WHO Immunological Basis for Immunization Series

Module 4: Pertussis
Update 2017

Immunization, Vaccines and Biologicals

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Contents

Abbreviations and acronyms

AC	adenylate cyclase
ACT	adenylate cyclase toxin
AGG	agglutinogens
aP	acellular pertussis (vaccine)
BA	bacterial agglutination
BrkA	Bordetella resistance to killing genetic locus, frame A
BvgAS	complex virulence expression system
CHO	Chinese hamster ovary (cells)
CMI	cell-mediated immunity
DNT	dermonecrotic toxin
DTaP	diphtheria–tetanus–acellular pertussis
DTP	diphtheria-tetanus-pertussis vaccine
DTwP	diphtheria–tetanus whole-cell pertussis (vaccine)
ELISA	enzyme-linked immunosorbent assay
ELISPOT	enzyme-linked immunospot assay
EPI	Expanded Programme on Immunization
ESEN	European Sero-Epidemiology Network
FHA	filamentous haemagglutinin
FIM	fimbriae
GMT	geometric mean titre
GSK	GlaxoSmithKline
HCW	health-care worker
Hib	Haemophilus influenzae type b
HLT	heat-labile toxin
ICS	intracellular cytokine secretion
Ig	immunoglobulin
IPV	inactivated polio vaccine
Lf	flocculation units of toxoid

LOS	lipooligosaccharide
LPS	lipopolysaccharide
MRC	Medical Research Council
NACI	National Advisory Committee on Immunization
NIBSC	National Institute for Biological Standards & Control
NIH	National Institutes of Health
NT	neutralization test
OMP	outer membrane protein
PRN	pertactin
PRP	polyribosyl-ribitol-phosphate
PT	pertussis toxin
RGD	arginine-glycine-aspartic acid
RTX	repeats-in-toxin
SphB1	serine-protease/lipoprotein
TCT	tracheal cytotoxin
Tdap	DTaP with reduced antigen content
VE	vaccine efficacy
WHO	World Health Organization
wP	whole-cell pertussis (vaccine)

Preface

This module is part of the WHO series The Immunological Basis for Immunization, which was initially developed in 1993 as a set of eight modules, comprising one module on general immunology and seven modules each devoted to one of the vaccines recommended for the Expanded Programme on Immunization, i.e. vaccines against diphtheria, measles, pertussis, polio, tetanus, tuberculosis and yellow fever. Since then, this series has been updated and extended to include other vaccines of international importance.

The main purpose of the modules is to provide national immunization managers and vaccination professionals with an overview of the scientific basis of vaccination against a range of important infectious diseases. The modules developed since 1993 continue to be vaccine-specific, reflecting the biological differences in immune responses to the individual pathogens and the differing strategies employed to create the best possible level of protection that can be provided by vaccination. The modules also serve as a record of the immunological basis for the WHO recommendations on vaccine use, published in the WHO vaccine position papers.[*]

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[*] See: http://www.who.int/immunization/documents/positionpapers_intro/en/index.html, accessed 10 August 2017.

1. Pertussis

Bordetella pertussis is a strictly human pathogen with multiple biological activities. The bacteria are transmitted by droplets and the infectious dose is small in immunologically naïve patients. Pertussis infection begins with the attachment of *B. pertussis* to the ciliated epithelium of the respiratory tract; the subsequent manifestations are thought to be the result of the interplay between various virulence factors (toxins and adhesins) of the organism (Table 1). Irrespective of high vaccination coverage in infants and toddlers, *B. pertussis* circulates in all countries; reinfections are common and occur throughout a person's lifetime.

The genomes of the three classical *Bordetella* species – *B. pertussis, B. parapertussis and B. bronchiseptica* – as well as of some other *Bordetella* spp. have been sequenced and are publicly available (Parkhill et al., 2003; Sebaihia et al., 2006; Bouchez & Guiso, 2013; Harvill et al., 2014; Gross et al., 2008). *B. pertussis* and *B. parapertussis* appear to have emerged relatively recently from a common *B. bronchiseptica*-like ancestor (Diavatopoulos et al., 2005). Changes in virulence factor expression in *Bordetella* spp. have also been studied at the genomic level (Linz et al., 2016).

Large parts of the genome of *B. pertussis* and *B. parapertussis* were inactivated or lost during adaptation to the human host a result of an expansion of insertion sequence elements. Compared to other human pathogens, isolates of *B. pertussis* show only small genomic heterogeneity, suggesting a more recent development as a human pathogen, but the population structure of *B. pertussis* is constantly evolving (Bart et al., 2014, Linz et al., 2016).

Table 1: Virulence factors of *B. pertussis* and *B. parapertussis*

Virulence factor	Function/structure	Vaccine component	Antibody isotypes	B. pertussis	B. parapertussis
FHA	Adhesion	wP, most aP	IgG, IgA	yes	yes
	Surface associated + secreted				
Fimbriae	Adhesion	wP, many aP	IgG, IgA, IgM	yes	no
	Surface associated				
Pertactin	Cell binding, adhesion	wP, many aP	IgG, IgA	yes	yes
	Surface protein				
BrkA	Possible adhesion	wP	IgG, *		no
	Surface protein				
PT	Suppression of host response	wP, all aP	IgG, IgA	yes	no
	Adhesion				
	Surface associated and secreted				
ACT	Suppression of host response	wP	IgG, *	yes	yes
	Surface associated and secreted				
Dermonecrotic toxin	Induction of host cell necrosis	wP	IgG, *	yes	
	Secreted				
Tracheal cytotoxin	Damage to cilia	wP			
	Peptidoglycan by-product				
LOS/LPS	Induces fever + other LPS responses	wP	IgM, IgG	yes	yes
	Gram-negative cell surface				

Note: * = antibody isotypes are not entirely known

2. Antigens of *Bordetella pertussis*

The structure and in vitro function of many *B. pertussis* virulence factors are quite well understood and this has led to the development of acellular pertussis (aP) vaccines. For a review of the biology of *B. pertussis*, see Melvin et al., 2014.

2.1 Regulation of antigen production

The virulence factors are controlled by a complex virulence expression system (BvgAS). BvgA is a DNA-binding response regulator, and BvgS is a 135-kDa transmembrane sensor kinase. The virulence factors under the regulation of the BvgAS system may be functionally characterized as adhesins and autotransporters (filamentous haemagglutinin, fimbriae, pertactin and tracheal colonization factor), toxins (pertussis toxin, adenylate cyclase toxin, dermonecrotic toxin, tracheal cytotoxin and lipopolysaccharide), and other antigens. Decades ago, it was observed that *B. pertussis* can display different "phases" (referred to as phases I, II, III and IV) in response to the environment. Thus, *B. pertussis*, like other *Bordetella* spp, is capable of responding to the environment by switching from the X mode (all virulence factors expressed) to the I mode, in which some virulence factors are suppressed, and theoretically also to the C mode, in which almost no virulence factors are expressed. The C mode has been demonstrated as a starvation survival mode in *B. bronchiseptica*. For a summary see Melvin et al. (2014).

2.2 Pertussis toxin

The best-known toxin of *B. pertussis* is pertussis toxin (PT), which has several biological activities and is secreted by a type IV secretion system. PT, like other bacterial toxins, is a typical AB toxin consisting of two main subunits – an enzymatically active A (S1) subunit and a B (S2–S5) oligomer which binds to receptors on target cells. The B oligomer has no enzymatic activity but is required for efficient binding of the toxin to cells and allows the S1 enzymatic subunit to reach the site of action within the target cell. S1 is an ADP-ribosyltransferase which ribosylates G proteins (Pittman, 1979; Burns, 1988; Kerr & Matthews, 2000). PT can be inactivated chemically or genetically but still retains its immunogenicity (Edwards et al., 1995). Other biological activities of PT include histamine sensitization, induction of lymphocytosis, insulin secretion and modification of immune responses. PT is produced only by *B. pertussis* although the genome of other *Bordetella* spp. such as *B. parapertussis* and *B. bronchiseptica* contain a nonfunctional *ptx* locus. Thus, PT is the only antigen specific for *B. pertussis*.

Sequencing of the *ptx* locus in circulating and historic strains has shown that PT displays some degree of polymorphism with different *ptx* genes (*ptx1*, *ptx2* etc.). Some polymorphism is also observed in the promoter of the PT-operon (*ptxP*), which could modify the expression of the toxin (Mooi et al., 2009). Current isolates worldwide mainly are of the *ptxP3* type (Bart et al., 2014). Antibodies to PT are induced by infection and vaccination and are used for diagnostic serology. PT is critical to the action of *B. pertussis* and is a component of all aP vaccines.

2.3 Adenylate cyclase toxin

B. pertussis adenylate cyclase toxin (ACT), a haemolysin with enzymatic activity, is secreted in high concentration into the extracytoplasmatic space via a type I secretion system. It is produced by both *B. pertussis* and *B. parapertussis*, and belongs to the family of bacterial activated repeats-in-toxin (RTX) toxins. ACT is activated by calmodulin. By close contact between the bacteria and the host cells, ACT enters the cells and inhibits the microbicidal and cytotoxic function of neutrophils, monocytes and natural killer cells. ACT probably contributes to clinical pertussis through impairment of host defenses or through a direct effect on the respiratory mucosa (Hewlett et al., 2006; Sebo et al., 2014). ACT is produced during pertussis infection in humans, but antibody to ACT has not been consistently shown after infection or after vaccination with whole-cell pertussis vaccines (Farfel et al., 1990; Cherry et al., 2004). It is a conserved toxin and isolates of *B. pertussis* or *B. parapertussis* without ACT production have not been found.

Inactivated ACT toxin is not a component of aP vaccines.

2.4 Lipopolysaccharide

Like other gram-negative bacteria, *B. pertussis* organisms produce a lipopolysaccharide endotoxin (LPS). By contrast with other *Bordetella* spp., however, the *B. pertussis* LPS lacks a long O-antigenic chain, and is also called lipooligosaccharide (LOS). In contrast, *B. parapertussis* LPS has a long O antigen which plays an important role in the virulence of the bacterium (Zhang et al., 2009). LOS is probably responsible for some of the adverse reactions in children following whole-cell pertussis immunization, and has antigenic (although not protective) and adjuvant properties. The amount of LOS in wP vaccines has been shown to be largely associated with the frequency of fever after vaccination (Baraff et al., 1989). LOS was also recognized as one of the agglutinogens, formerly called AGG1.

LPS induces antibodies after infection and vaccination with wP vaccines.

