., 2003. Pertussis resurgence in Canada largely caused by a cohort effect. Pediatr Infect Dis J 22, 22–27.

[90] Skowronski, D.M., De Serres, G., MacDonald, D., Wu, W., Shaw, C., Macnabb, J., Champagne, S., Patrick, D.M., Halperin, S.A., 2002. The changing age and seasonal profile of pertussis in Canada. J Infect Dis 185, 1448–1453.

[91] Tanaka, M., Vitek, C.R., Pascual, F.B., Bisgard, K.M., Tate, J.E., Murphy, T.V., 2003. Trends in pertussis among infants in the United States, 1980-1999. JAMA 290, 2968–2975.

[92] Yih, W.K., Lett, S.M., des Vignes, F.N., Garrison, K.M., Sipe, P.L., Marchant, C.D., 2000. The increasing incidence of pertussis in Massachusetts adolescents and adults, 1989-1998. J.Infect.Dis. 182, 1409–1416.

[93] McIntyre, P., Gidding, H., Gilmour, R., Lawrence, G., Hull, B., Horby, P., Wang, H., Andrews, R., Burgess, M., National Centre for Immunisation Research and Surveillance of Vaccine Preventable Diseases, 2002. Vaccine preventable diseases and vaccination coverage in Australia, 1999 to 2000. Commun Dis Intell Q Rep Suppl, i–xi, 1–111.

[94] Rosewell, A., Spokes, P.J., Gilmour, R.E., 2012. NSW Annual vaccine-preventable disease report, 2011. N S W Public Health Bull 23, 171–178.

[95] de Melker, H.E., Schellekens, J.F., Neppelenbroek, S.E., Mooi, F.R., Rümke, H.C., Conyn-van Spaendonck, M.A., 2000. Reemergence of pertussis in the highly vaccinated population of the Netherlands: observations on surveillance data. Emerg Infect Dis 6, 348–357.

[96] Elomaa, A., Advani, A., Donnelly, D., Antila, M., Mertsola, J., Hallander, H., He, Q., 2005. Strain variation among Bordetella pertussis isolates in finland, where the whole-cell pertussis vaccine has been used for 50 years. J Clin Microbiol 43, 3681–3687.

[97] ECDC, 2023. Disease data from ECDC surveillance Atlas for pertussis. http://ecdc.europa.eu/en/pertussis/surveillance-and-disease-data/atlas, accessed 18.8.2023

[98] Sheridan, S.L., Frith, K., Snelling, T.L., Grimwood, K., McIntyre, P.B., Lambert, S.B., 2014. Waning vaccine immunity in teenagers primed with whole cell and acellular pertussis vaccine: recent epidemiology. Expert Rev.Vaccines 13, 1081–1106.

[99] Amirthalingam, G., Andrews, N., Campbell, H., Ribeiro, S., Kara, E., Donegan, K., Fry, K., Miller, E., Ramsay, M., 2014. Effectiveness of maternal pertussis vaccination in England: an observational study. Lancet. 384:1521–1528.

[100] Huovila R., 1981. Hinkuyskä ja sen esiintyminen Suomessa vuosina 1920–1970. Helsingin yliopistollisen keskussairaalan lastentautien klinikka.

[101] THL, 2023b. https://thl.fi/fi/web/infektiotaudit-ja-rokotukset/seurantajarjestelmat-ja-rekisterit/tartuntatautirekisteri/tartuntatautien-esiintyvyystilastot/tartuntatautien-esiintyvyys-suomessa-raportit, accessed 18.8.2023

[102a] Paireau J., Guillot S., Aït El Belghiti F., Matczak S., Trombert-Paolantoni S., Jacomo V., Taha MK., Salje H., Brisse S., Lévy-Bruhl D., Cauchemez S., Toubiana J., 2022. Effect of change in vaccine schedule on pertussis epidemiology in France: a modelling and serological study. Lancet Infect Dis. 22(2):265-273.

[102b] Zöldi, V., Sane, J., Nohynek, H., Virkki, M., Hannila-Handelberg, T., Mertsola, J., 2017. 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 35, 5249–5255.

[103] Gustafsson, L., Hessel, L., Storsaeter, J., Olin, P., 2006. Long-term follow-up of Swedish children vaccinated with acellular pertussis vaccines at 3, 5, and 12 months of age indicates the need for a booster dose at 5 to 7 years of age. Pediatrics 118, 978–984.

[104] Romanus, V., Jonsell, R., Bergquist, S.O., 1987. Pertussis in Sweden after the cessation of general immunization in 1979. Pediatr Infect Dis J 6, 364–371.

[105] CDC, https://www.cdc.gov/pertussis/surv-reporting/cases-by-year.htmL, , accessed 18.8.2023

[106] Bordet J, Gengou O., 1906. Le microbe de la coqueluche. Ann. Inst. Pasteur. 20, 731–741.

[107] Gerlach, G., von Wintzingerode, F., Middendorf, B., Gross, R., 2001. Evolutionary trends in the genus Bordetella. Microbes Infect 3, 61–72.

[108] Gilligan, P.H., Fisher, M.C., 1984. Importance of culture in laboratory diagnosis of Bordetella pertussis infections. J Clin Microbiol 20, 891–893.

[109] Hoffman, C.L., Gonyar, L.A., Zacca, F., Sisti, F., Fernandez, J., Wong, T., Damron, F.H., Hewlett, E.L., 2019. Bordetella pertussis Can Be Motile and Express Flagellum-Like Structures. mBio 10, e00787-19.

[110] Macewen, H.A., 1908. THE CONVEYANCE OF WHOOPING-COUGH FROM MAN TO ANIMALS BY DIRECT EXPERIMENT. Br Med J 1, 146–147.

[111] Wollstein, M., 1909. THE BORDET-GENGOU BACILLUS OF PERTUSSIS. J Exp Med 11, 41–54.

[112] Elahi, S., Brownlie, R., Korzeniowski, J., Buchanan, R., O'Connor, B., Peppler, M.S., Halperin, S.A., Lee, S.F., Babiuk, L.A., Gerdts, V., 2005. Infection of newborn piglets with Bordetella pertussis: a new model for pertussis. Infect Immun 73, 3636–3645

[113] Khelef, N., Bachelet, C.M., Vargaftig, B.B., Guiso, N., 1994. Characterization of murine lung inflammation after infection with parental Bordetella pertussis and mutants deficient in adhesins or toxins. Infect Immun 62, 2893–2900.

[114] Parton, R., Hall, E., Wardlaw, A.C., 1994. Responses to Bordetella pertussis mutant strains and to vaccination in the coughing rat model of pertussis. J Med Microbiol 40, 307–312.

[115] Warfel, J.M., Beren, J., Kelly, V.K., Lee, G., Merkel, T.J., 2012. Nonhuman primate model of pertussis. Infect Immun 80, 1530–1536.

[116] Bart, M.J., Harris, S.R., Advani, A., Arakawa, Y., Bottero, D., Bouchez, V., Cassiday, P.K., Chiang, C.S., Dalby, T., Fry, N.K., Gaillard, M.E., van Gent, M., Guiso, N., Hallander, H.O., Harvill, E.T., He, Q., van der Heide, H.G., Heuvelman, K., Hozbor, D.F., Kamachi, K., Karataev, G.I., Lan, R., Lutynska, A., Maharjan, R.P., Mertsola, J., Miyamura, T., Octavia, S., Preston, A., Quail, M.A., Sintchenko, V., Stefanelli, P., Tondella, M.L., Tsang, R.S., Xu, Y.,

Yao, S.M., Zhang, S., Parkhill, J., Mooi, F.R., 2014. Global population structure and evolution of Bordetella pertussis and their relationship with vaccination. MBio 5, 1074.

[117] Bjørnstad, O.N., Harvill, E.T., 2005. Evolution and emergence of Bordetella in humans. Trends Microbiol 13, 355–359.

[118] Parkhill, J., Sebaihia, M., Preston, A., Murphy, L.D., Thomson, N., Harris, D.E., Holden, M.T.G., Churcher, C.M., Bentley, S.D., Mungall, K.L., Cerdeño-Tárraga, A.M., Temple, L., James, K., Harris, B., Quail, M.A., Achtman, M., Atkin, R., Baker, S., Basham, D., Bason, N., Cherevach, I., Chillingworth, T., Collins, M., Cronin, A., Davis, P., Doggett, J., Feltwell, T., Goble, A., Hamlin, N., Hauser, H., Holroyd, S., Jagels, K., Leather, S., Moule, S., Norberczak, H., O'Neil, S., Ormond, D., Price, C., Rabbinowitsch, E., Rutter, S., Sanders, M., Saunders, D., Seeger, K., Sharp, S., Simmonds, M., Skelton, J., Squares, R., Squares, S., Stevens, K., Unwin, L., Whitehead, S., Barrell, B.G., Maskell, D.J., 2003. Comparative analysis of the genome sequences of Bordetella pertussis, Bordetella parapertussis and Bordetella bronchiseptica. Nat Genet 35, 32–40.

[119] van der Zee, A., Mooi, F., Van Embden, J., Musser, J., 1997. Molecular evolution and host adaptation of Bordetella spp.: phylogenetic analysis using multilocus enzyme electrophoresis and typing with three insertion sequences. J Bacteriol 179, 6609–6617.

[120] Barkoff, A.M., Mertsola, J., Pierard, D., Dalby, T., Hoegh, S.V., Guillot, S., Stefanelli, P., van Gent, M., Berbers, G., Vestrheim, D., Greve-Isdahl, M., Wehlin, L., Ljungman, M., Fry, N.K., Markey, K., He, Q., 2019. Pertactin-deficient Bordetella pertussis isolates: evidence of increased circulation in Europe, 1998 to 2015. Euro Surveill. 24, 10.2807/1560-7917.ES.2019.24.7.1700832.

[121] Bouchez, V., Brun, D., Cantinelli, T., Dore, G., Njamkepo, E., Guiso, N., 2009. First report and detailed characterization of B. pertussis isolates not expressing Pertussis Toxin or Pertactin. Vaccine 27, 6034–6041.

[122] Lam, C., Octavia, S., Ricafort, L., Sintchenko, V., Gilbert, G.L., Wood, N., McIntyre, P., Marshall, H., Guiso, N., Keil, A.D., Lawrence, A., Robson, J., Hogg, G., Lan, R., 2014. Rapid increase in pertactin-deficient Bordetella pertussis isolates, Australia. Emerg.Infect.Dis. 20, 626–633.

[123] Otsuka, N., Han, H.J., Toyoizumi-Ajisaka, H., Nakamura, Y., Arakawa, Y., Shibayama, K., Kamachi, K., 2012. Prevalence and genetic characterization of pertactin-deficient Bordetella pertussis in Japan. PLoS One 7, e31985.

