Conference report

Pertussis: Biology, epidemiology and prevention

Mitra Saadatian-Elahi [a,*], Stanley Plotkin [b], Kingston H.G. Mills [c], Scott A. Halperin [d], Peter B. McIntyre [e], Valentina Picot [f], Jacques Louis [f], David R. Johnson [g]

[a] Pôle Santé, Recherche, Risques et Vigilances Groupement Hospitalier Edouard Herriot, Unité d'Hygiène, Epidémiologie et Prévention, 5 Place d'Arsonval, 69437 Lyon cedex 03, France
[b] University of Pennsylvania and Vaxconsult, LLC, USA
[c] School of Biochemistry and Immunology, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin 2, Ireland
[d] Canadian Centre for Vaccinology, Dalhousie University, The IWK Health Centre and Nova Scotia Health Authority, Halifax, Nova Scotia, Canada
[e] National Centre for Immunisation Research and Surveillance, The Children's Hospital at Westmead, Sydney, NSW, Australia
[f] Fondation Mérieux, 17 rue Bourgelat, 69002 Lyon, France
[g] Global Medical Affairs, Sanofi Pasteur, Swiftwater, PA, USA

ARTICLE INFO

ABSTRACT

Despite long-standing vaccination programs, substantial increases in reported cases of pertussis have been described in several countries during the last 5 years. Cases among very young infants who are at greatest risk of pertussis-related hospitalizations and mortality are the most alarming. Multiple hypotheses including but not limited to the availability of more sensitive diagnostic tests, greater awareness, and waning vaccine-induced immunity over time have been posited for the current challenges with pertussis. The conference "Pertussis: biology, epidemiology and prevention" held in Annecy-France (November 11–13, 2015) brought together experts and interested individuals to examine these issues and to formulate recommendations for optimal use of current vaccines, with a particular focus on strategies to minimize severe morbidity and mortality among infants during the first months of life. The expert panel concluded that improving vaccination strategies with current vaccines and development of new highly immunogenic and efficacious pertussis vaccines that have acceptable adverse event profiles are currently the two main areas of investigation for the control of pertussis. Some possible pathways forward to address these main challenges are discussed in this report.

1. Introduction

Pertussis, commonly called whooping cough, is a highly infectious disease that was previously a universal rite of passage for older infants and young children. The discovery in 1906 of its causative organism, *Bordetella pertussis*, led to the development of whole-cell pertussis (wP) vaccines, which by the late 1940s were combined with diphtheria (D) and tetanus (T) toxoids. Countries that instituted broad DTwP vaccination programs beginning in the mid-20th century saw pertussis dramatically decrease over subsequent decades. However, concerns over reactogenicity prompted some parents to refuse wP-containing vaccines for their children and some countries to discontinue their programs [1]. Less reactogenic acellular pertussis (aP) vaccines were developed to address these concerns. They were deployed in Japan

approximately 35 years ago; in North America, Australia and some European countries about 15–20 years ago; and more recently in other middle- and high-income countries.

During the last 5 years, multiple countries (e.g., Australia, the United Kingdom, and the United States of America) have experienced substantial increases in reported cases of pertussis [2,3]. Cases among very young infants who are at greatest risk of pertussis-related hospitalizations and mortality are the most alarming. Multiple hypotheses have been posited for the current challenges with pertussis, including:

- More sensitive diagnostic tests combined with greater pertussis disease awareness;
- Inadequate vaccination schedules and poor compliance with vaccination recommendations;
- Evolution of circulating pertussis strains to evade vaccine-induced immunity;
- Suboptimal priming by and decreased duration of protection from aP compared to wP vaccines.

The Fondation Mérieux organized a conference (11–13 November 2015) entitled: "Pertussis: biology, epidemiology and prevention" in Annecy, France ("Les Pensières" Conference Centre). The purpose of this symposium was to bring together experts and interested individuals to:

- Explore the latest trends in pertussis epidemiology;
- Better understand the reasons for these trends;
- Discuss potential ways in which pertussis vaccines might be improved and the practicalities of their introduction into routine use;
- Formulate recommendations for optimal use of current vaccines, with a particular focus on strategies to minimize severe morbidity and mortality among infants during the first months of life.

This report provides a summary of the issues discussed, key findings and areas for future research and development.

2. Pertussis epidemiology and vaccine impact: some examples

While the long-standing use of pertussis vaccines has greatly reduced the disease burden, pertussis continues to be a public health concern, even in some countries with well-established childhood vaccination programs. The following sections give an overview of the observed changes in the epidemiology of pertussis in a number of countries.

2.1. The United States of America

DTwP vaccine was introduced in the USA in the late 1940s, leading to a substantial drop in the annual number of case reports of pertussis, reaching a nadir in 1976 [1]. It has been experiencing regular (every 3–4 years) epidemic peaks in reports of pertussis since the late 1970s, with these peaks substantially increasing in magnitude beginning in 2004–05. The USA transitioned from DTwP- to DTaP-containing vaccines from 1991 to 2001, first with the school-entry and toddler doses, later with the infant doses. The Tdap booster for adolescents and adults was introduced in 2005, and coverage rates with this vaccine among adolescents surpassed 80% by 2012 [4]. However, Tdap vaccine uptake among adults has been much lower [5]. Overall and adolescent-specific case reports decreased for several years following the introduction of Tdap vaccine, but rose again in 2010. Reported cases exceeded 48,000 in 2012, the highest number since 1955 [6]. In addition to a considerable number of deaths in young infants, high incidence rates were observed in children 7–10 years of age and in adolescents 13–14 year olds. Age-group specific trends observed in 2014 were similar to those in 2012, but there was also a peak in 16 year olds [6]. The increased pertussis cases among 13–14 years olds in 2012 and among 16 year olds in 2014 raise concerns about the duration of Tdap's effectiveness when given to adolescents whose previous pertussis vaccinations were exclusively acellular [7,8]. In the past, mothers have been the most commonly cited source of infection in the United States [9] while siblings are now identified as the major source of transmission to young infants [10]. This epidemiology shift supports the change in recommendations in the US to include Tdap vaccination during every pregnancy.