B. pertussis LOS is not a declared component of aP vaccines.

2.5 Dermonecrotic toxin, heat-labile toxin

Dermonecrotic toxin (DNT), one of the first virulence factors of *B. pertussis* to be discovered, induces dermal necrosis in mice when injected intradermally. It also induces necrosis of various other cell types in vitro. This heat-labile toxin (HLT) is a 160 kDa protein, the structure of which is compatible with an A-B model of bacterial toxins. However, in a mouse model, variants of *B. pertussis* lacking DNT are no less virulent than the parental strain. It is not secreted. DNT is not contained in aP vaccines.

2.6   Tracheal cytotoxin

Tracheal cytotoxin (TCT) is a fragment of bacterial peptidoglycan that causes loss of ciliated cells and reduction of cilial activity in vitro, possibly related to an increase in nitric oxide and/or IL-1ɑ. The structure of TCT resembles that of the biological response modifier muramyl dipeptide (Flak & Goldman, 1999). Due to its molecular size, it does not induce antibodies, but is recognized by peptidoglycan-binding-proteins (PGRP) (Swaminathan et al., 2006).

TCT is not contained in aP vaccines.

2.7   Filamentous haemagglutinin

Filamentous haemagglutinin (FHA) is a large hairpin-shaped (molecular-weight 220 kDa) surface-associated and secreted protein. FHA has no enzymatic activity but plays a major role in the initial colonization of *B. pertussis* by mediating the adhesion of *B. pertussis* to the ciliated epithelium of the upper respiratory tract. FHA belongs to the "two-partner secretion" systems of bacterial excreted proteins, in which a transporter protein, subtilisin-like serine-protease/lipoprotein (SphB1), is responsible for the recognition and transport of FHA. FHA is produced by *B. pertussis*, *B. parapertussis* and *B. bronchiseptica*, and it cross-reacts with structures from other bacteria (Scheller & Cotter, 2015). Genomic studies of the *fha* genes have shown almost no heterogeneity among different clinical isolates (Mooi & Greef, 2007).

FHA induces antibodies after infection and vaccination.

FHA is contained in most aP vaccines.

2.8   Pertactin

Pertactin (PRN), an autotransporter, is a 68–70 kDa surface protein that mediates eukaryotic cell-binding by its Arg-Gly-Asp (RGD) motif, and is also produced by classical *Bordetella* spp.

The *prn* genes are among the most polymorphic in the *B. pertussis* genome, and various *prn* types (*prn1–prn11*) have been identified (Mooi & Greef, 2007). Changes in *prn* types were suspected to contribute to the reduced vaccine effectiveness of the Dutch whole-cell vaccines (Mooi et al., 2001). However, studies in France showed a similar duration of protection induced by whole-cell pertussis or acellular pertussis vaccines when circulating isolates produced different PRNs (PRN2 or 3) than the vaccine strain (PRN1) (Guiso et al., 2007; Guiso et al., 2008) During the last decade, pertactin-deficient isolates of *B. pertussis* and of *B. parapertussis* have been found in France and many other countries (Bouchez et al., 2009; Hegerle & Guiso, 2014). In a murine respiratory model, these isolates interfered with the efficacy of the aP vaccines (Hegerle et al., 2014; Safarchi et al., 2015). A cohort study in humans showed a better fitness of PRN-deficient isolates in vaccinated versus non-vaccinated subjects (Martin et al., 2015) whereas, in a case-control study, no difference in vaccine effectiveness was seen (Breakwell et al., 2016). Given the broad differences in circulation of PRN-deficient isolates, the overall relevance of these variants remains to be elucidated.

PRN induces antibodies after infection and vaccination.

PRN is an antigen in many aP vaccines. Low amounts of PRN, sufficient to induce immune responses, are present in some two-component (PT and FHA) aP vaccines thought not to contain PRN (Edwards & Decker, 2013).

2.9 Fimbriae

Fimbriae (FIM) types 2 and 3 also represent serotype-specific agglutinogens (AGG) and are important surface components involved in colonizing the respiratory mucosa (Scheller & Cotter, 2015). FIM2 and FIM3 contained in whole-cell pertussis vaccines are believed to contribute to protective efficacy, and the WHO requirements for pertussis vaccine licensure require the presence of such AGG to be demonstrated (WHO, 1990). Most manufacturers use several strains of *B. pertussis* in the production of whole-cell pertussis vaccines to ensure that both types of fimbriae are present (Kudelski et al., 1978), although some manufacturers base their production on only one strain (Huovila et al., 1982).

Isolates of *B. pertussis* can display FIM2, FIM3 or both on their surface. It has long been observed that the FIM type of circulating isolates could change over time (Mooi et al., 2007). Although the *fim* genes are rather preserved, polymorphisms have been found among FIM antigens, and one structure, FIMD, is common to all fimbriae.

FIM induce the synthesis of antibodies, such as agglutinins after infection and vaccination. FIM2 and FIM3 are antigens in some aP vaccines. FIM antigens may be present in minute amounts in antigen preparations of aP vaccines that are thought not to contain FIM (Edwards & Decker, 2013).

2.10 Animal models of *Bordetella pertussis* infection

Our understanding of the role of specific components of *B. pertussis* in the pathogenesis of, and immunity to, the disease is impaired by the limited availability of suitable animal models that are equivalent to clinical pertussis in humans. However, the emergence of the baboon model can be helpful in elucidating various aspects of transmission.

Rodent models: the murine model has been used for several decades because of the availability of murine reagents, knock-out mice and their reasonable cost. Mice are infected either systemically (intravenous, intraperitoneal) or by the respiratory tract. Some aspects of human disease can be reproduced; however, mice do not cough and do not transmit. Nevertheless, most virulence factors of *Bordetella* spp, as well as the immune responses, were characterized using the murine model. Furthermore, the potency tests of wP and aP vaccines are made using intracerebral murine models. Another advantage of mice is that they are not infected by *B. bronchiseptica* (Mills & Gerdts, 2014)

Swine models: the swine model can reproduce many parameters of human disease, including transmission. The model was also useful in generating data concerning maternal immunization (Mills & Gerdts, 2014).

Baboon model: the baboon seems to reproduce the human disease and transmission quite effectively (Warfel et al., 2012). In this model, aP vaccination provided protection against disease symptoms but not against colonization or transmission (Warfel et al., 2013). However, it was also shown that baboons may be infected by *B. bronchiseptica* (Nguyen et al., 2016), which may impair the interpretation of data. Thus, this model requires extremely careful handling.

3. Pertussis vaccines

The pertussis working group of the WHO Strategic Advisory Group of Experts (SAGE) on Immunization has produced a background paper that highlights many aspects of pertussis surveillance and vaccine use (WHO, 2014a).

3.1   Whole-cell pertussis vaccines

Whole-cell pertussis (wP) vaccines contain various amounts of whole nonviable bacterial cells. All antigens and virulence factors described above – such as PT, ACT, LOS, FHA, PRN and FIM – can be components of wP vaccines.

wP vaccines are produced in many countries, and WHO has established quality requirements for production and lot release (WHO, 1990). wP vaccines are produced by growing bacteria in standardized liquid synthetic media. The bacteria are then killed chemically or by heating, adjusted to a certain density (i.e. number of cells), mostly adsorbed to aluminium salts, and a preservative is added. The production process and the composition of strains may vary from producer to producer. The potency of wP vaccines is usually controlled by an intracerebral mouse challenge test developed in the 1940s (Kendrick et al., 1947). Although this test has been used for a long time, it is not clear what type of murine immune response it measures.

Considerable variation has been found in the amount of FHA and PT in different wP vaccines. Measured as antigen, FHA ranges between 0 and 1.6 µg per dose, and total biologically- active PT has been reported to be in the range of 0.02 to 0.68 µg per dose (Ashworth et al., 1983). The amount of FIM2 in Wellcome wP vaccine was estimated to be 4.7 µg per single dose (Ashworth et al., 1983).

The amount of LOS in wP vaccines ranges from 0.9 to 2.8 µg per mL, and most has been found to exist as free, not cell-bound toxin. The release of LOS from cells during storage of vaccine is quite rapid; in the first few weeks 35–50% of the LOS is released, and after 5–6 months 60–80% of LOS is released (Ibsen et al., 1988).

Although the production process of wP vaccines appears to be simple and standardized, significant differences have been observed in the immunogenicity and efficacy of wP vaccines from different producers (Bellalou & Relyveld, 1984). wP vaccines were included as a comparator in the trials on aP vaccines during the 1990s. The studies used different designs, and so the estimates of efficacy for the wP groups cannot be compared directly. A German wP vaccine showed vaccine efficacy estimates (VE) of 98% and 96% in two studies, while one American vaccine had a VE of 83% in another study done in Germany. A French-made vaccine had a VE estimate of 96% in a study in Senegal. By contrast, an American wP vaccine that had passed the potency tests was found to be only 36% efficacious in Italy and only 48% in Sweden after three primary doses.

Similar effectiveness estimates were reported from other countries by using surveillance data. In the Netherlands, data from 1997 on the local vaccine against bacteriologically proven pertussis suggested an effectiveness of 51% (de Melker et al., 2000a). In a case-control study in Canada in children aged 4 years and over, vaccine effectiveness against laboratory-confirmed pertussis was only 57% (Bentsi-Enchil et al., 1997). Although aP vaccines have replaced wP vaccines in many industrialized countries, the majority of infants worldwide are primed with wP vaccines, and estimates for effectiveness of these products in lower- and middle-income countries (LMIC) are lacking.

wP vaccines are not licensed for routine use in adolescents and adults.

3.2   Acellular pertussis vaccines

Recognition of the roles of PT, FHA, PRN and AGG/FIM in the pathogenesis of, and immunity against, pertussis, together with concerns about frequent local side-effects, as well as public anxiety about the safety of wP vaccines, prompted the development of aP vaccines. All aP vaccines are associated with significantly fewer and less serious side-effects, and thus the replacement of the wP vaccines was mainly driven by the safety profile of these vaccines. The other important advantage of the aP vaccines is the reproducible production process with its use of purified antigens and the removal of LPS and other parts of the bacterial cell wall during the purification of soluble antigenic material.

The first aP vaccines were prepared through a co-purification process; they contained a substantial predominance of FHA over PT (30–40 μg of FHA and about 5 μg of PT per dose), and a small amount of AGG (about 1 μg per dose) (Aoyama et al., 1989). These aP vaccines were studied in Japan and in Europe (Aoyama et al., 1989; Mortimer et al., 1990; Kimura & Kuno-Saki, 1990; Tomoda et al., 1991; Stehr et al., 1998). The second type of aP vaccines purified the antigens separately and combined with equal amounts of FHA and PT (usually 12.5 to 24 μg per dose). They were initially licensed and used in Japan for children over 2 years of age on the basis of immunogenicity data without an efficacy study.