[124] Pawloski, L.C., Queenan, A.M., Cassiday, P.K., Lynch, A.S., Harrison, M.J., Shang, W., Williams, M.M., Bowden, K.E., Burgos-Rivera, B., Qin, X., Messonnier, N., Tondella, M.L., 2014. Prevalence and molecular characterization of pertactin-deficient Bordetella pertussis in the United States. Clin.Vaccine Immunol. 21, 119–125.

[125] Stefanelli, P., Fazio, C., Fedele, G., Spensieri, F., Ausiello, C.M., Mastrantonio, P., 2009. A natural pertactin deficient strain of Bordetella pertussis shows improved entry in human monocyte-derived dendritic cells. New Microbiol 32, 159–166.

[126] Roy, C.R., Miller, J.F., Falkow, S., 1989. The bvgA gene of Bordetella pertussis encodes a transcriptional activator required for coordinate regulation of several virulence genes. J Bacteriol 171, 6338–6344.

[127] Coutte, L., Locht, C., 2015. Investigating pertussis toxin and its impact on vaccination. Future Microbiol 10, 241–254

[128] Preston, A., 2005. Bordetella pertussis: the intersection of genomics and pathobiology. CMAJ 173, 55–62.

[129] Aricò, B., Rappuoli, R., 1987. Bordetella parapertussis and Bordetella bronchiseptica contain transcriptionally silent pertussis toxin genes. J Bacteriol 169, 2847–2853.

[130] Marchitto, K.S., Smith, S.G., Locht, C., Keith, J.M., 1987. Nucleotide sequence homology to pertussis toxin gene in Bordetella bronchiseptica and Bordetella parapertussis. Infect Immun 55, 497–501.

[131] Tamura, M., Nogimori, K., Murai, S., Yajima, M., Ito, K., Katada, T., Ui, M., Ishii, S., 1982. Subunit structure of islet-activating protein, pertussis toxin, in conformity with the A-B model. Biochemistry (N.Y.) 21, 5516–5522.

[132] Locht, C., Keith, J.M., 1986. Pertussis toxin gene: nucleotide sequence and genetic organization. Science 232, 1258–1264.

[133] Nicosia, A., Perugini, M., Franzini, C., Casagli, M.C., Borri, M.G., Antoni, G., Almoni, M., Neri, P., Ratti, G., Rappuoli, R., 1986. Cloning and sequencing of the pertussis toxin genes: operon structure and gene duplication. Proc Natl Acad Sci U S A 83, 4631–4635.

[134] Farizo, K.M., Cafarella, T.G., Burns, D.L., 1996. Evidence for a ninth gene, ptlI, in the locus encoding the pertussis toxin secretion system of Bordetella pertussis and formation of a PtlI-PtlF complex. J Biol Chem 271, 31643–31649.

[135] Pizza, M., Bugnoli, M., Manetti, R., Covacci, A., Rappuoli, R., 1990. The subunit S1 is important for pertussis toxin secretion. J Biol Chem 265, 17759–17763.

[136] Weiss, A.A., Johnson, F.D., Burns, D.L., 1993. Molecular characterization of an operon required for pertussis toxin secretion. Proc Natl Acad Sci U S A 90, 2970–2974.

[137] Carbonetti, N.H., Artamonova, G.V., Mays, R.M., Worthington, Z.E.V., 2003. Pertussis toxin plays an early role in respiratory tract colonization by Bordetella pertussis. Infect Immun 71, 6358–6366.

[138] Saukkonen, K., Burnette, W.N., Mar, V.L., Masure, H.R., Tuomanen, E.I., 1992. Pertussis toxin has eukaryotic-like carbohydrate recognition domains. Proc.Natl.Acad.Sci.U.S.A. 89, 118–122.

[139] de Gouw, D., Diavatopoulos, D.A., Bootsma, H.J., Hermans, P.W.M., Mooi, F.R., 2011. Pertussis: a matter of immune modulation. FEMS Microbiol Rev 35, 441–474.

[140] Tuomanen, E., Weiss, A., 1985. Characterization of two adhesins of Bordetella pertussis for human ciliated respiratory-epithelial cells. J Infect Dis 152, 118–125.

[141] Katada, T., Ui, M., 1982. ADP ribosylation of the specific membrane protein of C6 cells by islet-activating protein associated with modification of adenylate cyclase activity. The Journal of biological chemistry 257, 7210–7216.

[142] Tamura, M., Nogimori, K., Yajima, M., Ase, K., Ui, M., 1983. A role of the B-oligomer moiety of islet-activating protein, pertussis toxin, in development of the biological effects on intact cells. The Journal of biological chemistry 258, 6756–6761.

[143] Lobet, Y., Feron, C., Dequesne, G., Simoen, E., Hauser, P., Locht, C., 1993. Site-specific alterations in the B oligomer that affect receptor-binding activities and mitogenicity of pertussis toxin. J.Exp.Med. 177, 79–87.

[144] Millen, S.H., Lewallen, D.M., Herr, A.B., Iyer, S.S., Weiss, A.A., 2010. Identification and characterization of the carbohydrate ligands recognized by pertussis toxin via a glycan microarray and surface plasmon resonance. Biochemistry 49, 5954–5967.

[145] Raze, D., Veithen, A., Sato, H., Antoine, R., Menozzi, F.D., Locht, C., 2006. Genetic exchange of the S2 and S3 subunits in pertussis toxin. Mol Microbiol 60, 1241–1250.

[146] Armstrong, G.D., Howard, L.A., Peppler, M.S., 1988. Use of glycosyltransferases to restore pertussis toxin receptor activity to asialoagalactofetuin. J Biol Chem 263, 8677–8684.

[147] Brennan, M.J., David, J.L., Kenimer, J.G., Manclark, C.R., 1988. Lectin-like binding of pertussis toxin to a 165-kilodalton Chinese hamster ovary cell glycoprotein. J Biol Chem 263, 4895–4899.

[148] Locht, C., Coutte, L., Mielcarek, N., 2011. The ins and outs of pertussis toxin. FEBS J 278, 4668–4682.

[149] Cortina, G., Krueger, K.M., Barbieri, J.T., 1991. The carboxyl terminus of the S1 subunit of pertussis toxin confers high affinity binding to transducin. J Biol Chem 266, 23810–23814.

[150] Rossjohn, J., Buckley, J.T., Hazes, B., Murzin, A.G., Read, R.J., Parker, M.W., 1997. Aerolysin and pertussis toxin share a common receptor-binding domain. EMBO J 16, 3426–3434.

[151] Kaslow, H.R., Burns, D.L., 1992. Pertussis toxin and target eukaryotic cells: binding, entry, and activation. FASEB J 6, 2684–2690.

[152] Bokoch, G.M., Katada, T., Northup, J.K., Hewlett, E.L., Gilman, A.G., 1983. Identification of the predominant substrate for ADP-ribosylation by islet activating protein. The Journal of biological chemistry 258, 2072–2075.

[153] Cortina, G., Barbieri, J.T., 1989. Role of tryptophan 26 in the NAD glycohydrolase reaction of the S-1 subunit of pertussis toxin. J Biol Chem 264, 17322–17328..

[154] Locht, C., Capiau, C., Feron, C., 1989. Identification of amino acid residues essential for the enzymatic activities of pertussis toxin. Proc Natl Acad Sci U S A 86, 3075–3079.

[155] Burnette, W.N., Cieplak, W., Mar, V.L., Kaljot, K.T., Sato, H., Keith, J.M., 1988. Pertussis toxin S1 mutant with reduced enzyme activity and a conserved protective epitope. Science 242, 72–74.

[156] Locht, C., Lobet, Y., Feron, C., Cieplak, W., Keith, J.M., 1990. The role of cysteine 41 in the enzymatic activities of the pertussis toxin S1 subunit as investigated by site-directed mutagenesis. J Biol Chem 265, 4552–4559.

[157] Antoine, R., Locht, C., 1994. The NAD-glycohydrolase activity of the pertussis toxin S1 subunit. Involvement of the catalytic HIS-35 residue. J Biol Chem 269, 6450–6457.

[158] Xu, Y., Barbançon-Finck, V., Barbieri, J.T., 1994. Role of histidine 35 of the S1 subunit of pertussis toxin in the ADP-ribosylation of transducin. J Biol Chem 269, 9993–9999.

[159] Antoine, R., Tallett, A., van Heyningen, S., Locht, C., 1993. Evidence for a catalytic role of glutamic acid 129 in the NAD-glycohydrolase activity of the pertussis toxin S1 subunit. J Biol Chem 268, 24149–24155.

[160] Melvin, J.A., Scheller, E.V., Miller, J.F., Cotter, P.A., 2014. Bordetella pertussis pathogenesis: current and future challenges. Nat Rev Microbiol 12, 274–288.

[161] Carbonetti, N.H., Artamonova, G.V., Andreasen, C., Dudley, E., Mays, R.M., Worthington, Z.E.V., 2004. Suppression of serum antibody responses by pertussis toxin after respiratory tract colonization by Bordetella pertussis and identification of an immunodominant lipoprotein. Infect Immun 72, 3350–3358.

[162] Connelly, C.E., Sun, Y., Carbonetti, N.H., 2012. Pertussis toxin exacerbates and prolongs airway inflammatory responses during Bordetella pertussis infection. Infect Immun 80, 4317–4332.

[163] Diehl, S.A., McElvany, B., Noubade, R., Seeberger, N., Harding, B., Spach, K., Teuscher, C., 2014. G proteins Gαi1/3 are critical targets for Bordetella pertussis toxin-induced vasoactive amine sensitization. Infect Immun 82, 773–782.

[164] Morse, S.I., Morse, J.H., 1976. Isolation and properties of the leukocytosis- and lymphocytosis-promoting factor of Bordetella pertussis. J Exp Med 143, 1483–1502.