2.2. Australia

The vaccination schedule in Australia has been the subject of several changes over time in an attempt to improve the control of pertussis [11,12]. However, pertussis continues to be a public health concern in the country. During the last 10 years, the average annual notification rate was more than 3 times that of the previous decade [13]. In contrast, early infant hospitalization and mortality rates have remained unchanged, largely attributable to increased diagnosis of milder disease due to the availability of PCR testing [14]. The pattern of age-specific notifications has changed substantially, with cases aged <15 years representing an increasing proportion of all cases during the 2008–2011 epidemics [11]. Although the infant pertussis mortality rate has not changed much over time, there has been a progressive increase in deaths during early infancy, with all 10 infant deaths occurring among those <2 months of age in the period 2006–2012 [13]. Starting in March 2009, parents and general practitioners in successive Australian jurisdictions were asked to bring the first infant vaccine dose forward to 6 weeks of age, as advancing that first dose by 2 weeks was estimated to reduce the number of notified cases and hospitalizations by 8–9% [15]. Although 'cocoon' doses for parents were recommended nationally in 2003 to provide indirect protection to newborns, cocoon doses were not free of charge until 2009, by when most states and territories provided Tdap vaccine to parents in response to epidemics. However, subsequent evaluations found only modest benefit in reducing pertussis risk in early infancy [16–18]. Lack of impact of cocooning was in part related to recently vaccinated siblings (3–4 years of age) emerging as the most common source of transmission following discontinuation of the 18-month booster dose in 2003 [19,20]. Following the availability from England of robust effectiveness data on maternal antepartum vaccination [21], this was formally endorsed as the preferred strategy in April 2015. From mid-2015, maternal vaccination during pregnancy has been fully funded by all jurisdictions separately, with strong consumer and professional support. A decision about cost-effectiveness of this intervention for the National Immunisation Program is expected soon.

2.3. England

Routine pertussis immunisation has been introduced into the national immunisation schedule in 1957 and has undergone a number of changes to optimise the control of infant disease. These included the introduction of an accelerated infant schedule (3 doses of wP vaccine at 2, 3 and 4 months of age) in 1990, the inclusion of aP vaccine in the early preschool booster dose in 2001 and the switch from wP to aP vaccine in the primary infant schedule in 2004. Despite sustained high vaccine coverage, England experienced a sizeable increase in infant disease and deaths during 2012 [22]. In response to this dramatic increase, the department of health recommended that pregnant women receive a dose of Tdap-IPV vaccine, ideally at 28–32 week's gestation [21]. Vaccine coverage was over 55% in the first year of the programme and reached a steady rate of above 60% in 2015. Vaccine effectiveness measured by screening and case-control methods was high, exceeding 90% [21,23]. The impact of the programme, as measured by annual age-specific laboratory-confirmed pertussis incidence rate showed that cases in infants <3 months have been held at low levels, suggesting that this strategy could be considered in other countries with large number of early infant pertussis notifications. Evaluations are on-going and, if continued through the next UK epidemic, should further increase understanding of the programme impact.

2.4. Africa

There is a paucity of data regarding the burden of *B. pertussis* in South Africa, and in Africa in general. Since July 2009, immunisation against pertussis in South Africa involves DTaP-IPV/Hib at 6, 10, and 14 weeks and again at +18 months. The high prevalence

of maternal human immunodeficiency virus (HIV) infection in this part of the world is of particular concern because it might increase the vulnerability of infants and children to other infectious diseases such as pertussis [24,25]. The impact of HIV-infection or in utero HIV-exposure on pertussis infection has recently been the subject of several studies. A community-based cohort study of HIV-uninfected and HIV-infected mothers in Khayelitsha, South Africa, reported 40% reduction in the transplacental transfer of *B. pertussis* antibody from HIV-infected mothers to their HIV-uninfected newborns, suggesting that these infants could be at higher risk of pertussis infection before receipt of their own pertussis vaccines [24]. Similar findings have been reported in a longitudinal cohort study in Cape Town [25]. Higher incidence rates of pertussis in HIV-infected pregnant women could also contribute to an increased vulnerability of their offspring to pertussis infection. In a cohort study including 2116 HIV-uninfected and 194 HIV-infected women, and their 2049 and 188 infants, respectively, there were 31 cases of pertussis-illness in the HIV-unexposed infants and 7 cases in the HIV-exposed infants (2.8 vs. 7.4 cases per 1000 child-months, p = 0.02), at median ages of 83 days (interquartile range IQR: 51–108) and 67 days (IQR: 28–76), respectively. In total, 40 pertussis cases were detected in the HIV-uninfected women compared to 11 cases in the HIV-infected women (2.4 vs. 7.5 cases per 1000 woman-months, p < 0.001); 54% of the women in both groups were pregnant at the time of the illness episode. In a hospital surveillance study, 1033 infants <6 months of age were enrolled and pertussis was detected in 32 of these. Infants infected with pertussis were a median of 52 days old (IQR: 34–70) and 36% were HIV-exposed (unpublished data: Marta C Nunes, Johannesburg, South Africa).