In order to evaluate multiple new aP vaccine candidates, a multicentre aP trial was conducted to assess the safety and immunogenicity of 13 candidate aP vaccines and two wP vaccines (Decker & Edwards, 1995). Taking into account the results of this immunogenicity study, an array of field studies was performed in subsequent years (Table 2) (Edwards & Decker, 2013). Although the efficacy trials differed significantly in vaccination times, design, case definition and technical aspects such as culture and serology, an attempt was made to put the results of all the studies into a synopsis (Edwards & Decker, 2013; Zhang et al., 2014). Unfortunately, all the trials failed to identify reliable serological correlates for clinical protection of the individual (see below).

As a result of these studies, aP vaccines were licensed in most countries for primary immunization and for booster immunization. Most licensed aP vaccines contain between one and four or five separately-purified antigens (PT, FHA, PRN, FIM2/3). Long-term surveillance of the effectiveness of the aP vaccines was initiated in Sweden, starting after the completion of efficacy trials there. Although the vaccines used in different parts of Sweden differed, the overall reduction of cases in all vaccinated cohorts was maintained (https://www.folkhalsomyndigheten.se/contentassets/dbd8cd9e157c47189d72dd8ad9f6c94b/pertussis-eighteen-year-report-16109.pdf).

Table 2: Composition of some acellular vaccines and efficacy estimates (%VE) after three doses for primary immunization (Edwards & Decker, 2013)

Composition	Producer	Study site	% VE
5 μ PT, 2.5 μg FHA, 2.5 μg PRN, 25 Lf D, 10 Lf T	Chiron, now Novartis	Italy	84% (76–90%)
23.4 μg PT, 23.4 μg FHA, 6.7 Lf D, 5 Lf T	Aventis Pasteur, now Sanofi	Germany	93% (63–99%)
25 μg PT, 25 mg FHA, 8 μg PRN, 25 Lf D, 10 Lf T	GlaxoSmithKline Biologicals	Italy	84% (76–89%)
25 μg PT, 25 mg FHA, 8 μg PRN, 25 Lf D, 10 Lf T	GlaxoSmithKline Biologicals	Germany	89% (77–95%)
10 μg PT, 5 μg FHA, 3 μ PRN, 3 μg FIM, 15 Lf D, 6 Lf T	Aventis Pasteur, now Sanofi	Sweden	85% (81–89%)
40 μg PT, 15 Lf D, 6 Lf T	Baxter	Sweden	71% (63–78%)
3.5 μg PT, 35 mg FHA, 2 μg PRN, 0.8 μg FIM, 9 Lf D, 5 Lf T	Wyeth	Germany	78% (60–88%)

Compared to wP vaccines, aP vaccines are associated with a significantly reduced frequency of systemic reactions (fever, vomiting, fretfulness, anorexia) and local reactions (swelling, redness, warmth, tenderness). Various efficacy trials in the 1990s and the subsequent post-marketing surveillance, as well as national surveillance systems such as the Vaccine Adverse Event Reporting System (VAERS) in the USA, have produced a large amount of data on the reduced reactogenicity of aP vaccines. Various reviews have summarized the side-effects of aP vaccines in infants (Zhou et al., 2003). Of particular concern was the observation of entire limb swelling after vaccination, which was not painful and did not interfere with overall health but which troubled parents. A systematic review (Rennels, 2003) showed that this type of side-effect was not typical for aP vaccines, but that all paediatric vaccines produced limb swelling in varying frequency. However, this reaction was seen more frequently with aP vaccines than with wP vaccines. One study gave a fifth dose of aP vaccine to recipients that had experienced limb swelling after the fourth dose, and only 20% experienced a recurrence of limb swelling (Rennels et al., 2008). Some cohorts of aP vaccine study participants have now received up to six doses of aP vaccine, and the frequency of limb swelling was not reported to increase after the sixth dose (Zepp et al., 2006).

Due to their safety profile, aP vaccines also offer the possibility of vaccinating older children, adolescents and adults. Further developments focus on aP vaccines with reduced antigen content (i.e. 50% or less of the infant formulation) to further decrease unwanted side- effects. These vaccines have also undergone extensive studies relating to their immunogenicity and side-effects. One of the reduced-dose aP vaccines was tested for efficacy in a trial among adolescents and adults in the USA and was found to have a point estimate of efficacy of 92% (95% CI: 32–99%) (Le at al., 2004). In another study in adolescents in the United Kingdom, both reduced-dose vaccines, in combination with tetanus and diphtheria toxoid or polio vaccine (Tdap and Tdap-IPV, respectively), were immunogenic and safe (Southern et al., 2005). The effectiveness of giving combined Tdap-vaccines was shown in Australia and, with a point estimate of 85%, it was similar to the efficacy trial mentioned above (Rank et al., 2009). When Tdap was recommended for adolescents, it was observed that the frequency of post-vaccination syncope was slightly higher in female vaccinees (CDC, 2008).

3.3   Combination vaccines

The term "monovalent" is used to indicate that the vaccine contains only pertussis antigens, and "monocomponent" is used to indicate that the vaccine contains only one single pertussis antigen.

wP vaccines have, for a long time, been combined with tetanus and diphtheria toxoid in the diphtheria–tetanus whole-cell pertussis (DTwP) vaccine. However, monovalent wP vaccines are still available in a few countries. Most aP vaccines are combined with other antigens in combination vaccines, and no monovalent aP vaccine is available in the Americas or the European Union (EU). A licensed monovalent aP vaccine for booster immunizations is available in Thailand.

Apart from problems in the production of combination vaccines, other concerns arise from possible interferences between antigens as, for instance, it has been shown that the geometric mean titres of antibodies against *H. influenzae* type b polysaccharide are significantly lower in vaccinees who received combination vaccines. Secondly, combination vaccines have generated regulatory concerns because their safety and effectiveness may be more difficult to monitor and regulate than with single component vaccines (Decker, Edwards & Bogaerts, 2013). Immunological theory suggests that the simultaneous exposure of the immune system to multiple conjugate antigens (such as Hib, *Streptococcus pneumoniae* and *Neisseria meningitidis*), could result in either enhanced or suppressed immune responses. Suppression is assumed to occur when a specific carrier for a polysaccharide is given more than once, and this phenomenon is called carrier-induced epitopic suppression (Findlow & Borrow, 2016).

Combination vaccines are licensed in the assumption that combining their antigens does not interfere with their safety, immunogenicity and effectiveness. This is chiefly monitored not by efficacy studies but by non-inferiority studies that compare the immunogenicity of separately-administered licensed vaccines with the same antigens when administered in a combination vaccine. One example illustrates possible problems: DTaP-Hib-combination vaccines were introduced in the United Kingdom when another combination with wP was not available. The United Kingdom used a 2-, 3- and 4-month schedule for primary immunization without a booster in the second year of life. Surveillance showed that invasive Hib disease increased, especially in the recipients of the DTaP-Hib combination. However, when a booster dose was introduced, invasive Hib disease fell rapidly to low levels, as was the experience of countries, such as Germany that used a booster in the second year of life (Public Health England, 2017; Kalies et al., 2004). These and other experiences suggest that sufficient post-marketing surveillance will be necessary if vaccination plans are changed from separately-administered to combination vaccines.

Safety of combination vaccines has, so far, been reassuring, as no combination vaccine has produced side-effects that were not observed with any of its components. Overall, combination vaccines tend to have slightly more local side-effects when compared with separate injections of their antigens. However, the reduction in the number of injections, especially in infants, is regarded as a significant advantage for these products. Furthermore, it was observed that the use of combination vaccines improved the timeliness of vaccination in both American and German infants (Kalies et al., 2006; Happe at al., 2009).

Overall, combination vaccines, especially for primary immunization of infants, have been very successful, with a good safety profile, and are used in most parts of the world (i.e. van Wijhe et al., 2016).

3.4   Combination vaccines with whole-cell pertussis components

The amount of pertussis antigens is low compared with the levels of protein in the tetanus and diphtheria toxoids in a dose of DTwP vaccine. A dose of DTwP vaccine normally contains 20 Lf of diphtheria toxoid and 10 Lf or more of tetanus toxoid. These amounts of toxoid provide 80 µg of diphtheria antigen and 40 µg of tetanus antigen per vaccine dose.

wP vaccines, together with tetanus and diphtheria toxoids, were the "building blocks" for all other infant combination vaccines. DTwP vaccines have been combined with *H. influenzae* type b polysaccharide, with HBs-antigen, with inactivated polio vaccines (IPV) and, experimentally, with *Neisseria meningitidis* type C vaccine. Many of these combination products are used for primary immunization of infants (Decker, Edwards & Bogaerts, 2008). Most immune responses to the different antigens were similar when antigens were injected either separately or as a combination. Antibodies to the polyribosyl-ribitol-phosphate (PRP) of Hib, however, were reproducibly lower when the antigen was given in a combination vaccine. These differences may be clinically irrelevant, or relevant, depending on the immunization scheme.

3.5   Combination vaccines with acellular pertussis vaccine

aP vaccines were initially combined with only tetanus and diphtheria toxoids. However, as in the case of wP vaccines, aP vaccines have also been combined with *H. influenzae* type b polysaccharide, with HBs-antigen, with IPV vaccines and, experimentally, with *N. meningitis* type C vaccine. Many of these combination products, with antigens from five or six different microorganisms, are used for primary immunization of infants. For a more detailed discussion on the immunogenicity of aP combination vaccines, see Decker, Edwards & Bogaerts, 2013.

Apart from combination vaccines for primary immunization, reduced dose combination vaccines have been developed for booster immunization, particularly in adolescents and adults. These booster vaccines contain about one third of the antigen content of those products for primary immunization; they are combined with a reduced dose of diphtheria toxoid to form Tdap products. For certain purposes, the Tdap vaccines are combined with IPV or other antigens.

Although the efficacy study of an aP vaccine for adults and adolescents was done with a non-combined vaccine (Le at al., 2004), all immunogenicity data and an effectiveness study of reduced-dose combination vaccines with pertussis components (Rank et al., 2009) suggest that they are as effective as the separate products.

4. Measuring the immune response to *Bordetella pertussis* antigens

Although wP vaccines have been used successfully for decades, there are no reliable correlates of protective immunity to pertussis. Furthermore, although many of the serological techniques have proved useful as diagnostic procedures, it is unclear whether any of them, or a combination of which, is a measure of protection from pertussis in the individual (Table 3).