[165] Munoz, J.J., Arai, H., Bergman, R.K., Sadowski, P.L., 1981. Biological activities of crystalline pertussigen from Bordetella pertussis. Infect Immun 33, 820–826.
[166] Parfentjev, I.A., Goodline, M.A., 1948. Histamine shock in mice sensitized with Hemophilus pertussis vaccine. J Pharmacol Exp Ther 92, 411–413.
[167] Parfentjev, I.A., Schleyer, W.L., 1949. The influence of histamine on the blood sugar level of normal and sensitized mice. Arch Biochem 20, 341–346.
[168] Sato, Y., Arai, H., Suzuki, K., 1973. Leukocytosis-promoting factor of Bordetella pertussis. II. Biological properties. Infect Immun 7, 992–999.
[169] Verschueren, H., Dewit, J., Van der Wegen, A., De Braekeleer, J., Van Heule, G., Dekegel, D., De Baetselier, P., 1991. The lymphocytosis promoting action of pertussis toxin can be mimicked in vitro. Holotoxin but not the B subunit inhibits invasion of human T lymphoma cells through fibroblast monolayers. J Immunol Methods 144, 231–240.
[170] Alfano, M., Schmidtmayerova, H., Amella, C.A., Pushkarsky, T., Bukrinsky, M., 1999. The B-oligomer of pertussis toxin deactivates CC chemokine receptor 5 and blocks entry of M-tropic HIV-1 strains. J Exp Med 190, 597–605.
[171] Schneider, O.D., Weiss, A.A., Miller, W.E., 2009. Pertussis toxin signals through the TCR to initiate cross-desensitization of the chemokine receptor CXCR4. J Immunol 182, 5730–5739.
[172] Carbonetti, N.H., Artamonova, G.V., Van Rooijen, N., Ayala, V.I., 2007. Pertussis toxin targets airway macrophages to promote Bordetella pertussis infection of the respiratory tract. Infect Immun 75, 1713–1720.
[173] Hou, W., Wu, Y., Sun, S., Shi, M., Sun, Y., Yang, C., Pei, G., Gu, Y., Zhong, C., Sun, B., 2003. Pertussis toxin enhances Th1 responses by stimulation of dendritic cells. J Immunol 170, 1728–1736.
[174] Fan, H., Peck, O.M., Tempel, G.E., Halushka, P.V., Cook, J.A., 2004. Toll-like receptor 4 coupled GI protein signaling pathways regulate extracellular signal-regulated kinase phosphorylation and AP-1 activation independent of NFkappaB activation. Shock 22, 57–62.
[175] Wang, Z.Y., Yang, D., Chen, Q., Leifer, C.A., Segal, D.M., Su, S.B., Caspi, R.R., Howard, Z.O.M., Oppenheim, J.J., 2006. Induction of dendritic cell maturation by pertussis toxin and its B subunit differentially initiate Toll-like receptor 4-dependent signal transduction pathways. Exp Hematol 34, 1115–1124.
[176] Nasso, M., Fedele, G., Spensieri, F., Palazzo, R., Costantino, P., Rappuoli, R., Ausiello, C.M., 2009. Genetically detoxified pertussis toxin induces Th1/Th17 immune response through MAPKs and IL-10-dependent mechanisms. J Immunol 183, 1892–1899.
[177] Hofstetter, H.H., Shive, C.L., Forsthuber, T.G., 2002. Pertussis toxin modulates the immune response to neuroantigens injected in incomplete Freund's adjuvant: induction of Th1 cells and experimental autoimmune encephalomyelitis in the presence of high frequencies of Th2 cells. J Immunol 169, 117–125.
[178] Mu, H.H., Sewell, W.A., 1994. Regulation of DTH and IgE responses by IL-4 and IFN-gamma in immunized mice given pertussis toxin. Immunology 83, 639–645.
[179] Chen, X., Howard, O.M.Z., Oppenheim, J.J., 2007. Pertussis toxin by inducing IL-6 promotes the generation of IL-17-producing CD4 cells. J Immunol 178, 6123–6129.
[180] Hofstetter, H.H., Grau, C., Buttmann, M., Forsthuber, T.G., Gaupp, S., Toyka, K.V., Gold, R., 2007. The PLPp-specific T-cell population promoted by pertussis toxin is characterized by high frequencies of IL-17-producing cells. Cytokine 40, 35–43.
[181] Murphey, C., Chang, S., Zhang, X., Arulanandam, B., Forsthuber, T.G., 2011. Induction of polyclonal CD8+ T cell activation and effector function by Pertussis toxin. Cell Immunol 267, 50–55.

[182] van den Berg, B.M., Beekhuizen, H., Willems, R.J., Mooi, F.R., van Furth, R., 1999. Role of Bordetella pertussis virulence factors in adherence to epithelial cell lines derived from the human respiratory tract. Infect Immun 67, 1056–1062.

[183] Alonso, S., Pethe, K., Mielcarek, N., Raze, D., Locht, C., 2001. Role of ADP-ribosyltransferase activity of pertussis toxin in toxin-adhesin redundancy with filamentous hemagglutinin during Bordetella pertussis infection. Infect Immun 69, 6038–6043.

[184] Pittman, M., 1984. The concept of pertussis as a toxin-mediated disease. Pediatr Infect Dis 3, 467–486.

[185] Scanlon, K., Skerry, C., Carbonetti, N., 2019. Association of Pertussis Toxin with Severe Pertussis Disease. Toxins (Basel) 11, 373.

[186] Kimura, A., Mountzouros, K.T., Relman, D.A., Falkow, S., Cowell, J.L., 1990. Bordetella pertussis filamentous hemagglutinin: evaluation as a protective antigen and colonization factor in a mouse respiratory infection model. Infect Immun 58, 7–16.

[187] Charles, I.G., Dougan, G., Pickard, D., Chatfield, S., Smith, M., Novotny, P., Morrissey, P., Fairweather, N.F., 1989. Molecular cloning and characterization of protective outer membrane protein P.69 from Bordetella pertussis. Proc Natl Acad Sci U S A 86, 3554–3558.

[188] Blom, J., Hansen, G.A., Poulsen, F.M., 1983. Morphology of cells and hemagglutinogens of Bordetella species: resolution of substructural units in fimbriae of Bordetella pertussis. Infect Immun 42, 308–317.

[189] Kilgore, P.E., Salim, A.M., Zervos, M.J., Schmitt, H.-J., 2016. Pertussis: Microbiology, Disease, Treatment, and Prevention. Clin Microbiol Rev 29, 449–486.

[190] Coutte, L., Alonso, S., Reveneau, N., Willery, E., Quatannens, B., Locht, C., Jacob-Dubuisson, F., 2003. Role of adhesin release for mucosal colonization by a bacterial pathogen. J Exp Med 197, 735–742.

[191] Irie, Y., Mattoo, S., Yuk, M.H., 2004. The Bvg virulence control system regulates biofilm formation in Bordetella bronchiseptica. J Bacteriol 186, 5692–5698.

[192] Serra, D., Bosch, A., Russo, D.M., Rodríguez, M.E., Zorreguieta, A., Schmitt, J., Naumann, D., Yantorno, O., 2007. Continuous nondestructive monitoring of Bordetella pertussis biofilms by Fourier transform infrared spectroscopy and other corroborative techniques. Anal Bioanal Chem 387, 1759–1767.

[193] Edwards, J.A., Groathouse, N.A., Boitano, S., 2005. Bordetella bronchiseptica Adherence to Cilia Is Mediated by Multiple Adhesin Factors and Blocked by Surfactant Protein A. Infect Immun 73, 3618–3626..

[194] Inatsuka, C.S., Xu, Q., Vujkovic-Cvijin, I., Wong, S., Stibitz, S., Miller, J.F., Cotter, P.A., 2010. Pertactin Is Required for Bordetella Species To Resist Neutrophil-Mediated Clearance. Infect Immun 78, 2901–2909.

[195] Shahin, R.D., Brennan, M.J., Li, Z.M., Meade, B.D., Manclark, C.R., 1990. Characterization of the protective capacity and immunogenicity of the 69-kD outer membrane protein of Bordetella pertussis. J Exp Med 171, 63–73.

[196] Robinson, A., Ashworth, L.A., Irons, L.I., 1989. Serotyping Bordetella pertussis strains. Vaccine 7, 491–494.

[197] Funnell, S.G., Robinson, A., 1993. A novel adherence assay for Bordetella pertussis using tracheal organ cultures. FEMS Microbiol Lett 110, 197–203.

[198] Mooi, F.R., Jansen, W.H., Brunings, H., Gielen, H., van der Heide, H.G., Walvoort, H.C., Guinee, P.A., 1992. Construction and analysis of Bordetella pertussis mutants defective in the production of fimbriae. Microb Pathog 12, 127–135.

[199] Fernandez, R.C., Weiss, A.A., 1994. Cloning and sequencing of a Bordetella pertussis serum resistance locus. Infect Immun 62, 4727–4738.
[200] Finn, T.M., Stevens, L.A., 1995. Tracheal colonization factor: a Bordetella pertussis secreted virulence determinant. Mol Microbiol 16, 625–634.
[201] Finn, T.M., Amsbaugh, D.F., 1998. Vag8, a Bordetella pertussis bvg-regulated protein. Infect Immun 66, 3985–3989.
[202] Cherry, J.D., 1996. Historical review of pertussis and the classical vaccine. J Infect Dis 174 Suppl 3, S259-263.
[203] Syed, YY., 2019. DTaP-IPV-HepB-Hib Vaccine (Hexyon®): An Updated Review of its Use in Primary and Booster Vaccination. Paediatr Drugs. 21(5):397–408.
[204] Lääkintöhallitus, 1957. Lääkintöhallituksen v.1957 antamat rokotusohjeet. Valtioneuvoston kirjapaino.
[205] Lääkintöhallitus, 1977. Lääkintöhallituksen kiertokirje. DNo 2102/55/77.
[206] THL, 2023a. https://thl.fi/fi/web/infektiotaudit-ja-rokotukset/tietoa-rokotuksista/kansallinen-rokotusohjelma/milloin-eri-rokotukset-ovat-alkaneet-suomessa-, accessed 18.8.2023
[207] Versteegen, P., Valente Pinto, M., Barkoff, A.M., van Gageldonk, P.G.M., van de Kassteele, J., van Houten, M.A., Sanders, E.A.M., de Groot, R., Diavatopoulos, D.A., Bibi, S., Luoto, R., He, Q., Buisman, A.M., Kelly, D.F., Mertsola, J., Berbers, G.A.M., 2021. 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, 103247.
[208] Versteegen, P., Berbers, G.A.M., Smits, G., Sanders, E.A.M., van der Klis, F.R.M., de Melker, H.E., van der Maas, N.A.T., 2021. 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 100196.
[209] Trollfors, B., Taranger, J., Lagergard, T., Lind, L., Sundh, V., Zackrisson, G., Lowe, C.U., Blackwelder, W., Robbins, J.B., 1995. A placebo-controlled trial of a pertussis-toxoid vaccine. N.Engl.J.Med. 333, 1045–1050.
[210] Hviid, A., Stellfeld, M., Andersen, P.H., Wohlfahrt, J., Melbye, M., 2004. Impact of routine vaccination with a pertussis toxoid vaccine in Denmark. Vaccine 22, 3530–3534.
[211] Taranger, J., Trollfors, B., Bergfors, E., Knutsson, N., Lagergård, T., Schneerson, R., Robbins, J.B., 2001. Immunologic and epidemiologic experience of vaccination with a monocomponent pertussis toxoid vaccine. Pediatrics 108, E115.
[212] Guiso, N., 2014. Bordetella pertussis: why is it still circulating? J Infect 68 Suppl 1, S119-124.
[213] World Health Organization, 2005. Pertussis vaccines--WHO position paper. Wkly Epidemiol Rec80(4): 31-9
[214] World Health Organization, 2010. Pertussis vaccines: WHO position paper. Weekly Epidemiol Record 2010;85(40):385–400.
[215] Isomura, S., Suzuki, S., Sato, Y., 1985. Clinical efficacy of the Japanese acellular pertussis vaccine after intrafamiliar exposure to pertussis patients. Dev Biol Stand 61, 531–537.
[216] Sato, H., Sato, Y., 1999. Experience with diphtheria toxoid-tetanus toxoid-acellular pertussis vaccine in Japan. Clin Infect Dis 28 Suppl 2, S124-130.
[217] Dakic, G., Kallonen, T., Elomaa, A., Pljesa, T., Vignjevic-Krastavcevic, M., He, Q., 2010. Bordetella pertussis vaccine strains and circulating isolates in Serbia. Vaccine 28, 1188–1192.
[218] Zawadka, M., Mosiej, E., Polak, M., Krysztopa-Grzybowska, K., Moskała, B., Rokosz-Chudziak, N., Rabczenko, D., Augustynowicz, E., Lutyńska, A., 2014. Consistency of

Bordetella pertussis vaccine seed strains and potency of whole-cell pertussis vaccine still in use in Poland. Biologicals 42, 123–127.