Low pertussis antibody levels at birth in HIV-exposed-uninfected children and high incidence rates of pertussis among HIV-infected pregnant women could be potential explanations for the higher pertussis morbidity and mortality among African HIV-exposed infants, mostly too young to be fully protected by direct immunisation. Vaccination of pregnant women, which has been shown to be efficacious against pertussis in young infants, might be a valuable strategy in such settings.

2.5. Position of the World Health Organisation

To examine the likely causes of the recent resurgence of pertussis in some industrialised countries and the role that aP vaccine may have played in this, the World Health Organisation (WHO) through its Strategic Advisory Group of Experts (SAGE) established a pertussis working group in March 2013. The working group reviewed (i) new data on the effectiveness of targeted vaccination strategies aimed at reducing infant mortality and commissioned a systematic review of the effectiveness of different primary and booster vaccination schedules, (ii) epidemiological data from countries with and without a resurgence using wP or aP vaccines in order to understand the role of aP vaccines in disease resurgence, (iii) data from animal models designed to test the effect of aP and wP vaccines on protection from infection and disease and (iv) mathematic models of pertussis transmission that were designed to explore the cause of the resurgence in specific countries [26]. The SAGE working group concluded that the between-country differences in the incidence of pertussis is due to multiple factors related to the vaccine (type, composition, schedule, coverage, boosters), to the population (age distribution, mixing, transmission patterns), to surveillance systems and diagnostic methods. Except for 5 countries (i.e. Australia, Chile, England and Wales, Portugal, and USA) with convincing evidence of a true resurgence, there was no evidence of a broad resurgence at a global level. The majority of increased incidence is likely associated with natural cyclic patterns along with greater awareness and more sensitive diagnostic testing. Key conclusions from modelling studies suggested shorter duration of aP than wP immunity. Following this work, SAGE recommended that (i) national programs currently using wP should continue using it for primary infant immunisation and (ii) countries using aP may also continue but should consider additional booster and other strategies to reduce childhood mortality [27].

3. Factors associated with pertussis resurgence

B. pertussis is a highly homogeneous pathogen with very low levels of variation between strains. Most observed changes are single base changes referred to as single nucleotide polymorphism (SNPs). *B. pertussis* contains many toxins and other virulence factors that interfere with the innate immune response and participate in the infectious process. However, clinical illness is primarily due to pertussis toxin (PT) and the hypothesized but yet unknown "cough toxin" [28].

Pathogen adaptation, possibly resulting from immune-driven selective pressure of aP vaccines, has been considered as one of the plausible explanations for the resurgence of pertussis [29,30]. Selective pressure could result in bacteria with increased virulence or the ability to evade protective immune responses. Evolutionary studies using SNPs classified *B. pertussis* isolates into 6 main clusters named I through VI [29,31]. Genotyping studies have shown that the predominant strains currently circulating in developed countries belong to cluster I, defined by the presence of certain SNPs. The expansion of cluster I was associated with genetic changes in the PT promoter and the emergence of pertactin (Prn) deficient strains. Importantly, the PT and Prn protein variants found in cluster I strains are different from those of the strain used to manufacture aP vaccines in many countries. Changes in the PT promoter (from *ptxP*1to *ptxP3*) have been linked to increased production of PT and several other virulence factors [31]. Mixed infection in a mouse model demonstrated that Prn-negative strains can evade immunity induced by aP more effectively than Prn-positive strains [32]. Prn-deficient strains, first reported in France in 2012 [33], have now been described in many countries [34,35]. Emergence of Prn-deficient strains has been suggested to play a role in the resurgence of pertussis in the USA. Screening of a large number of pertussis isolates throughout the USA provided evidence of a substantial increase in the prevalence of Prn-deficient isolates to more than 50% of those collected in 2012 [36]. The odds ratio of having pertussis disease by Prn-deficient strains was significantly higher (adjusted OR = 2.2; 95% confidence interval [CI], 1.3–4.0) in vaccinated compared with unvaccinated cases of pertussis [34], providing evidence for a possible selective advantage of Prn-deficient strains. No correlation between Prn-negative strains and disease symptoms was observed.

Suboptimal priming by and decreased duration of protection induced by aP compared with wP vaccines could also contribute to the resurgence of pertussis [37–40]. A meta-analysis of studies that have measured long-term immunity to pertussis after 3 or 5 doses of DTaP showed that protection against pertussis waned overtime, the odds of acquiring pertussis being increased by an average of 1.33 times (95% confidence interval: 1.23–1.43) per year [41]. Studies in animal models have shown that innate immune mechanisms – involving dendritic cells, macrophages, neutrophils, natural killers (NK) cells and antimicrobial peptides – help to control primary infection with *B. pertussis*, while complete bacterial clearance requires cellular immunity mediated by T helper (Th)1 and Th17 cells. In previously infected or wP-vaccinated animals, protective adaptive immunity is mediated mainly by Th1 and Th17 cells, while aP vaccination induces more prominent Th2 responses [42–44]. The Th1/Th17 response prevents both disease