Table 3: Methods for detection of antibodies to *B. pertussis* antigen

Method	Quantification	Antigens	Isotypes	Reported unit	Standardized	Commercially available
ELISA	Yes	PT, FHA, PRN, FIM	IgG, IgA	IU/mL	Yes	Yes
Flow cytometry	Yes	PT, FHA, PRN	IgG, IgA	IU/mL	Yes	Yes
CHO-cell assay	Semiquantitative	PT	IgG	Titres	No	No
Agglutination	Semiquantitative	Whole cells	IgM (IgG)	Titres	Partly	No

Note: CHO = Chinese hamster ovary.

Another crucial point is the standardization of detection methods to make results comparable. These standardizations would include methodology, purity of antigens and reference materials, which so far have been achieved only for enzyme-linked immunosorbent assay (ELISA) methodology (Giammanaco et al., 2008; Tondella et al., 2009). A WHO reference preparation for human pertussis serology has been developed and is available from the National Institute for Biological Standards & Control (NIBSC) (Xing et al., 2009) in the United Kingdom. All methods used for measuring the immune response to *B. pertussis* antigens are also being used as tools to diagnose the disease, and commercially distributed tests are available.

4.1　Bacterial agglutination test

The bacterial agglutination (BA) test, the first method developed to measure pertussis antibodies, employs a simple technique for measuring mainly IgM-antibodies induced by the fimbriae, PRN and LPS of *B. pertussis*. Early studies by Miller et al. (1943) and Sako (1947) suggested some correlation of agglutinins with immunity; vaccinated children with high agglutinin titres were protected from household exposure to pertussis. Studies have neither confirmed nor refuted this observation. Agglutinins are not regularly produced after infection. After vaccination with wP vaccines, however, agglutinins are often produced, although vaccinees without agglutinating antibody have been shown to be protected from disease. For example, the first "acellular" vaccine (based on sonically disintegrated *B. pertussis* cells called Pillemer antigen) was shown to provide strong protection in children although it had a weak capacity to stimulate production of agglutinins in mice and children (MRC, 1959).

The BA test suffers from low sensitivity and has not been standardized. The agglutinin titres strongly depend on the bacterial strain used (Wilkins et al., 1971; Blumberg et al., 1992). BA antibodies correlate best with antibodies to FIM determined by the ELISA test. There is a better correlation between the results of these tests when the BA titre is above 1:320 than at lower BA titres.

4.2　Enzyme-linked immunosorbent assay

The enzyme-linked immunosorbent assay (ELISA) uses purified protein antigens of *B. pertussis* (such as FHA, PT, PRN or FIM2/3) to measure serum immunoglobulin IgG and IgA responses following disease or vaccination (Granstrom et al., 1982; Ashworth et al., 1983; Burstyn et al., 1983; Mertsola et al., 1983; Baraff et al., 1984; Granstrom et al., 1988; Stroffolini et al., 1989; Thomas et al., 1989b; Zackrisson et al., 1990; Lynn et al., 1996). In addition, ELISAs have also been used to measure antibodies in saliva (Litt et al., 2006). The ELISA test is sensitive, specific, relatively cheap, and requires only a small amount of serum. The accuracy of the test depends on the purity of the antigens involved, and proof of the purity in commercial tests may be obtained from the manufacturers. With mixed preparations (whole bacteria, sonicate or extract of bacteria), it is not possible to identify the specific antigens to which the antibody response is directed (Thomas et al., 1989a).

The use of ELISA to quantify anti-pertussis toxin (PT) antibody levels can be performed with paired (acute and convalescent phase) or single serum samples (Guiso et al., 2011). Paired sample serology is a standardized method of diagnosing pertussis, being the most sensitive and specific. However, the need to collect two samples and to wait several weeks for the result makes it impractical for routine diagnosis. For this reason, single-sample serology has been developed and IgG-anti-PT serological cut-off values have been determined in a number of laboratories; this technique has been shown to provide good sensitivity and specificity in determining cases in adolescents and adults (Table 4) (Marchant et al., 1994; Wirsing von König et al., 1999 and 2002; de Melker et al., 2000; Baughman et al., 2004; Prince et al., 2006).

All studies that gave recommendations on the use of serology in pertussis diagnosis were performed in populations vaccinated with DTwP vaccines. Diphtheria–tetanus–acellular pertussis (DTaP) vaccines are now being used in many countries. DTaP vaccines induce immune responses different from those of DTwP, resulting in higher titres of antibody (Greco et al., 1996; Olin et al., 1997). Antibody responses to specific antigens may result in higher titres in DTaP vaccines compared with DTwP vaccines and may last for some years (Greco et al., 1996; Olin et al., 1997; Guiso et al., 2007; Riffelmann et al., 2009). For this reason, recommendations regarding serological cut-offs for single-sample serology may need to be monitored for their sensitivity and specificity when the vaccination schedule is changed.

Recommendations on what to do and what not to do in pertussis serology have been compiled by the European Union reference laboratories and are publicly available (Guiso et al., 2011).

4.3   Immunoblot assays

Immunoblot techniques for measuring antibodies to *B. pertussis* were developed in the late 1980s (Thomas et al., 1989a). Since then, these assays have been used in pertussis diagnosis (Redd et al., 1988; Guiso et al., 1993) but have limitations. Immunoblots cannot readily quantify the amount of antibodies. In most assays, purified pertussis antigens are used since they may be used more easily in an ELISA format; no typical pattern of immunoblot reactivity has been evaluated when a whole-cell lysate is used for this technique. Because of their lack of quantified results, immunoblot assays are not recommended by the European Union reference laboratories (Guiso et al., 2011) and, in comparison with ELISA, their performance was poor (Kennerknecht et al., 2011).

4.4   Other tests for measuring antibodies

Flow cytometry-based serological tests using multicoloured beads have been applied to pertussis serology, offering the advantage of measuring various antibody specificities in a single test. These tests were found to correlate well with standardized ELISA procedures (Pickering et al., 2002; Prince et al., 2006; Reder et al., 2008; van Gageldonk et al., 2008).

The in vitro neutralization test for antibodies to PT is conducted using microtitre cultures of Chinese hamster ovary (CHO) cells. PT induces a distinct cytopathogenic effect that results in the clustering of CHO cells in the microplate culture. Only a small amount of PT (about 1 ng) is needed to produce the clustering of CHO cells. The addition of sera to the microcultures allows the measurement of in vitro neutralization of the toxin (Gillenius et al., 1985; Granstrom et al., 1985). The neutralization test (NT) is laborious, requires tissue-culture facilities and involves subjective readings. Although the titres of NT tests correlate well with the results of IgG-anti-PT ELISA (Dalby et al., 2010), the NT is significantly less sensitive for the diagnosis of pertussis than measuring the IgG response to PT by ELISA. Furthermore, not all patients develop measurable neutralizing antibodies after clinical and culture-confirmed whooping cough (Granstrom et al., 1988).

Other serological methods, such as indirect haemagglutination, immunofluorescence, bactericidal reaction, immunodiffusion and complement fixation have also been used, but overall these have not gained wide acceptance and are not recommended by the European Union reference laboratories (Guiso et al., 2011).

4.5   Tests for cell-mediated immunity

As antibody-testing does not predict protective immunity reliably, many studies have focused on measuring cell-mediated immunity to antigens of *B. pertussis* using various methods (Ryan et al., 1997; Fedele et al., 2015).

Lymphocyte proliferation assays have been used primarily to measure cell-mediated immunity to pertussis. Mononuclear cells are cultured with various pertussis antigens and with polyclonal stimulants as controls. The proliferation of the cells is measured by the ingestion of radiolabeled nucleotides into the cells. Results are given as fold increases when compared with the control without stimulants.

Various tests using cytokine secretion, either by directly measuring the cytokines in the culture supernatant, intracellular cytokine secretion (ICS), or by counting the cytokine producing cells by enzyme-linked immunospot assay (ELISPOT), have been described. These assays have also been used for testing immunity to *B. pertussis* antigens (He et al., 1998; Tran Minh et al., 1999; Higgs et al., 2012; Rieber et al., 2008).

Other assays measuring cell-mediated immunity (CMI), such as tetramer assays and polychromatic flow cytometry, have also been applied to study the response to *B. pertussis* antigens in humans (Han et al., 2015;

The findings of the different assays are difficult to compare, not only because various biological activities are measured but also because the assays are not very well standardized and can be influenced by, among other parameters, the age and stability of the cells, the method by which the cells are purified and stored, the anticoagulant, the storage of the cells, the type and source of antigen used and other factors.

As measured by most methods, CMI seems to be long-lived but it has not been found to be a reliable correlate of protection against reinfection (Brummelman et al., 2015; Fedele et al., 2015; van Twillert et al., 2015).

5. Immune responses after exposure to *Bordetella pertussis*

The natural course of pertussis disease is influenced by the age-specific proportion of susceptible and resistant persons in the community (Galazka, 1992). It is also important to bear in mind that neither infection nor vaccination confers long-lasting immunity to subsequent infection or disease.

Although no specific level of antibody against antigens of *B. pertussis* has been convincingly shown to confer protection against the disease, the prevalence of these antibodies at different ages can be used as an index of the exposure to pertussis antigens. A number of sero-epidemiological studies (Barkoff, 2015) have shown convincingly that antibodies to *B. pertussis* antigens can be detected in the population irrespective of the local immunization schedule, indicating that the circulation of *B. pertussis* in populations is maintained regardless of current vaccination programmes (see below: Serosurveys for *B. pertussis* antibodies).

5.1 Development of antibodies after primary infection

The development of pertussis antibodies following disease has been studied by various authors (Aleksandrowicz & Pstragowska, 1980; Nagel & Poot-Scholtens, 1983; Granstrom et al., 1988; Trollfors et al., 1999; Ward et al., 2006, Watanabe et al., 2006). There is a significant rise of IgG and IgA antibodies to PT, FHA and other antigens (Nagel & Poot-Scholtens, 1983; Granstrom et al., 1988). In infants, six to seven weeks are needed for the serum IgA antibody to reach a high level (Nagel & Poot-Scholtens, 1983). As outlined above, PT is the only antigen specific to *B. pertussis*, and antibodies to FHA (Vincent et al., 2000) may be produced, resulting from different stimulation by non-pertussis antigens. However, antibodies to PT are produced only in about 80–85% of patients after natural infection (Zackrisson et al., 1989 and 1990; Thomas et al., 1989a and 1989b).