[219] Vidor E, Soubeyrand B. Manufacturing DTaP-based combination vaccines: industrial challenges around essential public health tools. Expert Rev Vaccines. 2016 Dec;15(12):1575-1582.

[220] Plotkin, S., Robinson, J.M., Cunningham, G., Iqbal, R., Larsen, S. The complexity and cost of vaccine manufacturing - An overview. Vaccine. 2017 35(33):4064-4071.

[221] Greco, D., Salmaso, S., Mastrantonio, P., Giuliano, M., Tozzi, A.E., Anemona, A., Ciofi degli Atti, M.L., Giammanco, A., Panei, P., Blackwelder, W.C., Klein, D.L., Wassilak, S.G., 1996. A controlled trial of two acellular vaccines and one whole-cell vaccine against pertussis. Progetto Pertosse Working Group. N.Engl.J.Med. 334, 341–348.

[222] Gustafsson, L., Hallander, H.O., Olin, P., Reizenstein, E., Storsaeter, J., 1996. A controlled trial of a two-component acellular, a five-component acellular, and a whole-cell pertussis vaccine. N.Engl.J.Med. 334, 349–355.

[223] Schmitt, H.J., von König, C.H., Neiss, A., Bogaerts, H., Bock, H.L., Schulte-Wissermann, H., Gahr, M., Schult, R., Folkens, J.U., Rauh, W., Clemens, R., 1996. Efficacy of acellular pertussis vaccine in early childhood after household exposure. JAMA 275, 37–41.

[224] Minh, N.N.T., He, Q., Ramalho, A., Kaufhold, A., Viljanen, M.K., Arvilommi, H., Mertsola, J., 1999. Acellular vaccines containing reduced quantities of pertussis antigens as a booster in adolescents. Pediatrics 104, e70.

[225] Gupta, R.K., Sharma, S.B., Ahuja, S., Saxena, S.N., 1987. Glutaraldehyde inactivated pertussis vaccine: a less histamine sensitizing vaccine. J Biol Stand 15, 159–164.

[226] Pizza, M., Covacci, A., Bartoloni, A., Perugini, M., Nencioni, L., De Magistris, M.T., Villa, L., Nucci, D., Manetti, R., Bugnoli, M., 1989. Mutants of pertussis toxin suitable for vaccine development. Science 246, 497–500..

[227] Edwards, K.M., Meade, B.D., Decker, M.D., Reed, G.F., Rennels, M.B., Steinhoff, M.C., Anderson, E.L., Englund, J.A., Pichichero, M.E., Deloria, M.A., 1995. Comparison of 13 acellular pertussis vaccines: overview and serologic response. Pediatrics 96, 548–557.

[228] Ad Hoc Group for the Study of Pertussis Vaccines, 1988. Placebo-controlled trial of two acellular pertussis vaccines in Sweden--protective efficacy and adverse events. Lancet 1, 955–960.

[229] Sato, Y., Kimura, M., Fukumi, H., 1984. Development of a pertussis component vaccine in Japan. Lancet 1, 122–126.

[230] Chong, P., Zobrist, G., Sia, C., Loosmore, S., Klein, M., 1992. Identification of T-Cell and B-Cell Epitopes of the S2-Subunit and S3-Subunit of Pertussis Toxin by Use of Synthetic Peptides. Infect.Immun. 60, 4640–4647.

[231] Fish, F., Cowell, J.L., Manclark, C.R., 1984. Proliferative response of immune mouse T-lymphocytes to the lymphocytosis-promoting factor of Bordetella pertussis. Infect Immun 44, 1–6.

[232] Sekura, R.D., Zhang, Y.L., Roberson, R., Acton, B., Trollfors, B., Tolson, N., Shiloach, J., Bryla, D., Muir-Nash, J., Koeller, D., 1988. Clinical, metabolic, and antibody responses of adult volunteers to an investigational vaccine composed of pertussis toxin inactivated by hydrogen peroxide. J Pediatr 113, 806–813.

[233] Ruuskanen, O., Noel, A., Putto-Laurila, A., Pêtre, J., Capiau, C., Delem, A., Vandevoorde, D., Simoen, E., Teuwen, D.E., Bogaerts, H., 1991. Development of an acellular pertussis vaccine and its administration as a booster in healthy adults. Vaccine 9, 117–121.

[234] Vanura, H., Just, M., Ambrosch, F., Berger, R.M., Bogaerts, H., Wynen, J., Vandevoorde, D., Wiedermann, G., 1994. Study of pertussis vaccines in infants: comparison of response to acellular pertussis DTP vaccines containing 25 micrograms of FHA and either 25 or 8 micrograms of PT with response to whole-cell pertussis DTP vaccine. Vaccine 12, 210–214.

[235] Lang, A.B., Ganss, M.T., Cryz, S.J., 1989. Monoclonal antibodies that define neutralizing epitopes of pertussis toxin: conformational dependence and epitope mapping. Infect Immun 57, 2660–2665.

[236] Oh, H., Kim, B.G., Nam, K.T., Hong, S.H., Ahn, D.H., Choi, G.S., Kim, H., Hong, J.T., Ahn, B.Y., 2013. Characterization of the carbohydrate binding and ADP-ribosyltransferase activities of chemically detoxified pertussis toxins. Vaccine 31, 2988–2993.

[237] Yuen, C.T., Asokanathan, C., Cook, S., Lin, N., Xing, D., 2016. Effect of different detoxification procedures on the residual pertussis toxin activities in vaccines. Vaccine 34, 2129–2134.

[238] Metz, B., Kersten, G.F.A., Hoogerhout, P., Brugghe, H.F., Timmermans, H.A.M., de Jong, A., Meiring, H., ten Hove, J., Hennink, W.E., Crommelin, D.J.A., Jiskoot, W., 2004. Identification of formaldehyde-induced modifications in proteins: reactions with model peptides. J Biol Chem 279, 6235–6243.

[239] Gomez, S.R., Xing, D.K.-L., Corbel, M.J., Coote, J., Parton, R., Yuen, C.-T., 2006. Development of a carbohydrate binding assay for the B-oligomer of pertussis toxin and toxoid. Anal Biochem 356, 244–253.

[240] Richards, F.M., Knowles, J.R., 1968. Glutaraldehyde as a protein cross-linking reagent. Journal of Molecular Biology 37, 231–233.

[241] Silva, C., Sousa, F., Bitz, G.G., Cavaco-Paulo, A., 2004. Chemical modifications on proteins using glutaraldehyde. Food Technology and Biotechnology 42, 51–56.

[242] Tummala, M., Lee, S.-M., Chess, E., Hu, P., 2010. Characterization of pertussis toxoid by two-dimensional liquid chromatography-tandem mass spectrometry. Anal Biochem 401, 295–302.

[243] Mielcarek, N., Debrie, A.-S., Raze, D., Bertout, J., Rouanet, C., Younes, A.B., Creusy, C., Engle, J., Goldman, W.E., Locht, C., 2006. Live attenuated B. pertussis as a single-dose nasal vaccine against whooping cough. PLoS Pathog 2, e65.

[244] Habeeb, A.J., Hiramoto, R., 1968. Reaction of proteins with glutaraldehyde. Arch Biochem Biophys 126, 16–26.

[245] Fowler, S., Byron, O., Jumel, K., Xing, D., Corbel, M.J., Bolgiano, B., 2003. Novel configurations of high molecular weight species of the pertussis toxin vaccine component. Vaccine 21, 2678–2688.

[246] Nencioni, L., Volpini, G., Peppoloni, S., Bugnoli, M., Magistris, T.D., Marsili, I., Rappuoli, R., 1991. Properties of pertussis toxin mutant PT-9K/129G after formaldehyde treatment. Infect.Immun. 59, 625–630.

[247] Locht, C., Antoine, R., 1995. A proposed mechanism of ADP-ribosylation catalyzed by the pertussis toxin S1 subunit. Biochimie 77, 333–340.

[248] Loosmore, S., Zealey, G., Cockle, S., Boux, H., Chong, P., Yacoob, R., Klein, M., 1993. Characterization of pertussis toxin analogs containing mutations in B-oligomer subunits. Infect Immun 61, 2316–2324.

[249] Brown, D.R., Keith, J.M., Sato, H., Sato, Y., 1991. Construction and characterization of genetically inactivated pertussis toxin. Dev Biol Stand 73, 63–73.

[250] Nencioni, L., Pizza, M., Bugnoli, M., De Magistris, T., Di Tommaso, A., Giovannoni, F., Manetti, R., Marsili, I., Matteucci, G., Nucci, D., 1990. Characterization of genetically

inactivated pertussis toxin mutants: candidates for a new vaccine against whooping cough. Infect Immun 58, 1308–1315.

[251] Robbins, J.B., Schneerson, R., Kubler-Kielb, J., Keith, J.M., Trollfors, B., Vinogradov, E., Shiloach, J., 2014. Toward a new vaccine for pertussis. Proc.Natl.Acad.Sci.U.S.A. 111, 3213–3216.

[252] Mielcarek, N., Debrie, A.-S., Mahieux, S., Locht, C., 2010. Dose response of attenuated Bordetella pertussis BPZE1-induced protection in mice. Clin Vaccine Immunol 17, 317–324.

[253] Feunou, P.F., Bertout, J., Locht, C., 2010. T- and B-cell-mediated protection induced by novel, live attenuated pertussis vaccine in mice. Cross protection against parapertussis. PLoS One 5, e10178.