and infection, and gives longer protection. Studies in mice suggested that Th2 responses were redundant to protection induced by aP vaccination [43]. While aP vaccines do induce good antibody responses, recent evidence suggests that T follicular helper (Tfh) cells, rather than Th2 cells, play a critical role in the generation of long-lived plasma cells and memory B cells [44]. However, aP vaccines have limited ability to induce Tfh cells which may in part explain waning antibody responses after aP vaccination. [Unpublished data: Anne-Marie Buisman et al. National Institute of Public Health and the Environment, The Netherlands, and Mills et al. Trinity College Dublin, Ireland]. Furthermore, aP vaccine fail to induce lung tissue resident memory T (T$_{RM}$) cells that ensure immunological memory in the respiratory tract [Unpublished data: Mills et al. Trinity College Dublin, Ireland]. The long-term immune responses against pertussis were investigated in a longitudinal study of children 4 through 10 years old who had been primed with either wP or aP vaccine at 2,3,4 and 11 months of age and boosted with aP vaccine at 4 and 9 years [45]. At 4 years of age, i.e. 3 years after the infant vaccination, antibody levels have waned in both groups but the level of antibodies to PT, FHA and Prn were significantly higher in aP-primed children than wP-primed children. After the school-entry booster, antibody levels and memory B-cell responses reached significantly higher levels in aP vaccine-primed children compared to wP vaccine-primed children [46]. All pre-booster T-cell cytokines responses were already high in aP-primed children and remained or decreased post-booster vaccination, whereas those in wP-primed children increased. At 9 years of age, i.e. 5 years after the school-entry booster, there was however a shift in immunity between the 2 groups in favour of wP-vaccine priming (Unpublished data: Anne-Marie Buisman, National Institute of Public Health and the Environment, The Netherlands). These data suggest that a late childhood/early adolescent booster may induce lesser protection in those primed with aP vaccines than in those primed with wP vaccines.

Differences in the type of immune response generated by aP vaccine as compared to wP vaccine have also been suggested to contribute to the increase in infection and transmission of *B. pertussis*. The baboon model has increased our understanding of pertussis vaccines, particularly the observation that aP vaccines protect from disease but not colonization [42]. This model also showed that wP vaccine provide some protection from colonization, while previous disease gives sterilizing protection. This allows aP-vaccinated animals to transmit pertussis to naïve animals. Transmission of *B. pertussis* may be thus greater in aP vaccinated populations than wP-vaccinated populations.

Other factors such as variable vaccine uptake, the availability of more sensitive diagnostic methods, increased awareness of disease, household transmission, increasing number of non-medically exempted children and inadequate adult booster dose coverage also contribute to the resurgence of pertussis in various populations.

4. The way forward

Improving vaccination strategies with current vaccines and development of new highly immunogenic and efficacious pertussis vaccines are currently the two main areas of investigation for the control of pertussis.

Vaccination of women during pregnancy may protect their infants during several months post-partum. Vaccination of pregnant women with Tdap has already been implemented in several countries (e.g. Argentina, Australia, Belgium, Brazil, Ireland, Mexico, New Zealand, the UK, USA), as a means to protect young infants from severe disease. Recommendation of pertussis

vaccination in the second or third trimester of pregnancy has been based so far on the beneficial impact of transplacental transfer of pertussis antibody (i.e. immunoglobulin G) to the foetus. Indeed, Tdap vaccination during pregnancy offers increased antibody levels at birth, lasting at least until the infant's first vaccination, thus helping to close the susceptibility gap for infection in young infants [47–50]. Vaccination during each pregnancy is recommended in Australia [51], Ireland [52], New Zealand [53], the USA [54], and the UK [55]. Secretory immunoglobulin (SIgA) of the breast milk can also provide protection to the infant by binding the pathogenic microorganisms, thus inhibiting the colonization and invasion of the mucosal membranes of the child. In contrast to mothers with no recent (>5 years) pertussis vaccination, higher levels of anti-PT SIgA were measured in breast milk of vaccinated mothers [56], still detected at 8 weeks [57].

Further research on pertussis vaccination during pregnancy is warranted. Blunting of the infant's antibody responses to her/his own pertussis vaccination by high concentrations of maternal antibodies is one of the remaining concerns in the research on the immunological effects of this strategy. Infants of women who received Tdap or Tdap-IPV during pregnancy achieved lower levels of antibodies to PT, FHA and FIM [49]; but not to PRN [50] after receiving 3 doses of aP-containing vaccine. Factors influencing this interference include: type and composition of vaccine used in mothers and offspring, vaccination schedule used in infants, and possibly the affinity of maternal antibodies [47,48]. Another challenge is to better understand the effect of Tdap vaccination during pregnancy on the anti-pertussis cellular immune responses in infants.

Vaccination of newborns very shortly after birth is another possible strategy to provide anti-pertussis protection in the first months of life. Neonatal vaccination with DTaP was safe, but was associated with lower geometric mean concentrations of anti-PT antibody and reduced responses to subsequent booster doses in one study [58]. In contrast, administration of a standalone aP vaccine at birth followed by DTaP combination vaccines was associated with enhanced antibody responses against pertussis antigens [59–61], suggesting that DTaP at birth has a 'bystander effect' not seen with aP at birth [62]. Findings from a large randomized controlled safety and immunogenicity trial carried out among term newborns who received standalone aP vaccine concurrently with hepatitis B vaccine support the potential for standalone aP to protect against severe early pertussis, especially if the mother has not received Tdap in pregnancy [Unpublished data: Peter B. McIntyre, University of Sydney, Australia].