5.2 Immune responses after non-primary stimulation

All populations are continuously exposed to *B. pertussis* antigens to some extent and they maintain a certain level of antibodies to PT and other *B. pertussis* antigens. Reinfections with *B. pertussis* are characterized by a very rapid increase in antibodies, making diagnosis more difficult because titre increases may not be seen between acute and convalescent serum samples (Simondon et al., 1998). Consequently, titre decreases of more than 50% have been used to define recent contact with the bacteria (Trollfors et al., 1999). This makes the establishment of cut-offs for IgG-anti-PT in serum samples with recent contact to *B. pertussis* antigens somewhat problematic. Some proposed cut-offs are shown in Table 4.

Table 4: Proposed cut-off values of IgG-anti-PT for diagnostic serology to indicate recent contact

Country	Type of study	Cut-off	Sensitivity	Specificity	Reference
MA, USA	Population study	~ 100 IU/mL	78%	98%	Marchant et al., 1994
MA, USA	Population study	~200 IU/mL	67%	99.9%	Yih et al., 2000
NL	Population study	125 IU/mL	70%	99%	de Melker et al., 2000
	62 IU/mL	80%	95%		
D	Population study	50 IU/mL	80%	95%	Wirsing von König et al., 1999
European Union	Epidemiological survey	125 IU/mL	n.a.	n.a.	Pebody et al., 2005
USA	Epidemiological survey, model	94 IU/mL	n.a.	n.a.	Baughman et al., 2004
	46 IU/mL	n.a.			
AUS	Clinical validation	50 IU/mL	better than 100 IU/mL		Horby et al., 2005

MA = Massachusetts;
n.a. = not available.

5.3 Transplacental passage of antibodies

Newborns acquire antibodies passively from their mothers. IgG antibodies against FHA, PT, PRN, FIM2 and FIM3 have been detected in cord serum, or in serum from healthy children before their first DwPT immunization, which represents transplacentally-acquired maternal IgG (Granstrom et al., 1982; Baraff et al., 1984; Celko et al., 1984; Thomas et al., 1989b; Van Savage et al., 1990; Plans et al., 2008; Heininger et al., 2009). The infant's pertussis IgG antibody level against PT and FHA is comparable to the corresponding maternal level (Van Savage et al., 1990; Healy et al., 2004; Heininger et al., 2009). One study reported that 5% of infants had IgA-anti-PT (Thomas et al., 1989b), although other studies have not substantiated this. No IgM antibodies to pertussis antigens were detected in cord blood (Baraff et al., 1984). However, pertussis agglutinins have been found in cord serum in varying concentrations, and a correlation between high cord blood antibody levels and protection of the infant has been noted (Izurieta et al., 1996). Passively-acquired maternal antibodies fall to a nadir after several months (Baraff et al., 1984; Van Savage et al., 1990) with a half-life of anti-PT, anti-FHA and agglutinin antibodies estimated to be 36, 40 and 55 days respectively (Van Savage et al., 1990).

Although there is placental passage of pertussis antibodies, most infants do not seem to be protected against clinical disease during the first months of life. The susceptibility of young infants to life-threatening pertussis has been well documented, with a high incidence of pertussis in the first six months of life. Consequently, attempts have been made to protect newborns against pertussis through maternal immunization. In earlier studies, pregnant women were immunized with six doses of unadsorbed wP vaccine, with a total dose of 150 million pertussis organisms. Most of the newborns showed agglutinin and mouse protective antibody titres equal to or greater than those of their mothers (Cohen & Scadron, 1943). In most of the early studies with unadsorbed vaccine, the total dosage in terms of volume and numbers of organisms was large by today's standards. Maternal vaccination with TdaP vaccines during pregnancy has been documented as an effective and safe means of protecting young infants from pertussis (Amirthalingam et al., 2014).

Anti-pertussis antibodies have been found in samples of human milk in Nigeria and the USA, but IgG serum antibody levels were higher than breast-milk levels. On the other hand, the mean IgA antibody levels to pertussis (as well as to *H. influenzae* type b, *Streptococcus pneumoniae* and *N. meningitidis*) were higher in breast-milk than in either maternal or infant sera (Kassim et al., 1989). Colostrum samples contained pertussis antibodies – i.e. agglutinins, anti-PT or anti-FHA – as did samples of human breast-milk (Takahashi et al., 2002). Colostrum containing anti-PT antibodies or agglutinins was shown to protect suckling mice from aerosol challenge with *B. pertussis*, whereas colostrum lacking these antibodies but containing anti-FHA gave little protection (Oda et al., 1985). In infants, breast-milk pertussis antibodies had no significant influence on enhancing infant immunity to pertussis (Pandolfi et al., 2017).

5.4 Antibody decay after natural infection

Studies from Germany (Heininger et al., 2004), Japan (Tomoda et al., 1991), Netherlands (de Melker et al., 2000; Versteegh et al., 2005) and the USA (Hodder et al., 2000), measuring IgG-anti-PT after infection with *B. pertussis* in populations with high vaccine coverage, have shown that pertussis antibodies quickly increased to peak levels of more than 100 IU/mL, but with great individual variation. Subsequently, antibody levels decreased rapidly, so that after five years all subjects had levels of IgG-anti-PT <10 IU/mL. A mathematical model used for the Netherlands data predicted that, depending on the age of the patient, most of the patients would be below the usual cut-off level of 100 IU/mL after one year. Thus, in serosurveys, a ≥100 IU/mL cut-off is chiefly used for very recent contacts while levels between >40 and <100 IU/mL may be regarded as non-recent contacts with *B. pertussis* antigen

5.5 Duration of protection after natural infection

Few studies have attempted to determine the duration of protection after *B. pertussis* infection. Symptomatic reinfections are common in adolescents and adults and have also been found in children (Broutin et al., 2004). It is therefore difficult to distinguish between the duration of immunity induced by primary infection and the immunity induced by symptomatic or asymptomatic reinfections. While Gordon & Hood (1951) assumed a near lifelong protection, a cohort study in Germany (Wirsing von Koenig et al., 1995) assumed a protection of 15 years, modelling studies assumed a duration of 7–10 years (Miller & Gay, 1997), and a case series from the Netherlands assumed a protection of between 3 and 12 years (Versteegh et al., 2002). Case reports have been published of symptomatic reinfections as early as 3.5 years after a previous infection (Versteegh et al., 2002).

6. Immune responses to vaccination

6.1 Type of immunity induced by whole-cell and acellular vaccines

wP vaccination induces a broad immune response against many bacterial antigens since they are composed of killed entire bacteria. aP vaccines are composed of between one and five purified detoxified antigens and consequently induce immunity against only a few bacterial proteins involved in the virulence of the bacterium. Thus, the vaccine-induced immunity is different, with bacterial virulence factors becoming the major target after immunization with aP vaccines. Given these differences in immune responses, the replacement of wP vaccines by aP vaccines was accompanied by surveillance of disease to evaluate the consequences of this replacement on herd immunity, and also by surveillance of the bacterial population.

Various clinical case definitions of pertussis based on clinical symptoms and laboratory confirmation have been proposed for vaccine studies and for surveillance purposes (Table 5). The sensitivity and specificity of these clinical case definitions have been evaluated (Blackwelder et al., 1991; Patriarca et al., 1998; Ghanaie et al., 2010; Cherry et al., 2012).

Table 5. Selected case definitions for pertussis (adapted from Guiso & Wirsing von Koenig, 2016, with permission)

Organization/ country and year	Clinical criteria	Laboratory and epidemiological criteria	Comment
World Health Organization 2000	A case diagnosed as pertussis by a physician, or A person with a cough lasting at least two weeks with at the least one of the following symptoms: • paroxysms (i.e. fits) of coughing, • inspiratory "whooping", • post-tussive vomiting (i.e. vomiting immediately after coughing) without other apparent cause	Isolation of B. pertussis, or Detection of genomic sequences by polymerase chain reaction (PCR), or Positive paired serology	**Case classification:** **Clinical case:** A case that meets the clinical case definition, but is not laboratory-confirmed **Laboratory-confirmed case:** A case that meets the clinical case definition and is laboratory-confirmed
Centers for Disease Control and Prevention (CDC) 2010	A cough illness lasting at least 2 weeks with one of the following: paroxysms of coughing, inspiratory "whoop", or post-tussive vomiting, without other apparent cause (as reported by a health professional)	Isolation of B. pertussis from clinical specimen Polymerase chain reaction (PCR) positive for pertussis	**Case classification** *Probable:* In the absence of a more likely diagnosis, a cough illness lasting ≥2 weeks, with at least one of the following symptoms: • paroxysms of coughing, OR • inspiratory "whoop", OR • post-tussive vomiting *Confirmed:* Acute cough illness of any duration, with isolation of *B. pertussis* from a clinical specimen, OR cough illness lasting ≥2 weeks, with at least one of the following symptoms: • paroxysms of coughing, OR • inspiratory "whoop", OR • post-tussive vomiting AND, at least one of the following: • PCR positive for pertussis, OR • contact with a laboratory-confirmed case of pertussis

Organization/ country and year	Clinical criteria	Laboratory and epidemiological criteria	Comment
Canada 2009	**Suspect case** One or more of the following, with no other known cause: • paroxysmal cough of any duration, • cough with inspiratory "whoop", • cough ending in vomiting or gagging, or associated with apnea **Probable case** Cough lasting 2 weeks or longer in the absence of appropriate laboratory tests and not epidemiologically linked to a laboratory – confirmed case **AND** one or more of the following, with no other known cause: • paroxysmal cough of any duration, • cough with inspiratory "whoop", • cough ending in vomiting or gagging, or associated with apnea	**Confirmed case** Laboratory confirmation of infection: • isolation of *B. pertussis* from an appropriate clinical specimen, OR • detection of *B. pertussis* DNA form an appropriate clinical specimen AND one or more of the following: • cough lasting 2 weeks or longer, • paroxysmal cough of any duration, • cough with inspiratory "whoop", • cough ending with vomiting or gagging, or associated with apnea, OR Epidemiological link to a laboratory-confirmed case AND one or more of the following which there is no other known cause: • paroxysmal cough of any duration, • cough with inspiratory "whoop", • cough ending in vomiting or gagging, or associated with apnea	

Organization/ country and year	Clinical criteria	Laboratory and epidemiological criteria	Comment
European Union 2008	Cough≥ 2 weeks with at least one the following: paroxysms, inspiratory "whooping", post-tussive vomiting, OR any person diagnosed as pertussis by a physician, OR apnea episodes in infants	Isolation of *B. pertussis* Nucleic acids of *B. pertussis* *B. pertussis* specific antibody response Epidemiological link by human to human transmission	**Possible case:** Any person with clinical criteria Probable case: Person with clinical criteria and epidemiological link Confirmed case: Person meeting the clinical and laboratory criteria
Australia	Coughing two weeks or more OR paroxysms of coughing, OR inspiratory whoop, OR post-tussive vomiting	Culture of *B. pertussis* PCR for *B. pertussis* Seroconversion or significant increase of antibodies (without recent vaccination) Single IgA titre to whole cells Detection of *B. pertussis* by DFA	**Probable case:** Any person with clinical criteria Confirmed case: Person meeting the clinical and laboratory criteria Or epidemiological link
Global pertussis initiative (2012)	0–3 months: Cough and coryza with minimal fever PLUS whoop OR apnea OR post-tussive emesis OR cyanosis 4 months – 9 years: Paroxysmal cough with no or minimal fever PLUS whoop OR apnea OR post-tussive emesis ≥ 10 years: nonproductive parorysmal cough of ≥2 weeks duration without fever PLUS whoop OR apnea OR sweating episodes	Culture of *B. pertussis* PCR for *B. pertussis* IgG-anti-PT serology (if more than 1 year after the last vaccination)	

6.2 Response to whole-cell pertussis vaccine

Vaccination results in an increase in the ELISA antibody titres to a variety of antigens of *B. pertussis* organisms. Children vaccinated with wP pertussis vaccines may show increasing levels of antibodies against FHA, PT, AGG-FIM, LPS and outer membrane protein, depending on the wP vaccine and the immunization schedule (Ashworth et al., 1983; Baraff et al., 1984; Barkin et al., 1984; Halsey & Galazka, 1985; Wilkins et al., 1987; Blumberg et al., 1991; Grimprel et al., 1996).