[254] Feunou, P.F., Kammoun, H., Debrie, A.-S., Mielcarek, N., Locht, C., 2010. Long-term immunity against pertussis induced by a single nasal administration of live attenuated B. pertussis BPZE1. Vaccine 28, 7047–7053.

[255] Keech, C., Miller, V.E., Rizzardi, B., Hoyle, C., Pryor, M.J., Ferrand, J., Solovay, K., Thalen, M., Noviello, S., Goldstein, P., Gorringe, A., Cavell, B., He, Q., Barkoff, A.-M., Rubin, K., Locht, C., 2023. Immunogenicity and safety of BPZE1, an intranasal live attenuated pertussis vaccine, versus tetanus-diphtheria-acellular pertussis vaccine: a randomised, double-blind, phase 2b trial. Lancet 401, 843–855.

[256] Raeven, R.H.M., van der Maas, L., Pennings, J.L.A., Fuursted, K., Jørgensen, C.S., van Riet, E., Metz, B., Kersten, G.F.A., Dalby, T., 2019. Antibody Specificity Following a Recent Bordetella pertussis Infection in Adolescence Is Correlated With the Pertussis Vaccine Received in Childhood. Front Immunol 10, 1364.

[257] Nguyen, A.W., Wagner, E.K., Laber, J.R., Goodfield, L.L., Smallridge, W.E., Harvill, E.T., Papin, J.F., Wolf, R.F., Padlan, E.A., Bristol, A., Kaleko, M., Maynard, J.A., 2015. A cocktail of humanized anti-pertussis toxin antibodies limits disease in murine and baboon models of whooping cough. Science translational medicine 7, 316ra195

[258] Salmaso, S., Mastrantonio, P., Wassilak, S. G., Giuliano, M., Anemona, A., Giammanco, A., Tozzi, A. E., Ciofi degli Atti, M.L., Greco, D., 1998. Persistence of protection through 33 months of age provided by immunization in infancy with two three-component acellular pertussis vaccines. Stage II Working Group. Vaccine 16, 1270–1275.

[259] Plotkin, S.A., 2013. Complex correlates of protection after vaccination. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 56, 1458–1465.

[260] Ausiello, C.M., Lande, R., Urbani, F., Carlo, B.D., Stefanelli, P., Salmaso, S., Mastrantonio, P., Cassone, A., 2000. Cell-mediated immunity and antibody responses to Bordetella pertussis antigens in children with a history of pertussis infection and in recipients of an acellular pertussis vaccine. J.Infect.Dis. 181, 1989–1995.

[261] Barkoff, A.M., Knuutila, A., Mertsola, J., He, Q., 2021. Evaluation of Anti-PT Antibody Response after Pertussis Vaccination and Infection: The Importance of Both Quantity and Quality. Toxins (Basel) 13, 10.3390/toxins13080508.

[262] Edelman, K., Malmström, K., He, Q., Savolainen, J., Terho, E.O., Mertsola, J., 1999. Local reactions and IgE antibodies to pertussis toxin after acellular diphtheria-tetanus-pertussis immunization. Eur J Pediatr 158, 989–994.

[263] Ryan, E.J., Nilsson, L., Kjellman, N., Gothefors, L., Mills, K.H., 2000. Booster immunization of children with an acellular pertussis vaccine enhances Th2 cytokine production and serum IgE responses against pertussis toxin but not against common allergens. Clin.Exp.Immunol. 121, 193–200.

[264] Grüber, C., Lau, S., Dannemann, A., Sommerfeld, C., Wahn, U., Aalberse, R.C., 2001. Down-regulation of IgE and IgG4 antibodies to tetanus toxoid and diphtheria toxoid by covaccination with cellular Bordetella pertussis vaccine. J Immunol 167, 2411–2417.

[265] Dalby, T., Petersen, J.W., Harboe, Z.B., Krogfelt, K.A., 2010. Antibody responses to pertussis toxin display different kinetics after clinical Bordetella pertussis infection than after vaccination with an acellular pertussis vaccine. J.Med.Microbiol. 59, 1029–1036.

[266] Fry, N.K., Litt, D.J., Duncan, J., Vaghji, L., Warrener, L., Samuel, D., Andrews, N., Harnden, A., Harrison, T.G., 2013. Modelling anti-pertussis toxin IgG antibody decay following primary and preschool vaccination with an acellular pertussis vaccine in UK subjects using a modified oral fluid assay. J.Med.Microbiol. 62, 1281–1289.

[267] Hallander, H.O., Gustafsson, L., Ljungman, M., Storsaeter, J., 2005. 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 23, 5359–5364.

[268] Heininger, U., Cherry, J.D., Stehr, K., 2004. Serologic response and antibody-titer decay in adults with pertussis. Clin.Infect.Dis. 38, 591–594.

[269] Riffelmann, M., Littmann, M., Hulsse, C., von Konig, C.H., 2009. Antibody decay after immunisation of health-care workers with an acellular pertussis vaccine. Eur.J.Clin.Microbiol.Infect.Dis. 28, 275–279.

[270] Le, T., Cherry, J.D., Chang, S.J., Knoll, M.D., Lee, M.L., Barenkamp, S., Bernstein, D., Edelman, R., Edwards, K.M., Greenberg, D., Keitel, W., Treanor, J., Ward, J.I., APERT Study, 2004. Immune responses and antibody decay after immunization of adolescents and adults with an acellular pertussis vaccine: the APERT Study. J.Infect.Dis. 190, 535–544.

[271] Klein, N.P., Bartlett, J., Rowhani-Rahbar, A., Fireman, B., Baxter, R., 2012. Waning protection after fifth dose of acellular pertussis vaccine in children. N.Engl.J.Med. 367, 1012–1019.

[272] Liko, J., Robison, S.G., Cieslak, P.R., 2013. Priming with whole-cell versus acellular pertussis vaccine. N Engl J Med 368, 581–582.

[273] Witt, M.A., Katz, P.H., Witt, D.J., 2012. Unexpectedly limited durability of immunity following acellular pertussis vaccination in preadolescents in a North American outbreak. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 54, 1730–1735.

[274] Sheridan, S.L., Ware, R.S., Grimwood, K., Lambert, S.B., 2012. Number and order of whole cell pertussis vaccines in infancy and disease protection. JAMA 308, 454–456.

[275] Wendelboe, A.M., Van Rie, A., Salmaso, S., Englund, J.A., 2005. Duration of immunity against pertussis after natural infection or vaccination. Pediatr.Infect.Dis.J. 24, 58.

[276] Misegades, L.K., Winter, K., Harriman, K., Talarico, J., Messonnier, N.E., Clark, T.A., Martin, S.W., 2012. Association of childhood pertussis with receipt of 5 doses of pertussis vaccine by time since last vaccine dose, California, 2010. JAMA 308, 2126–2132.

[277] McGirr, A., Fisman, D.N., 2015. Duration of pertussis immunity after DTaP immunization: a meta-analysis. Pediatrics 135, 331–343.

[278] Granstrom, G., Askelof, P., Granstrom, M., 1988. Specific immunoglobulin A to Bordetella pertussis antigens in mucosal secretion for rapid diagnosis of whooping cough. J.Clin.Microbiol. 26, 869–874.

[279] Zackrisson, G., Lagergard, T., Trollfors, B., Krantz, I., 1990. Immunoglobulin A antibodies to pertussis toxin and filamentous hemagglutinin in saliva from patients with pertussis. J.Clin.Microbiol. 28, 1502–1505.

[280] Mills, K.H., 2001. Immunity to Bordetella pertussis. Microbes Infect. 3, 655–677.

[281] Hellwig, S.M., van Spriel, A.B., Schellekens, J.F., Mooi, F.R., van de Winkel, J.G., 2001. Immunoglobulin A-mediated protection against Bordetella pertussis infection. Infect Immun 69, 4846–4850.

[282] Hallander, H. O., Ljungman, M., Storsaeter, J., Gustafsson, L., 2009. Kinetics and sensitivity of ELISA IgG pertussis antitoxin after infection and vaccination with Bordetella pertussis in young children. APMIS 117, 797–807.

[283] Giammanco, A., Taormina, S., Genovese, M., Mangiaracina, G., Giammanco, G., Chiarini, A., 1997. Serological responses to infection with B. pertussis. Dev Biol Stand 89, 213–220.

[284] Higgs, R., Higgins, S.C., Ross, P.J., Mills, K.H., 2012. Immunity to the respiratory pathogen Bordetella pertussis. Mucosal Immunol. 5, 485–500.

[285] Lamberti, Y.A., Hayes, J.A., Perez Vidakovics, M.L., Harvill, E.T., Rodriguez, M.E., 2010. Intracellular trafficking of Bordetella pertussis in human macrophages. Infect Immun 78, 907–913.

[286] Jongerius, I., Schuijt, T.J., Mooi, F.R., Pinelli, E., 2015. Complement evasion by Bordetella pertussis: implications for improving current vaccines. J Mol Med (Berl) 93, 395–402.

[287] Lambris, J.D., Ricklin, D., Geisbrecht, B.V., 2008. Complement evasion by human pathogens. Nat Rev Microbiol 6, 132–142.

[288] Leef, M., Elkins, K.L., Barbic, J., Shahin, R.D., 2000. Protective immunity to Bordetella pertussis requires both B cells and CD4(+) T cells for key functions other than specific antibody production. J Exp Med 191, 1841–1852.

[289] Wilkins RJ., (2006). Oxford handbook of medical sciences. Oxford, Oxford University Press.

[290] Ross, P.J., Sutton, C.E., Higgins, S., Allen, A.C., Walsh, K., Misiak, A., Lavelle, E.C., McLoughlin, R.M., Mills, K.H.G., 2013. Relative contribution of Th1 and Th17 cells in adaptive immunity to Bordetella pertussis: towards the rational design of an improved acellular pertussis vaccine. PLoS Pathog 9, e1003264.

[291] Warfel, J.M., Merkel, T.J., 2013. Bordetella pertussis infection induces a mucosal IL-17 response and long-lived Th17 and Th1 immune memory cells in nonhuman primates. Mucosal Immunol 6, 787–796.

[292] Weaver KL., Blackwood CB., Horspool AM., Pyles GM., Sen-Kilic E., Grayson EM., Huckaby AB., Witt WT., DeJong MA., Wolf MA., Damron FH., Barbier M., 2022. Long-Term Analysis of Pertussis Vaccine Immunity to Identify Potential Markers of Vaccine-Induced Memory Associated With Whole Cell But Not Acellular Pertussis Immunization in Mice. Front Immunol 13:838504.

[293] Rieber, N., Graf, A., Hartl, D., Urschel, S., Belohradsky, B.H., Liese, J., 2011. Acellular pertussis booster in adolescents induces Th1 and memory CD8+ T cell immune response. PLoS One 6, e17271.