Another field of research aimed at controlling the re-emergence of pertussis is directed towards the development of new vaccines. There are several approaches to new pertussis vaccines, including the development of (i) less reactogenic DTwP vaccines, (ii) new DTaP vaccines with different adjuvants and (iii) live-attenuated pertussis vaccines.

Lipooligosaccharide (LOS) is the endotoxin from the bacterial outer membrane, an important component of the whole-cell vaccine and the major cause of DTwP vaccine-related adverse reactions [63]. On the other hand, LOS is a potent adjuvant of the immune system and changes in LOS composition or concentration could affect the vaccine-induced immune response. An approach to produce less reactogenic DTwP vaccine is to remove or modify the endotoxin genetically or chemically. Attempts to remove or modify this endotoxin in pertussis vaccine have already been performed [63]. No pertussis vaccine containing genetically detoxified components is in use today. One approach to accelerate the availability of such vaccines is to modify the endotoxin of an available DTwP vaccine, and to compare the immune response of the modified vaccine with the original vaccine in mice. Reactogenicity studies in animals

and humans could be subsequently performed. Bridging data could be used to support efficacy and to evaluate reactogenicity in field studies.

The currently used aluminium-adjuvanted aP vaccines have suboptimal efficacy. In particular, their failure to induce Th1/Th17 responses and memory T cells may explain their suboptimal efficacy and failure to induce more durable immunity. Possible solutions include adding a different non-alum adjuvant to existing aP-containing vaccines used for school-entry or adolescent boosting or creating next generation paediatric aP vaccine with Th1/Th17/Tfh/T$_{RM}$ cell-inducing adjuvant (+/- alum).

In addition to the foregoing, adding one or more new antigens to aP vaccines has been proposed:

- The adenylate cyclase toxin (ACT) is critical for colonization by *B. pertussis* [64], hence immunity to it may be protective [65–67]. Addition of ACT has been shown to improve performance of the aP vaccine in mice [66]. ACT could partly shift the polarization of the immune response from Th2 to a Th1-bias even when administered with aluminium as the adjuvant [68]. ACT is therefore a prime antigen candidate for inclusion into a next generation of aP vaccines.
- Pertussis toxin (PT) is the main virulence factor of *B. pertussis* and detoxified PT is a component of all aP vaccines. Detoxification of PT in all current vaccines is achieved by treatment with chemical agents, which alters dramatically the immunological properties of the toxin. However, detoxification can also be achieved by genetic mutagenesis of the enzymatic subunit of PT, leaving most - if not all - B and T cell epitopes intact. The PT-9K/129G mutant is a genetically detoxified derivative in which the substitution of the two key enzymatic residues do not affect all functional and immunological properties of PT, resulting in a non-toxic antigen and a superior immunogenicity compared to chemically detoxified PT [69]. In an efficacy trial, the PT-9K/129G based-vaccine induced earlier and longer lasting protection as compared to vaccines containing chemically inactivated PT. Assessment of safety and immunogenicity of PT-9K/129G-containing aP and Tdap formulations in a booster situation showed that genetically detoxified PT elicits improved and longer-lasting (at least 1 year) immune responses when compared with the chemically detoxified PT containing vaccine. These data further support the hypothesis that PT-9K/129G is an ideal candidate for future pertussis vaccine formulations either as infant vaccines or as booster for older children, adolescents and adults [unpublished data].

Research is also focused on the development of a live-attenuated vaccine for intranasal administration. BPZE1, is a live attenuated *B. pertussis* intranasal vaccine candidate that has been developed by the genetic removal or inactivation of dermonecrotic toxin, tracheal cytotoxin and pertussis toxin [70]. In mouse models BPZE1 was found to be safe [70] and induced strong and long-lasting protection in mice [71]. Interestingly, BPZE1 also protected mice against *B. parapertussis* [72] and showed important non-specific beneficial effects against inflammatory disorders induced by infections such as influenza virus [73] or respiratory syncytial virus [74] or from non-infectious origin such as allergic asthma [75] in mice. In a Phase I clinical trial, BPZE1 found to be safe and induced immune response in all colonized male volunteers [76]. Non-colonized subjects were found to have higher pre-existing anti-pertactin antibodies, suggesting that these antibodies may have prevented BPZE1 colonization. A second Phase I clinical trial is currently on-going to determine whether higher dose and/or higher volume may overcome the effect of pre-existing anti-pertactin antibodies.

5. Conclusions and recommendations

Despite the availability of effective pertussis vaccines since the 1940s and considerable improvements in vaccination coverage of infants/young children in a number of countries, *B. pertussis* continues to circulate in the human population and pertussis disease is certainly less than optimally controlled. Our ability to counteract pertussis resurgence is hampered by the fact that - despite intensive research on the pathogenesis of and immunity to *B. pertussis* - many important questions remain. The length of *B. pertussis* carriage, the efficiency with which asymptomatic carriers can transmit infection, immune correlates of protection, and the nature and duration of the immune response after infection and immunisation are among the unresolved issues. Human challenge studies with *B. pertussis* might be a way forward. Indeed, they may provide methods of studying infections and vaccination carefully and in depth, and in dissecting out underlying pathogenic and immunologic mechanisms. Human challenge studies for pertussis were not deemed acceptable as little as a decade ago. However, the landscape is changing. Initial discussions with regulatory authorities have been favourable and human challenge opportunities for pertussis being developed. An open, phase 1 clinical trial is on-going to determine the optimal dose and methods of *B. pertussis* challenge administered to healthy adults to recover *B. pertussis* in nasopharyngeal culture after challenge [Unpublished data, Scott Halperin; Dalhousie University, Canada].