The extent of the response was proportional to the number of doses administered. Elevated levels of antibodies to outer membrane protein (OMP) and LOS were also found in sera of unvaccinated children, presumably directed against cross-reacting non-pertussis antigens (Ashworth et al., 1983). Antibody responses to vaccination given immediately after birth have also been reported (Provenzano et al., 1965).

In most studies, more than 70% of children responded to three doses of DTwP vaccine with an agglutinin titre of 1:80 or more. However, wP pertussis vaccines from different manufacturers differ considerably in their immunogenicity. The mean agglutinin titre after three doses of DTwP vaccine ranged between 1:1826 (Barkin et al., 1984) and 1:87 (Blumberg et al., 1991). In a study in France, three doses of DTwP polio vaccine (adsorbed on calcium phosphate) failed to stimulate an agglutinin level of 1:10 in 25% of children, and the mean titre (1:23) was low (Relyveld et al., 1991). A clinical trial conducted at two different academic centres in the USA showed that two commercially available wP vaccines consistently differed in their ability to induce antibody to PT. Infants receiving the Lederle vaccine produced a 46-fold increase in antibody to pertussis toxin, when compared to only a 2.4-fold increase in PT antibody in infants receiving the Connaught vaccine. The FHA and FIM responses to the two wP vaccines were comparable (Edwards et al., 1991b). Antibodies to PT as measured by CHO-cell assay also increased following immunization. Three doses of the wP vaccine caused a moderate response in neutralizing antibody titres (Blennow et al., 1988;, Blumberg et al., 1991).

As outlined above, wP vaccines with similar production processes differ in their antigenic dose, and so differences in immunogenicity of different wP vaccines are not surprising.

Levels of antibody against PT, FHA, OMPs, AGG-FiIM and neutralizing antitoxins decline considerably during the first year after completion of a primary series (Barkin et al., 1984; Blennow & Grandstrom, 1989a; Blumberg et al., 1991; Edwards et al., 1991b; Relyveld et al., 1991).

Serological studies provide strong evidence for the booster effect of the fourth dose of DTwP vaccine administered at the end of the second year of life, since antibodies against PT, FHA, and agglutinins increase significantly after the booster dose (Chen et al., 1957; Barkin et al., 1984; Pichichero et al., 1987; Edwards et al., 1991a; Relyveld et al., 1991), but the levels of antibody still differ considerably (Barkin et al., 1984; Relyveld et al., 1991).

6.3 Antibody decay after immunization with whole-cell vaccines

Few studies have addressed the antibody decay after vaccination with wP vaccines (Grimprel et al., 1996). Overall, the relatively low levels of antibodies induced by wP vaccines decline rapidly below detection levels 1–2 years after vaccination (Blennow & Granström, 1990).

6.4 Effectiveness of whole-cell pertussis vaccine in infants and toddlers

The efficacy and effectiveness of wP vaccines has been shown repeatedly in vaccine trials (MRC) and in the field. As noted, it must always be kept in mind that wP vaccines are produced by similar methods but may differ significantly in their immunogenicity and their effectiveness. As also mentioned, significant differences in efficacy were observed in vaccine studies in the 1990s between wP vaccines that had all been cleared by the regulatory agencies using the usual tests for vaccine potency (mouse intracerebral protection) (Edwards & Decker, 2013). Given the array of clinical presentations of pertussis, assessing the effectiveness of wP vaccines is difficult and the pitfalls of using immunization registry data to determine vaccine effectiveness have been highlighted (Mahon et al., 2008)

In many countries, such as France, the effectiveness of wP vaccines appeared to remain unchanged at a high level for more than 30 years (Baron et al., 1998). Similarly, in Australia the effectiveness of the locally produced wP vaccine was estimated to be 0.91 (Torvaldsen et al., 2003). In an outbreak in the USA, the effectiveness of wP vaccine was estimated to be 0.76 (Kenyon et al., 1996). In Poland, it was observed that, for reasons unknown, the effectiveness decreased between 1996 and 2001 from 0.973 to 0.735 (Zielinski et al., 2004). In the Netherlands, the Health Council presented data on the reduced effectiveness of the locally-produced wP vaccine and instead recommended the use of aP vaccines (Visser, 2004).

In Austria, the effectiveness of a three-dose course of wP vaccine for the prevention of pertussis hospitalization was estimated to be 0.79 when compared to 0.92 after a three-dose course of aP vaccines (Rendi-Wagner et al., 2006). A similar decrease in hospitalization after changing from wP to aP vaccines was observed in Canada (Bettinger et al., 2007). In contrast, a study in rural Senegal reported that wP vaccines were more effective (0.67) than a two-component aP vaccine (0.32) (Preziosi & Halloran, 2003).

6.5 Effectiveness of incomplete primary series of whole-cell pertussis vaccine

No formal efficacy studies have addressed the effect of an incomplete primary vaccination series or of single doses. Estimates of the effectiveness of incomplete primary series result from surveillance data; some of these estimates, with their relative endpoints, are given in Table 6.

Table 6: Estimated effectiveness (VE) after different doses of pertussis vaccine

Year	/type of study	Vaccine	Dose	End-point	VE%	Country	Reference
1990	/surveillance	wP	1	Pertussis	44	USA	Onorato et al., 1992
			4	"	80		
2004	/case-control	Mostly aP	1	Pertussis (CDC)	51	USA	Bisgard et al., 2005
			2	"	80		
			3	"	93		
1996 ff	/surveillance	Mostly aP	1	Hospitalization	68	Germany	Juretzko et al., 2002
			2	"	92		
			3	"	99		
1990 ff	/surveillance	wP	1	Hospitalization	36	Denmark	Hviid, 2009
			2	"	66		
			3	"	87		
200 #-	/surveillance	wp	1	Pertussis	62	UK	Campbell et al., 2012
			2	"	85		
			3	"	95		
2012 ff	/surveillance	ap	1	Hospitalization	55	AUS	Quinn et al., 2014
			2	"	83		
			3	"	85		
1990 ff	/surveillance	wp/ap	1	Death	71	US	Tiwari et al., 2015
			1	Hospitalization	31		

6.6   Effectiveness of whole-cell vaccine in adolescents and adults

Few studies have been performed to evaluate the immunogenicity and safety of wP vaccines in adolescents and adults because pertussis was not perceived as a relevant problem in these age groups, and the reactogenicity of wP vaccines was thought to be too high for routine use in older children, adolescents and adults. Nevertheless, between 1933 and 1975, a number of immunogenicity studies, although no efficacy studies, were performed in adults and in pregnant women (Keitel, 1999).

6.7   Immune responses to acellular pertussis vaccines

Due to the use of purified antigens in aP pertussis vaccines, the PT response to primary and booster immunization with aP pertussis vaccine is usually more pronounced than the response after immunization with wP vaccine (Pichichero et al., 1987; Anderson et al., 1987; Morgan et al., 1990; Van Savage et al., 1990; Edwards et al., 1995). Compared with wP vaccine, significantly higher anti-PT and anti-FHA responses have been reported with aP vaccines containing these antigens (Edwards & Decker, 2013). Differences have also been found between responses to aP and wP vaccines in infants with various pre-immunization levels of IgG ELISA antibody to PT. The response to aP vaccine was independent of the pre-immunization antibody titre,

while the response to wP vaccine was strongly dependent on the pre-immunization titre. It is not known whether the better response to aP vaccine among those with higher anti-PT titres was due to greater immunogenicity of PT in the aP product, the absence of some component of the wP vaccine, or some other as yet unidentified factors (Van Savage et al., 1990). IgG-anti-PT levels do not differ between natural infection or vaccination (Giammanco et al., 2003). In respect to isotypes and subtypes of antibodies, aP was reported to induce relatively more IgG4 to vaccine antigens than wP did (Hendricks et al., 2011). IgE responses occur in relevant proportions after natural infection, after wP vaccination and after aP vaccination (Hedenskog et al., 1989), and these responses to aP vaccines have gained renewed attention (Holt et al., 2016).

The diphtheria and tetanus responses in children receiving aP-pertussis and wP-pertussis component DPT vaccines were similar in some studies (Pichichero et al., 1987; Anderson et al., 1987; Edwards et al., 1991a) and lower in the National Institutes of Health (NIH) trial (Edwards et al., 1995). The efficacy studies in Europe and Africa showed no relevant differences in the diphtheria and tetanus response between the DTwP and DTaP recipients (Edwards & Decker, 2013).

As in the case of infection and wP vaccination, aP vaccines not only induce antibodies against the vaccine antigens but also B and T cell responses. However, the cellular response induced by priming with aP is, to a greater or lesser extent, skewed towards a Th2 response, whereas infection and wP vaccination result in a response that is skewed, more or less, towards Th1 (Mascart et al., 2003; Dirix et al., 2009; Higgs et al., 2012; Edwards & Berbers, 2014). Additionally, aP and wP vaccinations differ in inducing Th17 cells (Fedele et al., 2015). Cellular immune responses also wane with time. Studies reviewed by van Twillert et al. (2015) indicated that both peak and maintenance of CMI could be influenced by vaccination type or infection history, as well as by age. Furthermore, pertussis-specific memory B- and T-cell responses may follow different dynamics and vaccine types differ in the induction of B-memory cells (van Twillert et al., 2015). It has been suggested that aP vaccines containing genetically modified PT, as opposed to chemically inactivated PT, may alleviate some of these differences in immune responses between aP and wP vaccines (Seubert et al., 2014).