[294] Dirix, V., Verscheure, V., Vermeulen, F., De Schutter, I., Goetghebuer, T., Locht, C., Mascart, F., 2012 Both $CD4^+$ and $CD8^+$ lymphocytes participate in the IFN-γ response to filamentous hemagglutinin from Bordetella pertussis in infants, children, and adults. Clin Dev Immunol. 2012:795958.

[295] Mills, K.H.G., Ross, P.J., Allen, A.C., Wilk, M.M., 2014. Do we need a new vaccine to control the re-emergence of pertussis? Trends Microbiol 22, 49–52.

[296] Ausiello, C.M., Urbani, F., la Sala, A., Lande, R., Cassone, A., 1997. Vaccine- and antigen-dependent type 1 and type 2 cytokine induction after primary vaccination of infants with whole-cell or acellular pertussis vaccines. Infect Immun 65, 2168–2174.

[297] Mascart, F., Hainaut, M., Peltier, A., Verscheure, V., Levy, J., Locht, C., 2007. Modulation of the infant immune responses by the first pertussis vaccine administrations. Vaccine 25, 391–398.

[298] Higgins, S.C., Jarnicki, A.G., Lavelle, E.C., Mills, K.H.G., 2006. TLR4 mediates vaccine-induced protective cellular immunity to Bordetella pertussis: role of IL-17-producing T cells. J Immunol 177, 7980–7989.

[299] Ryan, M., Murphy, G., Ryan, E., Nilsson, L., Shackley, F., Gothefors, L., Oymar, K., Miller, E., Storsaeter, J., Mills, K.H., 1998. Distinct T-cell subtypes induced with whole cell and acellular pertussis vaccines in children. Immunology 93, 1–10.

[300] White OJ., Rowe J., Richmond P., Marshall H., McIntyre P., Wood N., Holt PG., 2010. Th2-polarisation of cellular immune memory to neonatal pertussis vaccination. Vaccine 28(14):2648-52.

[301] McGuirk, P., Mills, K.H., 2000. Direct anti-inflammatory effect of a bacterial virulence factor: IL-10-dependent suppression of IL-12 production by filamentous hemagglutinin from Bordetella pertussis. Eur J Immunol 30, 415–422.

[302] Meyer, C.U., Zepp, F., Decker, M., Lee, M., Chang, S.-J., Ward, J., Yoder, S., Bogaert, H., Edwards, K.M., 2007. Cellular immunity in adolescents and adults following acellular pertussis vaccine administration. Clin Vaccine Immunol 14, 288–292.

[303] Smits, K., Pottier, G., Smet, J., Dirix, V., Vermeulen, F., De Schutter, I., Carollo, M., Locht, C., Ausiello, C.M., Mascart, F., 2013. Different T cell memory in preadolescents after whole-cell or acellular pertussis vaccination. Vaccine 32, 111–118.

[304] Salminen, T., Knuutila, A., Barkoff, A.M., Mertsola, J., He, Q., 2018. A rapid lateral flow immunoassay for serological diagnosis of pertussis. Vaccine 36, 1429–1434.

[305] Kennerknecht, N., Riffelmann, M., Schmetz, J., Wirsing von König, C.H., 2011. Comparison of commercially available immunoblot assays measuring IgG and IgA antibodies to Bordetella pertussis antigens. Eur J Clin Microbiol Infect Dis 30, 1531–1535.

[306] Knuutila, A., Rautanen, C., Barkoff, A.-M., Mertsola, J., He, Q., 2022. Whole blood based point-of-care assay for the detection of anti-pertussis toxin IgG antibodies. Journal of Immunological Methods 510, 113361.

[307] Knuutila, A., Duncan, J., Li, F., Eletu, S., Litt, D., Fry, N., He, Q., 2023. Oral fluid-based lateral flow point-of-care assays for pertussis serology. J Med Microbiol 72.

[308] Knuutila, A., Barkoff, A.M., Mertsola, J., Osicka, R., Sebo, P., He, Q., 2021. Simultaneous Determination of Antibodies to Pertussis Toxin and Adenylate Cyclase Toxin Improves Serological Diagnosis of Pertussis. Diagnostics (Basel) 11, 10.3390/diagnostics11020180.

[309] Subissi, L., Rodeghiero, C., Martini, H., Litzroth, A., Huygen, K., Leroux-Roels, G., Pierard, D., Desombere, I., 2019. Assessment of IgA anti-PT and IgG anti-ACT reflex testing to improve Bordetella pertussis serodiagnosis in recently vaccinated subjects. Clin.Microbiol.Infect.

[310] May, M.L., Doi, S.A., King, D., Evans, J., Robson, J.M., 2012. Prospective evaluation of an Australian pertussis toxin IgG and IgA enzyme immunoassay. Clin.Vaccine Immunol. 19, 190–197.

[311] Vidarsson, G., Dekkers, G., Rispens, T., 2014. IgG subclasses and allotypes: from structure to effector functions. Front Immunol 5, 520.

[312] Zaitsev, E.M., Krasnoproshina, L.I., Mazurova, I.K., Petrova, M.S., Popova, O.P., Zakharova, N.S., 2011. Isotypes of anti-pertussis antibodies in pertussis patients at different stages of the disease. Zh.Mikrobiol.Epidemiol.Immunobiol. (3), 53–56.

[313] Sato, H., Ito, A., Chiba, J., Sato, Y., 1984. Monoclonal antibody against pertussis toxin: effect on toxin activity and pertussis infections. Infect.Immun. 46, 422–428.

[314] Sato, H., Sato, Y., Ito, A., Ohishi, I., 1987. Effect of monoclonal antibody to pertussis toxin on toxin activity. Infect Immun 55, 909–915.

[315] Sato, H., Sato, Y., 1990. Protective activities in mice of monoclonal antibodies against pertussis toxin. Infect.Immun. 58, 3369–3374.

[316] Sato, H., Sato, Y., Ohishi, I., 1991. Comparison of pertussis toxin (PT)-neutralizing activities and mouse-protective activities of anti-PT mouse monoclonal antibodies. Infect.Immun. 59, 3832–3835.

[317] Giammanco, A., Taormina, S., Chiarini, A., Dardanoni, G., Stefanelli, P., Salmaso, S., Mastrantonio, P., 2003. Analogous IgG subclass response to pertussis toxin in vaccinated children, healthy or affected by whooping cough. Vaccine 21, 1924–1931.

[318] Hendrikx, L. H., Schure, R.M., Ozturk, K., de Rond, L.G., de Greeff, S.C., Sanders, E.A., Berbers, G.A., Buisman, A.M., 2011. Different IgG-subclass distributions after whole-cell and acellular pertussis infant primary vaccinations in healthy and pertussis infected children. Vaccine 29, 6874–6880.

[319] van Twillert, I., Bonacic Marinovic, A.A., Kuipers, B., van Gaans-van den Brink, J.A., Sanders, E.A., van Els, C.A., 2017. Impact of age and vaccination history on long-term serological responses after symptomatic B. pertussis infection, a high dimensional data analysis. Sci.Rep. 7, 40328.

[320] Zackrisson, G., Lagergard, T., Trollfors, B., 1989. Subclass compositions of immunoglobulin G to pertussis toxin in patients with whooping cough, in healthy individuals, and in recipients of a pertussis toxoid vaccine. J.Clin.Microbiol. 27, 1567–1571.

[321] Rastawicki, W., Smietanska, K., Rokosz-Chudziak, N., Jagielski, M., 2013. Serum immunoglobulin IgG subclass distribution of antibody responses to pertussis toxin and filamentous hemagglutinin of Bordetella pertussis in patients with whooping cough. Med.Dosw.Mikrobiol. 65, 269–274.

[322] Schure, R.-M., Hendrikx, L.H., de Rond, L.G.H., Öztürk, K., Sanders, E.A.M., Berbers, G.A.M., Buisman, A.-M., 2013. 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. Clinical and Vaccine Immunology 20, 1388–1395.

[323] Diavatopoulos, D.A., Edwards, K.M., 2017. What Is Wrong with Pertussis Vaccine Immunity? Why Immunological Memory to Pertussis Is Failing. Cold Spring Harb Perspect Biol 9, a029553.

[324] Lagergard, T., Trollfors, B., Claesson, B.A., Karlberg, J., Taranger, J., 1992. Determination of neutralizing antibodies and specific immunoglobulin isotype levels in infants after vaccination against diphtheria. Eur.J.Clin.Microbiol.Infect.Dis. 11, 341–345.

[325] Palmer, S.R., Balfour, A.H., Jephcott, A.E., 1983. Immunisation of adults during an outbreak of diphtheria. Br.Med.J.(Clin.Res.Ed) 286, 624–626.

[326] Gillenius, P., Jäätmaa, E., Askelöf, P., Granström, M., Tiru, M., 1985. The standardization of an assay for pertussis toxin and antitoxin in microplate culture of Chinese hamster ovary cells. J.Biol.Stand. 13, 61–66.

[327] Hewlett, E.L., Sauer, K.T., Myers, G.A., Cowell, J.L., Guerrant, R.L., 1983. Induction of a novel morphological response in Chinese hamster ovary cells by pertussis toxin. Infect.Immun. 40, 1198–1203.

[328] Markey, K., Asokanathan, C., Feavers, I., 2019. Assays for Determining Pertussis Toxin Activity in Acellular Pertussis Vaccines. Toxins 11, 417.

[329] Millen, S.H., Bernstein, D.I., Connelly, B., Ward, J.I., Chang, S.-J., Weiss, A.A., 2004. Antibody-mediated neutralization of pertussis toxin-induced mitogenicity of human peripheral blood mononuclear cells. Infect Immun 72, 615–620.

[330] Blanchard Rohner, G., Chatzis, O., Chinwangso, P., Rohr, M., Grillet, S., Salomon, C., Lemaître, B., Boonrak, P., Lawpoolsri, S., Clutterbuck, E., Poredi, I.K., Wijagkanalan, W., Spiegel, J., Pham, H.T., Viviani, S., Siegrist, C.A., 2019. Boosting Teenagers With Acellular Pertussis Vaccines Containing Recombinant or Chemically Inactivated Pertussis Toxin: A Randomized Clinical Trial. Clin.Infect.Dis. 68, 1213–1222.

[331] Isbrucker, R., Daas, A., Wagner, L., Costanzo, A., 2016. Transferability study of CHO cell clustering assays for monitoring of pertussis toxin activity in acellular pertussis vaccines. Pharmeur Bio Sci Notes 2015, 97–114.

[332] Markey, K., Douglas-Bardsley, A., Hockley, J., Le Tallec, D., Costanzo, A., 2018. Calibration of pertussis toxin BRP batch 1 in a standardised CHO cell-based clustering assay. Pharmeur Bio Sci Notes 2018, 112–123.