Improved surveillance around the world, and especially in Africa, should enhance understanding of pertussis epidemiology. This enhanced understanding should be combined with findings from future human challenge studies and advanced molecular genomic and proteomic studies to expand the collective evidence-base for the development of new pertussis vaccines. These new vaccines should be able to induce higher levels of and longer-lasting immunity that can be boosted throughout the lifespan. And they should have very acceptable levels of reactogenicity. The development and deployment of pertussis vaccines with all of these virtues will very likely take decades. In the meantime, what can and what should be done?

In the shorter term, more limited improvements in current aP vaccines might be made through switching from chemically detoxified to genetically detoxified PT. Adding fimbriae, or increasing that antigen in vaccines that already contain it, may enhance effectiveness against Prn-deficient strains. Expanding infant/early childhood vaccination schedules is something that should be done in some countries, while improving timely uptake with these schedules is something that is needed in most.

For countries in which pertussis-related disease, hospitalizations and deaths are occurring in infants less than 6–8 weeks of age (ie, too young to have begun receiving their own wP- or aP-containing vaccinations), the use of Tdap vaccines in pregnant women during the 2nd or 3rd trimester is a very encouraging intervention. Considerable data supporting this intervention has recently been and continues to be generated, although several important questions are yet to be answered satisfactorily. Uptake of Tdap vaccination during pregnancy might be improved were the labels for these vaccines expanded to include more information about the benefits versus the risks. National regulatory authorities in certain countries may eventually allow a pregnancy use indication to be added to the posology section of the label, assuming of course that data from clinical trials and others studies are compiled, submitted and deemed adequate.

Neonates whose mothers have not received pertussis vaccination during pregnancy may benefit from receipt of a standalone aP vaccine administered shortly after birth. But such vaccines are not at present commercially available.

Lastly, there has been the suggestion that the USA reintroduce wP-containing vaccine to be used as the first dose in the primary infant series, the intention being to appropriately prime the pertussis-naïve immune system before embarking on aP vaccination [77]. For countries like the USA with long-standing aP vaccination programs, even a single wP vaccination may be deemed unacceptable by many parents [78], and may also be very difficult if not altogether impossible from a regulatory perspective. However, countries that have recently switched to aP-containing vaccines and still have access to wP-containing vaccines or countries that are anticipating such a switch may want to consider a sequential schedule of aP following one or more wP vaccinations.

Funding

Conflict of interest

DRJ is full-time employee of Sanofi Pasteur. KHGM has received research funding from Novartis Vaccines (now GSK). SAH has received research funding from GSK and Sanofi Pasteur, and has served on ad hoc advisory panels for both companies. Other authors declare that they have no conflicts of interest to report.

List of speakers and chairs

Gayatri Amirthalingam: UK | Public Health England
Norman Baylor: USA | Biologics Consulting Group, Inc
Anne-Marie Buisman: The Netherlands | National Institute of Public Health and the Environment
James Cherry: USA | David Geffen School of Medicine at UCLA
Scott Halperin: Canada | Dalhousie University
Eric Harvill: USA | The Pennsylvania State University
David Johnson: USA | Sanofi Pasteur
Ruiting Lan: Australia | University of New South Wales
Elke Leuridan: Belgium | University of Antwerpen
Camille Locht: France | Inserm
Jacques Louis: France | Fondation Mérieux
Stacey Martin: USA | Centers for Disease Control and Prevention
Peter McIntyre: Australia | University of Sydney
Jodie McVernon: Australia | University of Melbourne
Tod Merkel: USA | U.S. Food and Drug Administration
Elizabeth Miller: UK | Public Health England
Kingston Mills: Ireland | Trinity College Dublin
Pieter Neels: Belgium | Vaccine Advie BVBA
Marta Nunes: South Africa | University of Witwatersrand
Mariagrazia Pizza: Italy | GSK Vaccines
Stanley Plotkin: USA | University of Pennsylvania and Vaxconsult
Jan Poolman: The Netherlands | Janssen Pharmaceuticals

Helen Quinn: Australia | National Centre for Immunisation Research and Surveillance
Rene Raeven: The Netherlands | Intravacc

Acknowledgments

References

[1] Edwards KM, Decker MD. Pertussis vaccines. In: Vaccines. 6th ed.; 2013. p. 447–92.

[2] Tan T, Dalby T, Forsyth K, Halperin SA, Heininger U, Hozbor D, et al. Pertussis across the globe: recent epidemiologic trends from 2000 to 2013. Pediatr Infect Dis J 2015;34(9):e222–32. doi: http://dx.doi.org/10.1097/INF.0000000000000795.

[3] Sealey KL, Belcher T, Preston A. *Bordetella pertussis* epidemiology and evolution in the light of pertussis resurgence. Infect Genet Evol 2016;40:136–43. doi: http://dx.doi.org/10.1016/j.meegid.2016.02.032.

[4] Reagan-Steiner S, Yankey D, Jeyarajah J, Elam-Evans LD, Curtis CR, MacNeil J, et al. National, regional, state, and selected local area vaccination coverage among adolescents aged 13–17 years – United States, 2015. MMWR 2016;65 (33):850–8. doi: http://dx.doi.org/10.15585/mmwr.mm6533a4.

[5] Williams WW, Lu PJ, O'Halloran A, Bridges CB, Pilishvili T, Hales CM, et al. Noninfluenza vaccination coverage among adults – United States, 2012. MMWR 2014;63(5):95–102.