6.8   Antibody decay after immunization with acellular vaccines

In the case of primary immunization, the aP vaccine trial in Italy and Sweden also produced data about antibody decay in the study populations. Giuliano et al. (1998) showed that 15 months after immunization with three doses of aP vaccines, antibodies to PT, FHA and PRN had mostly fallen below the level of detection, irrespective of sustained vaccine efficacy (Salmaso et al., 2001).

With regard to booster immunizations, Tdap boosters result in rapid responses to pertussis antigens in adults (Kirkland et al., 2009). The APERT study (Le et al., 2004) suggested that these antibodies to PT will be above the level of detection for about five years. Other studies into the decay of antibodies after booster vaccination in adolescents and adults assume that, after a steep decline in the first year after vaccination, antibodies decline gradually and may be detectable for longer than five years after aP administration. In this study, after one month, a geometric mean titre (GMT) of 38 EU/mL of IgG-anti-PT was found, and after one year the mean IgG-anti-PT levels had decreased to 8 EU/mL. McIntyre et al. (2004) found a peak GMT of 83 EU/mL IgG-anti-PT that decreased to 30 EU/mL after one year. Edelman et al.

(2004 and 2007), using the same vaccine in adolescents, found a peak GMT of IgG-anti-PT of 116 EU/mL four weeks after vaccination, decreasing to 16 EU/mL after three years and to 8 EU/mL five years after vaccination. Riffelmann et al. (2009) vaccinated health-care workers and found a higher peak value, with a rapid decline in antibody over the first year after vaccination and a slower decrease in the three consecutive years. Modelling the decay of antibodies after vaccination suggested Tdap booster doses every 10 years would be necessary (Bailleux et al., 2008).

Despite more than 20 years of clinical experience, our basic understanding about aP vaccines is still incomplete. The current positions in this ongoing discussion are summarized by Burtin et al. (2017), Diavatopoulos & Edwards (2017) and Eberhardt & Siegrist (2017).

6.9 Effectiveness of acellular vaccines in infants and toddlers

After the successful introduction of acellular vaccines in Japan (Aoyama et al., 1988), a number of large vaccine efficacy studies with aP vaccines were performed in Africa and Europe. Although these studies used different designs, were performed in different populations, and employed different vaccines, they all used the WHO case definition for pertussis and therefore the results of the studies have been repeatedly compared and summarized (Edwards & Decker, 2013; Zhang et al., 2014). An example of the various estimates of efficacy is given in Table 2. Meanwhile, effectiveness estimates are available as a result of the broad use of aP vaccines (Elliott et al., 2004; Edwards & Decker, 2013; Zhang et al., 2014). Although the isolates of *B. pertussis* have undergone some changes in their genomic makeup and in the expression of virulence factors such as PRN when compared to the Tohama reference strain – as used for the production of some aP vaccines (He et al., 2003; Hallander et al., 2007) – no significant changes in the effectiveness of aP vaccines after the primary series have been observed over time. However, some studies in high income countries indicated waning in older children, but additional studies in different regions are needed (Burdin et al., 2017).

6.10 Effectiveness of incomplete primary series of acellular vaccines

As expected, no formal efficacy study has addressed the effect of an incomplete primary vaccination or of single doses. However, during the aP vaccine efficacy studies, some estimates of the effectiveness of incomplete series were performed. During prolonged enhanced surveillance in Sweden following the efficacy trials there, the rates of pertussis were 225 per 100 000 in unvaccinated infants aged 0–2 months, 212 per 100 000 after one dose in infants aged 3–4 months, 31 per 100 000 after two doses in infants aged 5–11 months and 19 per 100 000 after three doses (Gustaffson et al., 2005). A hospital-based survey in Germany estimated that even one dose of vaccine was 68% effective in reducing hospitalization in infants (Juretzko et al., 2002). A case-control study in the USA (Bisgard et al., 2005) found that the effectiveness of one dose of acellular vaccine was approximately 50%. Results of the studies are summarized in Table 6.

6.11 Effectiveness of acellular vaccines in adolescents and adults

During the last two decades, many reports have shown an increase of pertussis in adolescents and adults (Wirsing von König et al., 2002; Halperin, 2007). This may reflect a shift in the age distribution of pertussis since the disease is effectively controlled by vaccine among children. On the other hand, several authors have expressed concern that pertussis immunity may be only partial among adults (Fine & Clarkson, 1987). The decreased immunity among adults may be related to the reduced circulation of pertussis organisms in well-vaccinated populations, with subsequent less frequent exposure to *B. pertussis* and less natural boosting. The increase in cases among adolescents and adults may also be due to a higher awareness of the disease in adolescence and adulthood, or may be the effect of more sensitive laboratory methods (i.e. serology or PCR) to detect the infection (Cagney et al., 2008). It may, however, also be a function of a different community immunity.

Consequently, the importance of late booster doses of aP vaccines for maintaining immunity against pertussis in older children or adolescents has been discussed intensively and many countries have now recommended booster doses in adolescents and adults. A vaccine efficacy study conducted in the USA obtained a point estimate of vaccine efficacy in adolescents and adults of 0.92 (Ward et al., 2005). Another effectiveness study of an adolescent booster dose conducted in Australia had a point estimate of 0.78 (Rank et al., 2009). Given the intensive circulation of the bacteria, even one dose of an aP vaccine given to adolescents without a history of pertussis disease or vaccination induced an immune response in nearly all vaccinees (Knuf et al., 2006).

Many countries in the European Union, as well as Australia, Canada and the USA, recommend a preschool and/or adolescent booster. The USA and many other countries have also introduced a booster for the adult population using a Tdap combination vaccine (CDC, 2006; ECDC, 2017; Lee & Choi, 2017). Nevertheless, it should be noted that in all countries the vaccine coverage in adults in the general population or even among health-care workers is still very low (Lee & Choi, 2017).

6.12 Effectiveness of maternal immunization to prevent infant pertussis

Maternal vaccination with pertussis-containing vaccines for preventing infant diseases has been advocated for some time (Edwards, 2003; Mooi & Greef, 2007). Its effectiveness was studied in the United Kingdom, and it was shown by different methods that immunization with a Tdap vaccine in a whole-cell primed cohort of pregnant women was over 90% effective in reducing infant pertussis (Amirthalingam et al., 2014; Dabrera et al., 2015). Subsequent surveillance showed that, by maintaining a high coverage rate, the effects on infant pertussis remained unchanged (Amirthalingam et al., 2016). These effects were then also observed in other countries (Vizotti et al., 2016).

6.13 Correlates of protection for pertussis vaccines

No serological correlate for protection after vaccination with wP vaccines has been established, although the MRC trial already suggested a correlation between high agglutinin titres and protection (Table 7).

Table 7: Suggested correlates of protection after vaccination with wP or aP vaccines

Antibody type	Study type	Vaccine type	Correlation	Reference
Agglutinins (anti-FIM)	Vaccine trial	wP	High titres protect	Medical Research Council, 1959
Agglutinins (anti-FIM)	Household contact	wp (?)	High titres protect	Deen et al., 1995
Anti-PRN	Household contact	wp (?)	High titres protect	Deen et al., 1995
Anti-PRN	Vaccine trial	aP	High titres protect	Storsaeter et al., 1998
Anti-PRN	Vaccine trial	aP	High titres protect	Cherry et al., 1998
Anti-PT	Household contact	aP	Low titres make susceptible	Storsaeter et al., 2003
Anti-PT	Household study	aP	High titres protect	Taranger et al., 2000
Anti-FIM	Household contact	aP	High titres protect	Storsaeter et al., 1998
Anti-FHA	Cohort study	wP	High titres protect	He et al., 1996a

Various studies have attempted to find a serological correlate of protection after vaccination with aP vaccines (Table 7). For instance, using data derived from the Swedish NIH-sponsored trial (Storsaeter et al., 1998), it was shown that subjects with detectable IgG-anti-PT but with non-detectable anti-PRN and anti-FIM had an assumed vaccine efficacy of 46%. Those with anti-PT and anti-FIM had an estimated efficacy of 72%, those with anti-PT and anti-PRN had an assumed vaccine efficacy of 75%, and those with all three antibodies had an assumed vaccine efficacy of 85%. A German study (Cherry et al., 1998) used thresholds for antibodies and found that subjects with anti-PRN titres were best protected, that high anti-PT contributed to protection, but that anti-FHA and anti-FIM did not correlate with protection. In a Finnish study, IgG-anti-FHA at elevated levels correlated best with protection (He et al., 1994). Following the cohort of the Swedish vaccine study, it was assumed that low or undetectable levels of IgG-anti-PT would be the best predictor of susceptibility to reinfection (Storsaeter et al., 2003). The Gothenburg study, using a PT-only vaccine (Taranger et al., 2000) showed that the induction of anti-PT induced good protection.

No correlate of protection for cell-mediated immunity against the different pertussis antigens has been observed so far. Overall, it seems most probable that no single correlate of protection exists and that antibodies to many antigens in differing amounts, probably in conjunction with cell-mediated immunity, confer protection against symptomatic reinfection (Plotkin, 2013).

6.14 Interchangeability of pertussis vaccines

Few studies have addressed the interchangeability of aP or wP pertussis vaccines from different manufacturers during primary vaccination. However, it seems clearly advisable not to interchange wP or aP vaccines from different manufacturers during the primary series.

Tripedia® (Sanofi) and Infanrix® (GSK) were interchanged in one study (Greenberg et al., 2002) and no differences in immunogenicity were observed. Similar results were found in Canada, when Pentacel® (Sanofi) and Infanrix® (GSK) were interchanged during primary immunization (Halperin et al., 2006). Another study interchanging Acelimune® (Wyeth) and Tripedia® (Sanofi) again produced non-significant differences in immunogenicity (Wirsing von König et al., 2000). The Canadian Immunization Guide stated that, for primary immunization, a vaccine from the same manufacturer should be used whenever possible. For the 18-month booster, and for the preschool booster, experts agreed that aP containing combination vaccines can be interchanged without loss in immunogenicity (NACI, 2005; Canadian Immunization Guide, 2006).

6.15 Serosurveys for *Bordetella pertussis* antibodies

The prevalence of pertussis antibody in various age groups in the general population depends on the status of pertussis immunization, the extent of exposure to circulating *B. pertussis* organisms, and the methods used to measure them. As antibodies to PT are specific to *B. pertussis*, only these antibodies can be used in serosurveys as an estimate of the circulation of *B. pertussis*.