[333] Podda, A., Nencioni, L., De Magistris, M.T., Di Tommaso, A., Bossù, P., Nuti, S., Pileri, P., Peppoloni, S., Bugnoli, M., Ruggiero, P., 1990. Metabolic, humoral, and cellular responses in adult volunteers immunized with the genetically inactivated pertussis toxin mutant PT-9K/129G. J Exp Med 172, 861–868.

[334] Carlsson, R.M., Claesson, B.A., Selstam, U., Fagerlund, E., Granstrom, M., Blondeau, C., Hoffenbach, A., 1998. 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. 17, 1026–1033.

[335] Meade, B.D., Lynn, F., Reed, G.F., Mink, C.M., Romani, T.A., Deforest, A., Deloria, M.A., 1995. Relationships between functional assays and enzyme immunoassays as measurements of responses to acellular and whole-cell pertussis vaccines. Pediatrics 96, 595–600.

[336] Isacson, J., Trollfors, B., Lagergrd, T., Taranger, J., 1997. Comparison of a toxin neutralization assay and ELISA for determination of pertussis toxin antibodies. Serodiagnosis and Immunotherapy in Infectious Disease 8, 163–167.

[337] Zhang, Y., Li, Y., Chen, Z., Liu, X., Peng, X., He, Q., 2021. Determination of serum neutralizing antibodies reveals important difference in quality of antibodies against pertussis toxin in children after infection. Vaccine 39, 1826–1830.

[338] Ahmed, R., Gray, D., 1996. Immunological memory and protective immunity: understanding their relation. Science (New York, N.Y.) 272, 54–60.

[339] Denoel, P.A., Goldblatt, D., Vleeschauwer, I. de, Jacquet, J.M., Pichichero, M.E., Poolman, J.T., 2007. Quality of the Haemophilus influenzae type b (Hib) antibody response induced by diphtheria-tetanus-acellular pertussis/Hib combination vaccines. Clinical and vaccine immunology : CVI 14, 1362–1369.

[340] Goldblatt, D., Vaz, A.R., Miller, E., 1998. Antibody avidity as a surrogate marker of successful priming by Haemophilus influenzae type b conjugate vaccines following infant immunization. J.Infect.Dis. 177, 1112–1115.

[341] Goodnow, C.C., Vinuesa, C.G., Randall, K.L., Mackay, F., Brink, R., 2010. Control systems and decision making for antibody production. Nat.Immunol. 11, 681–688.

[342] McHeyzer-Williams, M.G., McLean, M.J., Lalor, P.A., Nossal, G.J., 1993. Antigen-driven B cell differentiation in vivo. J.Exp.Med. 178, 295–307.

[343] Schallert, N., Pihlgren, M., Kovarik, J., Roduit, C., Tougne, C., Bozzotti, P., Giudice, G.D., Siegrist, C.A., Lambert, P.H., 2002. Generation of adult-like antibody avidity profiles after early-life immunization with protein vaccines. Eur.J.Immunol. 32, 752–760.

[344] Berek, C., Berger, A., Apel, M., 1991. Maturation of the immune response in germinal centers. Cell 67, 1121–1129.

[345] Kepler, T.B., Perelson, A.S., 1993. Somatic hypermutation in B cells: an optimal control treatment. J.Theor.Biol. 164, 37–64.

[346] Eisen, H.N., 1964. Determination of Antibody Affinity for Haptens and Antigens by Means of Fluorescence Quenching. Methods Med.Res. 10, 115–121.

[347] French, D.L., Laskov, R., Scharff, M.D., 1989. The role of somatic hypermutation in the generation of antibody diversity. Science (New York, N.Y.) 244, 1152–1157.

[348] Dorner, T., Radbruch, A., 2007. Antibodies and B cell memory in viral immunity. Immunity 27, 384–392.

[349] Almanzar, G., Ottensmeier, B., Liese, J., Prelog, M., 2013. Assessment of IgG avidity against pertussis toxin and filamentous hemagglutinin via an adapted enzyme-linked immunosorbent assay (ELISA) using ammonium thiocyanate. J.Immunol.Methods 387, 36–42.

[350] Prelog, M., Almanzar, G., Rieber, N., Ottensmeier, B., Zlamy, M., Liese, J., 2013. Differences of IgG antibody avidity after an acellular pertussis (aP) booster in adolescents after a whole cell (wcP) or aP primary vaccination. Vaccine 31, 387–393.

[351] Barkoff, A.M., Grondahl-Yli-Hannuksela, K., Vuononvirta, J., Mertsola, J., Kallonen, T., He, Q., 2012. Differences in avidity of IgG antibodies to pertussis toxin after acellular pertussis booster vaccination and natural infection. Vaccine 30, 6897–6902.

[352] Hendrikx, L. H., Felderhof, M.K., Ozturk, K., de Rond, L.G., van Houten, M.A., Sanders, E.A., Berbers, G.A., Buisman, A.M., 2011b. Enhanced memory B-cell immune responses after a second acellular pertussis booster vaccination in children 9 years of age. Vaccine 30, 51–58.

[353] Hendrikx, L.H., Berbers, G.A., Veenhoven, R.H., Sanders, E.A., Buisman, A.M., 2009. IgG responses after booster vaccination with different pertussis vaccines in Dutch children 4 years of age: effect of vaccine antigen content. Vaccine 27, 6530–6536.

[354] Moriuchi, T., Otsuka, N., Hiramatsu, Y., Shibayama, K., Kamachi, K., 2017. A high seroprevalence of antibodies to pertussis toxin among Japanese adults: Qualitative and quantitative analyses. PLoS One 12, e0181181.

[355] Hovingh, E.S., Kuipers, B., Bonacic Marinovic, A. A., Jan Hamstra, H., Hijdra, D., Mughini Gras, L., van Twillert, I., Jongerius, I., van Els, C., Pinelli, E., 2018. Detection of opsonizing antibodies directed against a recently circulating Bordetella pertussis strain in paired plasma samples from symptomatic and recovered pertussis patients. Scientific reports 8, 12039–12038.

[356] Eby, J.C., Hoffman, C.L., Gonyar, L.A., Hewlett, E.L., 2015. Review of the neutrophil response to Bordetella pertussis infection. Pathog Dis 73, ftv081.

[357] Lesne, E., Cavell, BE., Freire-Martin, I., Persaud, R., Alexander, F., Taylor, S., Matheson, M., van Els, C., Gorringe, A., 2020. Acellular Pertussis Vaccines Induce Anti-pertactin Bactericidal Antibodies Which Drives the Emergence of Pertactin-Negative Strains. Front Microbiol. 11:2108.

[358] Halperin, S.A., Issekutz, T.B., Kasina, A., 1991. Epitope specificity of three anti-pertussis toxin monoclonal antibodies with dissimilar effects in assays of toxin neutralizing activity. Mol.Immunol. 28, 247–250.

[359] Sutherland, J.N., Chang, C., Yoder, S.M., Rock, M.T., Maynard, J.A., 2011. Antibodies Recognizing Protective Pertussis Toxin Epitopes Are Preferentially Elicited by Natural Infection versus Acellular Immunization. Clin. Vaccine Immunol. 18, 954–962.

[360] Sutherland, J.N., Maynard, J.A., 2009. Characterization of a key neutralizing epitope on pertussis toxin recognized by monoclonal antibody 1B7. Biochemistry (N.Y.) 48, 11982–11993.

[361] Kowalsky, C.A., Faber, M.S., Nath, A., Dann, H.E., Kelly, V.W., Liu, L., Shanker, P., Wagner, E.K., Maynard, J.A., Chan, C., Whitehead, T.A., 2015. Rapid fine conformational epitope mapping using comprehensive mutagenesis and deep sequencing. The Journal of biological chemistry 290, 26457–26470.

[362] Acquaye-Seedah, E., Huang, Y., Sutherland, J.N., DiVenere, A.M., Maynard, J.A., 2018. Humanised monoclonal antibodies neutralise pertussis toxin by receptor blockade and reduced retrograde trafficking. Cell.Microbiol. 20, e12948.

[363] Wagner, E.K., Wang, X., Bui, A., Maynard, J.A., 2016. Synergistic Neutralization of Pertussis Toxin by a Bispecific Antibody In Vitro and In Vivo. Clinical and vaccine immunology : CVI 23, 851–862.

[364] Nguyen, A.W., DiVenere, A.M., Papin, J.F., Connelly, S., Kaleko, M., Maynard, J.A., 2020. Neutralization of pertussis toxin by a single antibody prevents clinical pertussis in neonatal baboons. Science advances 6, eaay9258.

[365] Acquaye-Seedah, E., Reczek, E.E., Russell, H.H., DiVenere, A.M., Sandman, S.O., Collins, J.H., Stein, C.A., Whitehead, T.A., Maynard, J.A., 2018. Characterization of Individual Human Antibodies That Bind Pertussis Toxin Stimulated by Acellular Immunization. Infect.Immun. 86, 4.

[366] Vaughan, K., Seymour, E., Peters, B., Sette, A., 2014. Substantial gaps in knowledge of Bordetella pertussis antibody and T cell Cross Mark epitopes relevant for natural immunity and vaccine efficacy. Hum.Immunol. 75, 440–451.

[367] Lagstrom, H., Rautava, P., Kaljonen, A., Raiha, H., Pihlaja, P., Korpilahti, P., Peltola, V., Rautakoski, P., Osterbacka, E., Simell, O., Niemi, P., 2013. Cohort profile: Steps to the healthy development and well-being of children (the STEPS Study). Int.J.Epidemiol. 42, 1273–1284.

[368] Dalby, T., Seier-Petersen, M., Kristiansen, M.P., Harboe, Z.B., Krogfelt, K.A., 2009. Problem solved: a modified enzyme-linked immunosorbent assay for detection of human antibodies to pertussis toxin eliminates false-positive results occurring at analysis of heat-treated sera. Diagn.Microbiol.Infect.Dis. 63, 354–360.

[369] He, Q., Mertsola, J., Himanen, J.P., Ruuskanen, O., Viljanen, M.K., 1993. Evaluation of pooled and individual components of Bordetella pertussis as antigens in an enzyme immunoassay for diagnosis of pertussis. Eur.J.Clin.Microbiol.Infect.Dis. 12, 690–695.

[370] Fumimoto, R., Otsuka, N., Sunagawa, T., Tanaka-Taya, K., Kamiya, H., Kamachi, K., 2019b. Age-related differences in antibody avidities to pertussis toxin and filamentous hemagglutinin in a healthy Japanese population. Vaccine 37, 2463–2469.

[371] Abu-Raya, B., Giles, M.L., Kollmann, T.R., Sadarangani, M., 2020. Profiling avidity of antibodies elicited by vaccination using enzyme-linked immunosorbent assay-based elution - Insights into a novel experimental and analytical approach. Vaccine 38, 5389–5392.

[372] Kapil, P., Merkel, T.J., 2019. Pertussis vaccines and protective immunity. Curr Opin Immunol 59, 72–78.