[6] CDC. Pertussis outbreak trends: <http://www.cdc.gov/pertussis/outbreaks/trends.html> [accessed 25.05.2016].

[7] Acosta AM, DeBolt C, Tasslimi A, Lewis M, Stewart LK, Misegades LK, et al. Tdap vaccine effectiveness in adolescents during the 2012 Washington State pertussis epidemic. Pediatrics 2015;135(6):981–9. doi: http://dx.doi.org/10.1542/peds.2014-3358.

[8] Koepke R, Eickhoff JC, Ayele RA, Petit AB, Schauer SL, Hopfensperger DJ, et al. Estimating the effectiveness of tetanus-diphtheria-acellular pertussis vaccine (Tdap) for preventing pertussis: evidence of rapidly waning immunity and difference in effectiveness by Tdap brand. J Infect Dis 2014;210(6):942–53. doi: http://dx.doi.org/10.1093/infdis/jiu322.

[9] Wendelboe AM, Njamkepo E, Bourillon A, Floret DD, Gaudelus J, Gerber M, et al. Transmission of *Bordetella pertussis* to young infants. Pediatr Infect Dis J 2007;26(4):293–9.

[10] Skoff TH, Kenyon C, Cocoros N, Liko J, Miller L, Kudish K, et al. Sources of infant pertussis infection in the United States. Pediatrics 2015;136(4):635–41. doi: http://dx.doi.org/10.1542/peds.2015-1120.

[11] Campbell P, McIntyre P, Quinn H, Hueston L, Gilbert GL, McVernon J. Increased population prevalence of low pertussis toxin antibody levels in young children preceding a record pertussis epidemic in Australia. PLoS ONE 2012;7(4): e35874. doi: http://dx.doi.org/10.1371/journal.pone.0035874.

[12] Quinn HE, McIntyre PB. The impact of adolescent pertussis immunization, 2004–2009: lessons from Australia. Bull World Health Organ 2011;89 (9):666–74. doi: http://dx.doi.org/10.2471/BLT.11.086538.

[13] Pillsbury A, Quinn HE, McIntyre PB. Australian vaccine preventable disease epidemiological review series: pertussis, 2006–2012. Commun Dis Intell Q Rep 2014;38(3):E179–94.

[14] Kaczmarek MC, Valenti L, Kelly HA, Ware RS, Britt HC, Lambert SB. Sevenfold rise in likelihood of pertussis test requests in a stable set of Australian general practice encounters, 2000–2011. Med J Aust 2013;198(11):624–8.

[15] Foxwell AR, McIntyre P, Quinn H, Roper K, Clements MS. Severe pertussis in infants: estimated impact of first vaccine dose at 6 versus 8 weeks in Australia. Pediatr Infect Dis J 2011;30(2):161–3. doi: http://dx.doi.org/10.1097/INF.0b013e3181f43906.

[16] Carcione D, Regan AK, Tracey L, Mak DB, Gibbs R, Dowse GK, et al. The impact of parental postpartum pertussis vaccination on infection in infants: a population-based study of cocooning in Western Australia. Vaccine 2015;33 (42):5654–61. doi: http://dx.doi.org/10.1016/j.vaccine.2015.08.066.

[17] Quinn HE, Snelling TL, Macartney KK, McIntyre PB. Duration of protection after first dose of acellular pertussis vaccine in infants. Pediatrics 2014;133(3): e513–9. doi: http://dx.doi.org/10.1542/peds.2013-3181.

[18] Castagnini LA, Healy CM, Rench MA, Wootton SH, Munoz FM, Baker CJ. Impact of maternal postpartum tetanus and diphtheria toxoids and acellular pertussis immunization on infant pertussis infection. Clin Infect Dis 2012;54(1):78–84. doi: http://dx.doi.org/10.1093/cid/cir765.

[19] Bertilone C, Wallace T, Selvey LA. Finding the 'who' in whooping cough: vaccinated siblings are important pertussis sources in infants 6 months of age and under. Commun Dis Intell Q Rep 2014;38(3):E195–200.

[20] Jardine A, Conaty SJ, Lowbridge C, Staff M, Vally H. Who gives pertussis to infants? Source of infection for laboratory confirmed cases less than 12 months of age during an epidemic, Sydney, 2009. Commun Dis Intell Q Rep 2010;34(2):116–21.

[21] Amirthalingam G, Andrews N, Campbell H, Ribeiro S, Kara E, Donegan K, et al. Effectiveness of maternal pertussis vaccination in England: an observational study. Lancet 2014;384(9953):1521–8. doi: http://dx.doi.org/10.1016/S0140-6736(14)60686-3.

[22] Public Health England: <https://www.gov.uk/government/publications/whooping-cough-pertussis-statistics> [accessed 25.05.2016].

[23] Dabrera G, Amirthalingam G, Andrews N, Campbell H, Ribeiro S, Kara E, et al. A case-control study to estimate the effectiveness of maternal pertussis vaccination in protecting newborn infants in England and Wales, 2012–2013. Clin Infect Dis 2015;60(3):333–7. doi: http://dx.doi.org/10.1093/cid/ciu821.

[24] Jones CE, Naidoo S, De Beer C, Esser M, Kampmann B, Hesseling AC. Maternal HIV infection and antibody responses against vaccine-preventable diseases in uninfected infants. JAMA 2011;305(6):576–84. doi: http://dx.doi.org/10.1001/jama.2011.100.