Serological studies in Germany (Wirsing von König et al., 1999), in the Netherlands (De Melker et al., 2000) and in the USA (Marchant et al., 1994; Yih et al., 2000; Baughman et al., 2004) evaluated age-specific cut-offs for single-sample serological assays. From these studies it emerged that, in adolescents and adults, IgG-anti-PT antibodies from >100–125 EU/mL (= IU/mL) could be used as an indicator of recent pertussis exposure (Table 4). It was also observed that, in most patients, the IgG-anti-PT levels declined rapidly with time (Versteegh et al., 2005; Mertens et al., 2007). In countries such as Sweden, where vaccination against pertussis was stopped in 1979 and pertussis disease incidence was high for more than decade before the introduction of acellular vaccines, the prevalence of antibodies in children below 5 years of age increased with age (Zackrisson et al., 1990). Antibody titres increased in older teenagers, so that 90% of young adults had measurable antibody titres (Granstrom et al., 1982). This agrees with the results of studies in the prevaccination era, which showed that a high proportion of children had experienced pertussis infection by the age of 10 years (Fine & Clarkson, 1987). In Palermo, Italy, where the coverage rate with DTwP vaccine was very low, the results of a seroepidemiological study suggest a high exposure of children to *B. pertussis*, resulting in increasing seroprevalence of IgG-anti-PT antibodies with age. The overall prevalence of these antibodies determined by the ELISA test was 56%; it increased from 24% in 1–3-year-old children to 67% in 11–12-year-old children (Stroffolini et al., 1989).

Astonishingly, in vaccinating countries such as the USA, results of seroepidemiological studies showed similar results in older children, adolescents and adults (Cattaneo et al., 1996; Cherry et al., 1995). Another study in the USA using serum samples from a national nutrition survey found that antibodies to PT indicated that *B. pertussis* was circulating widely in the population (Baughman et al., 2004). The USA study also indicated that different cohorts of the population could be distinguished, and it was assumed that an IgG-anti-PT level of more that ~100 EU/mL would reflect recent contact with the bacteria. Across Europe, other serosurveys were performed that resulted in similar findings, as in the European Sero-Epidemiology Network (ESEN) study (Pebody et al., 2005).

In New Zealand, when measured after vaccination with wP vaccines, the percentage of recipients with ELISA IgG antibody against pertussis toxin also increased with age, from 16% in 5-year-olds to 63% in the 40–49-year age group. The percentage of individuals with antibody dropped to 45% in the to 65-year age group (Lau, 1989). In other countries, similar assumptions concerning the circulation of *B. pertussis* were derived from serosurveys (Maixnerova et al., 1979; Stroffolini et al., 1991; Park et al., 2005; Higa et al., 2008; Yildirim et al., 2008).

Because of their relative simplicity, serosurveys have been conducted in many countries as a primary tool for pertussis surveillance (Guiso & Wirsing von Koenig, 2016), and their use in tracking pertussis has been documented (Quinn et al., 2010; Barkoff et al., 2015).

6.16 Duration of protection after vaccination with whole-cell vaccines

Many studies have provided strong evidence that wP pertussis vaccines are effective in protecting against typical pertussis, either by preventing its occurrence altogether or by markedly reducing its severity (Griffiths, 1988; Wendelboe et al., 2005). However, the duration of immunity following pertussis vaccination is still an open question and may also depend on the intervals used during primary vaccination (Silfverdal et al., 2007). Usually the primary series consists of three doses of DTwP vaccine given during the first year of life. In the WHO African and South-East Asia Regions, most countries use the immunization schedule recommended by the Expanded Programme on Immunization (EPI), which calls for three doses of DTwP vaccine at 6, 10 and 14 weeks; however, some countries use a 3-, 4- and 5-month schedule (WHO, 2017). In the Region of the Americas, countries generally follow the schedule of 2, 4, and 6 months of age in the primary series, as used in the USA (WHO, 2017).

It is noteworthy that for 14–18% of countries in WHO's Americas, European, Eastern Mediterranean and Western Pacific Regions, the third dose of DTwP vaccine is recommended at a late age, generally after six months of age, so this may reflect a two-dose primary immunization with a booster. Various immunization schedules used by countries in the European Region are available on the ECDC website (ECDC, 2017). The WHO website contains information about schedules and coverage rates worldwide (WHO, 2017).

Epidemiological observations suggest that the efficacy of pertussis vaccine is high only for a limited period and falls gradually with time after immunization. In the United Kingdom, the vaccine efficacy fell from 100% in the first year following three doses of DTwP vaccine to 46% in the seventh year (Jenkinson, 1988). In another outbreak study in the USA it was estimated that protection lasted about 12 years after wP vaccination. In Sweden, the efficacy of three doses of unadsorbed wP pertussis vaccine declined from 89% in 6–11-month-old children to 76% in children at the end of the second year of life (Blennow et al., 1988). Other cohort and case-control studies suggest continuous decrease in vaccine efficacy with time (Fine & Clarkson, 1987).

About one third of countries in the Americas and Western Pacific Regions give a fifth dose of DTwP vaccine ("second booster"). The need for a fifth dose of DTwP vaccine and its importance in controlling pertussis in other regions remains to be proven. Serologically, this additional dose of DTwP vaccine seems to exert a clear booster effect (Edwards et al., 1991a; Morgan et al., 1990).

6.17 Duration of protection after vaccination with acellular vaccines

After completion of the aP vaccine studies in the 1990s, their long-time effectiveness was closely monitored. Studies in Italy (Salmaso et al., 2001), Sweden (Tinberg et al., 1999) and Germany (Lugauer et al., 2002) estimated that efficacy remained almost unchanged until six years of age. Gustafsson et al. (2005) showed that protection began to wane in 7–8-year-olds. Some studies, however, seem to indicate a shorter duration of sustained protection. During pertussis outbreaks in the USA, a yearly 42% increase of pertussis risk was described in 4–12-year-olds after receiving five doses (Klein et al., 2012), and a case-control study assumed a sustained effectiveness of four years (Misegades et al., 2012). Similar data were found when mainly PRN-negative isolates were prevalent (Breakwell et al., 2016). Using the test-negative approach, studies in Germany (Riffelmann et al., 2014) and Canada (Schwartz et al., 2016) also indicated a continuous decrease in effectiveness 3–4 years after vaccination, whereas in a New Zealand study effectiveness was sustained for at least four years (Radke et al., 2017). The studies used different methods (cohort study, surveillance, case-control, test-negatives) and had very different clinical endpoints (i.e. WHO case definition, PCR-positive patient), so direct comparison of the results is difficult.

It must be kept in mind that aP is not the only vaccine component and that, in addition to the effectiveness of the vaccines, implementation issues concerning vaccine coverage play an important role.

7. Conclusions and perspectives

Whereas aP vaccines have been used for the past two decades mainly in industrialized countries, most infants worldwide are still immunized primarily with wP vaccines. Surveillance indicates that wP and aP vaccines both protect newborns and toddlers effectively against severe disease. Cellular immune responses differ after priming with either wP or aP vaccines, and the duration of protection seems to be somewhat shorter with aP vaccines than with efficacious wP vaccines. However, the effectiveness of the wP vaccines that are currently used in most countries is not known. Consequently, WHO emphasizes that "there is a need for improved epidemiological data. Surveillance of the disease in infants is crucial and an etiology should be sought on any infant that dies. More solid laboratory data are needed" (WHO, 2017a). Laboratory methods should focus on enhanced specific diagnosis, for which a WHO manual is available (WHO, 2014)

In the medium term, effectiveness estimates should be established for currently used wP vaccines and the search for biomarkers that correlate with protection should be intensified. These are necessary to estimate more effectively the duration of protection induced by infection and vaccination. The first step is to standardize all techniques measuring avidity of antibodies, neutralizing antibodies or cell immunity.

In the longer term, the development of new vaccines (new adjuvants, additional antigens, different antigen preparations, other delivery methods) inducing a more effective immune response with longer duration of protection can be envisaged. In order to evaluate these vaccines, a broad consensus among scientists, regulators and vaccine producers will be required together with the standardization of technical aspects and animal models.

8. Summary

Bordetella pertussis produces an array of virulence factors that act together to induce the clinical symptoms of pertussis or whooping cough. The human immune response to antigens of *B. pertussis* can be measured by ELISA, by bacterial agglutination, and by various other tests, including those measuring cell-mediated immunity. Different pertussis vaccines are available and licensed; they consist either of whole bacterial cells (whole-cell = wP vaccines) or of purified virulence factors (acellular = aP vaccines). Both types of vaccines have been shown to be effective, but aP vaccines show fewer local and systemic side-effects, and their production process is more reproducible. Both wP and aP antigens are mostly combined with tetanus and diphtheria toxoid, and also additionally with other antigens such as Hib, hepatitis B or polio. Maternal immunization can protect newborns and young infants who bear the brunt pertussis mortality. Neither infection nor vaccination induces a life-long immunity. Reinfections are frequent and *B. pertussis* circulates cyclically all over the world. Surveillance data on pertussis are lacking for many parts of the world though they can be estimated by serosurveys and other methods.

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Conflict of interest

Carl Heinz Wirsing von König served as consultant to GSK in 2015 and to Sanofi-Pasteur in 2017. He gave presentations on epidemiology, diagnostics and prevention of pertussis at industry sponsored sessions of scientific meetings. These interests were assessed as personal, specific and financially insignificant.[1] He is a member of the steering committee of the Global Pertussis lnitiative (GPl) which currently receives a grant from Sanofi Pasteur. This interest was assessed as non-personal, specific and financially significant.[1]

Nicole Guiso served as consultant to GSK in 2017 and to Bionet in 2016 and 2017 on methods of surveillance, diagnosis and vaccine strategy of pertussis vaccines. These interests were assessed as personal, specific and financially insignificant.[1] She gave classes in the context of a master programme in 2016 which were funded by Sanofi Pasteur. This interest was assessed as personal, non-specific and financially insignificant.[1]

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[1]   According to WHO's Guidelines for Declaration of Interests (WHO expert), an interest is considered "personal" if it generates financial or nonfinancial gain to the expert, such as consulting income or a patent. "Specificity" indicates whether the declared interest is a subject matter of the meeting or work to be undertaken. An interest has "financial significance" if the honoraria, consultancy fees or other received funding, including those received by expert's organization, from any single vaccine manufacturer or other vaccine-related company exceeds US$ 5 000 in a calendar year. Likewise, a shareholding in any one vaccine manufacturer or other vaccine-related company in excess of US$ 1000 would also constitute a "significant shareholding".

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