[373] Ahvenainen, N.,, Dub, T., Knuutila, A., Barkoff, A.M., Sane, J., He, Q., 2021 Widespread circulation of pertussis in Finland during 1968-1972 when the whole cell vaccine was in use. Clin. Microbiol. Infect. 27, 1526–1528.

[374] Granström, M., Granström, G., 1993. Serological correlates in whooping cough. Vaccine 11, 445–448.

[375] Granstrom, M., Granstrom, G., Gillenius, P., Askelof, P., 1985. Neutralizing antibodies to pertussis toxin in whooping cough. J.Infect.Dis. 151, 646–649.

[376] Dimitrov, J.D., Lacroix-Desmazes, S., Kaveri, S.V., 2011. Important parameters for evaluation of antibody avidity by immunosorbent assay. Anal.Biochem. 418, 149–151.

[377] Luijkx, T., van Dijken, H., van Els, C., van den Dobbelsteen, G., 2006. Heterologous prime-boost strategy to overcome weak immunogenicity of two serosubtypes in hexavalent Neisseria meningitidis outer membrane vesicle vaccine. Vaccine 24, 1569–1577.

[378] G. R. Pullen, M. G. Fitzgerald, and C. S. Hosking, "Antibody avidity determination by ELISA using thiocyanate elution," J.Immunol.Methods, vol. 86, no. 1, pp. 83–87, 1986.

[379] Luijkx, T., van Gaans-van den Brink, J.A., van Dijken, H., van den Dobbelsteen, G., van Els, C., 2008. Hyperproliferation of B cells specific for a weakly immunogenic PorA in a meningococcal vaccine model. Clinical and vaccine immunology : CVI 15, 1598–1605.

[380] Cherry, J.D., 2013. Pertussis: challenges today and for the future. PLoS Pathog. 9, e1003418.

[381] Marchant, A., Pihlgren, M., Goetghebuer, T., Weiss, H.A., Ota, M.O., Schlegel-Hauter, S.E., Whittle, H., Lambert, P.H., Newport, M.J., Siegrist, C.A., Group, M.R.C.G.T.S., 2006. Predominant influence of environmental determinants on the persistence and avidity maturation of antibody responses to vaccines in infants. J.Infect.Dis. 193, 1598–1605.

[382] Matos, D.C., Silva, A.M., Neves, P.C., Martins, R.M., Homma, A., Marcovistz, R., 2009. Pattern of functional antibody activity against Haemophilus influenzae type B (Hib) in infants immunized with diphtheria-tetanus-pertussis/Hib Brazilian combination vaccine. Brazilian journal of medical and biological research = Revista brasileira de pesquisas medicas e biologicas 42, 1242–1247.

[383] CDC, 2023b. https://www.cdc.gov/pertussis/images/incidence-graph-age-2021.png, accessed 18.8.2023

[384] de Greeff, S.C., Mooi, F.R., Westerhof, A., Verbakel, J.M., Peeters, M.F., Heuvelman, C.J., Notermans, D.W., Elvers, L.H., Schellekens, J.F., de Melker, H.E., 2010. Pertussis disease burden in the household: how to protect young infants. Clin.Infect.Dis. 50, 1339–1345.

[385] Blennow, M., Granstrom, M., Jaatmaa, E., Olin, P., 1988. Primary immunization of infants with an acellular pertussis vaccine in a double-blind randomized clinical trial. Pediatrics 82, 293–299.

[386] Baker, J.D., Halperin, S.A., Edwards, K., Miller, B., Decker, M., Stephens, D., 1992. Antibody response to Bordetella pertussis antigens after immunization with American and Canadian whole-cell vaccines. J.Pediatr. 121, 523–527.

[387] Pichichero, M.E., Francis, A.B., Marsocci, S.M., Green, J.L., Disney, F.A., Meschievitz, C., 1993. 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 91, 756–760.

[388] Giuliano, M., Mastrantonio, P., Giammanco, A., Piscitelli, A., Salmaso, S., Wassilak, S.G., 1998. Antibody responses and persistence in the two years after immunization with two acellular vaccines and one whole-cell vaccine against pertussis. J.Pediatr. 132, 983–988.

[389] Carlsson, R.M., Claesson, B.A., Fagerlund, E., Knutsson, N., Lundin, C., 2002. Antibody persistence in five-year-old children who received a pentavalent combination vaccine in infancy. Pediatr.Infect.Dis.J. 21, 535–541.

[390] Knuutila, A., Barkoff, A.-M., Ivaska, L., Tenhu, E., Teräsjärvi, J., van Gageldonk, P., Buisman, A., Mertsola, J., He, Q., PERISCOPE consortium, 2023. Effect of immunization during pregnancy and pre-existing immunity on diphtheria-tetanus-acellular pertussis vaccine responses in infants. Emerg Microbes Infect 12, 2204146.

[391] Dalby, T., Sørensen, C., Petersen, J.W., Krogfelt, K.A., 2010. Pertussis serology: assessment of IgG anti-PT ELISA for replacement of the CHO cell assay. APMIS 118, 968–972.

[392] Trollfors, B., Krantz, I., Sigurs, N., Taranger, J., Zackrisson, G., Roberson, R., 1988. Toxin-neutralizing antibodies in patients with pertussis, as determined by an assay using Chinese hamster ovary cells. J.Infect.Dis. 158, 991–995.

[393] Blennow, M., Granstrom, M., 1990. Long term serologic follow-up after pertussis immunization. Pediatr.Infect.Dis.J. 9, 21–26.

[394] Paramonov, V., Sahlgren, C., Rivero-Müller, A., Pulliainen, A., 2020. iGIST-A Kinetic Bioassay for Pertussis Toxin Based on Its Effect on Inhibitory GPCR Signaling. ACS Sens. 25, 3438-3448.

[395] Versteegen, P., Barkoff, A.-M., Valente Pinto, M., van de Kasteele, J., Knuutila, A., Bibi, S., de Rond, L., Teräsjärvi, J., Sanders, K., de Zeeuw-Brouwer, M.-L., Luoto, R., Ten Hulscher, H., Clutterbuck, E.A., Sanders, E.A.M., Mertsola, J., Berbers, G.A.M., He, Q., Kelly, D.F., Buisman, A.-M., PERISCOPE Consortium, 2022. Memory B Cell Activation Induced by Pertussis Booster Vaccination in Four Age Groups of Three Countries. Front Immunol 13, 864674.

[396] Maertens, K., Hoang, T.H.T., Cabore, R.N., Leuridan, E., 2015. Avidity of maternal pertussis antibodies after vaccination during pregnancy. Vaccine 33, 5489.

[397] Marchant, A., Kollmann, T.R., 2015. Understanding the ontogeny of the immune system to promote immune-mediated health for life. Frontiers in immunology 6, 77.

[398] Prince, H.E., Lieberman, J.M., Cherry, J.D., 2012. Age-related differences in patterns of increased Bordetella pertussis antibodies. Clin.Vaccine Immunol. 19, 545–550.

[399] Mooi, F.R., van Loo, I.H., van Gent, M., He, Q., Bart, M.J., Heuvelman, K.J., de Greeff, S.C., Diavatopoulos, D., Teunis, P., Nagelkerke, N., Mertsola, J., 2009. Bordetella pertussis strains with increased toxin production associated with pertussis resurgence. Emerg.Infect.Dis. 15, 1206–1213.

[400] King, A.J., van der Lee, S., Mohangoo, A., van Gent, M., van der Ark, A., van de Waterbeemd, B., 2013. Genome-wide gene expression analysis of Bordetella pertussis isolates associated with a resurgence in pertussis: elucidation of factors involved in the increased fitness of epidemic strains. PLoS One 8, e66150.

[401] Cabore, R.N., Maertens, K., Dobly, A., Leuridan, E., Damme, P.V., Huygen, K., 2017. Influence of maternal vaccination against diphtheria, tetanus, and pertussis on the avidity of infant antibody responses to a pertussis containing vaccine in Belgium. Virulence 8, 1245–1254.

[402] Thorstensson R., Trollfors B., Al-Tawil N., Jahnmatz M., Bergström J., Ljungman M., Törner A., Wehlin L., Van Broekhoven A., Bosman F., Debrie AS., Mielcarek N., Locht C., 2014. 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. 9(1):e83449.

[403] Sirivichayakul, C., Chanthavanich, P., Limkittikul, K., Siegrist, C.A., Wijagkanalan, W., Chinwangso, P., Petre, J., Hong Thai, P., Chauhan, M., Viviani, S., 2017. Safety and immunogenicity of a combined Tetanus, Diphtheria, recombinant acellular Pertussis vaccine (TdaP) in healthy Thai adults. Hum.Vaccin Immunother. 13, 136–143.lon

[404] Agnolon, V., Bruno, C., Leuzzi, R., Galletti, B., D'Oro, U., Pizza, M., Seubert, A., O'Hagan, D.T., Baudner, B.C., 2015. The potential of adjuvants to improve immune responses against TdaP vaccines: A preclinical evaluation of MF59 and monophosphoryl lipid A. Int.J.Pharm. 492, 169–176.

[405] Davies, V., Vaughan, K., Damle, R., Peters, B., Sette, A., 2009. Classification of the universe of immune epitope literature: representation and knowledge gaps. PloS one 4, e6948.

[406] Bart, M.J., van Gent, M., van der Heide, H.G.J., Boekhorst, J., Hermans, P., Parkhill, J., Mooi, F.R., 2010. Comparative genomics of prevaccination and modern Bordetella pertussis strains. BMC Genomics 11, 627.

[407] Gent, M. van, Bart, M.J., Heide, H.G.J. van der, Heuvelman, K.J., Mooi, F.R., 2012. Small Mutations in Bordetella pertussis Are Associated with Selective Sweeps. PLOS ONE 7, e46407.

[408] Maharjan, R.P., Gu, C., Reeves, P.R., Sintchenko, V., Gilbert, G.L., Lan, R., 2008. Genome-wide analysis of single nucleotide polymorphisms in Bordetella pertussis using comparative genomic sequencing. Research in Microbiology 159, 602–608.

[409] Octavia, S., Maharjan, R.P., Sintchenko, V., Stevenson, G., Reeves, P.R., Gilbert, G.L., Lan, R., 2011. Insight into Evolution of Bordetella pertussis from Comparative Genomic Analysis: Evidence of Vaccine-Driven Selection. Molecular Biology and Evolution 28, 707–715.

TURUN
YLIOPISTO
UNIVERSITY
OF TURKU

Painosalama, Turku, Finland 2024

ISBN 978-951-29-9628-5 PRINT
ISBN 978-951-29-9629-2 PDF
ISSN 0355-9483 (Painettu/Print)
ISSN 2343-3213 (Sähköinen/Online)