[25] Reikie BA, Naidoo S, Ruck CE, Slogrove AL, de Beer C, la Grange H, et al. Antibody responses to vaccination among South African HIV-exposed and unexposed uninfected infants during the first 2 years of life. Clin Vaccine Immunol 2013;20(1):33–8. doi: http://dx.doi.org/10.1128/CVI.00557-12.

[26] WHO SAGE pertussis working group Background paper SAGE April 2014. <http://www.who.int/immunization/sage/meetings/2014/april/1_Pertussis_background_FINAL4_web.pdf> [accessed 25.05.2016].

[27] WHO. Pertussis vaccines: WHO position paper – September 2015. Wkly Epidemiol Rec 2015;90(35):433–60.

[28] Cherry JD, Paddock CD. Pathogenesis and histopathology of pertussis: implications for immunization. Expert Rev Vaccines 2014;13(9):1115–23. doi: http://dx.doi.org/10.1586/14760584.2014.935766.

[29] Octavia S, Maharjan RP, Sintchenko V, Stevenson G, Reeves PR, Gilbert GL, et al. Insight into evolution of *Bordetella pertussis* from comparative genomic analysis: evidence of vaccine-driven selection. Mol Biol Evol 2011;28(1):707–15. doi: http://dx.doi.org/10.1093/molbev/msq245.

[30] Kurniawan J, Maharjan RP, Chan WF, Reeves PR, Sintchenko V, Gilbert GL, et al. *Bordetella pertussis* clones identified by multilocus variable-number tandem-repeat analysis. Emerg Infect Dis 2010;16(2):297–300. doi: http://dx.doi.org/10.3201/eid1602.081707.

[31] Lam C, Octavia S, Bahrame Z, Sintchenko V, Gilbert GL, Lan R. Selection and emergence of pertussis toxin promoter ptxP3 allele in the evolution of *Bordetella pertussis*. Infect Genet Evol 2012;12(2):492–5. doi: http://dx.doi.org/10.1016/j.meegid.2012.01.001.

[32] Safarchi A, Octavia S, Luu LD, Tay CY, Sintchenko V, Wood N, et al. Pertactin negative *Bordetella pertussis* demonstrates higher fitness under vaccine selection pressure in a mixed infection model. Vaccine 2015;33(46):6277–81. doi: http://dx.doi.org/10.1016/j.vaccine.2015.09.064.

[33] Hegerle N, Paris AS, Brun D, Dore G, Njamkepo E, Guillot S, et al. Evolution of French *Bordetella pertussis* and *Bordetella parapertussis* isolates: increase of Bordetellae not expressing pertactin. Clin Microbiol Infect 2012;18(9):E340–6. doi: http://dx.doi.org/10.1111/j.1469-0691.2012.03925.x.

[34] Martin SW, Pawloski L, Williams M, Weening K, DeBolt C, Qin X, et al. Pertactin-negative *Bordetella pertussis* strains: evidence for a possible selective advantage. Clin Infect Dis 2015;60(2):223–7. doi: http://dx.doi.org/10.1093/cid/ciu788.

[35] Lam C, Octavia S, Ricafort L, Sintchenko V, Gilbert GL, Wood N, et al. Rapid increase in pertactin-deficient *Bordetella pertussis* isolates, Australia. Emerg Infect Dis 2014;20(4):626–33. doi: http://dx.doi.org/10.3201/eid2004.131478.

[36] Pawloski LC, Queenan AM, Cassiday PK, Lynch AS, Harrison MJ, Shang W, et al. Prevalence and molecular characterization of pertactin-deficient *Bordetella pertussis* in the United States. Clin Vaccine Immunol 2014;21(2):119–25. doi: http://dx.doi.org/10.1128/CVI.00717-13.

[37] Klein NP, Bartlett J, Rowhani-Rahbar A, Fireman B, Baxter R. Waning protection after fifth dose of acellular pertussis vaccine in children. N Engl J Med 2012;367(11):1012–9. doi: http://dx.doi.org/10.1056/NEJMoa1200850.

[38] Sheridan SL, Ware RS, Grimwood K, Lambert SB. Number and order of whole cell pertussis vaccines in infancy and disease protection. JAMA 2012;308(5):454–6.

[39] Misegades LK, Winter K, Harriman K, Talarico J, Messonnier NE, Clark TA, et al. Association of childhood pertussis with receipt of 5 doses of pertussis vaccine by time since last vaccine dose, California, 2010. JAMA 2012;308(20):2126–32. doi: http://dx.doi.org/10.1001/jama.2012.14939.

[40] Liko J, Robison SG, Cieslak PR. Priming with whole-cell versus acellular pertussis vaccine. N Engl J Med 2013;368(6):581–2.

[41] McGirr A, Fisman DN. Duration of pertussis immunity after DTaP immunization: a meta-analysis. Pediatrics 2015;135(2):331–43. doi: http://dx.doi.org/10.1542/peds.2014-1729.

[42] Warfel JM, Zimmerman LI, Merkel TJ. Acellular pertussis vaccines protect against disease but fail to prevent infection and transmission in a nonhuman primate model. Proc Natl Acad Sci USA 2014;111(2):787–92. doi: http://dx.doi.org/10.1073/pnas.1314688110.

[43] Ross PJ, Sutton CE, Higgins S, Allen AC, Walsh K, Misiak A, et al. Relative contribution of Th1 and Th17 cells in adaptive immunity to *Bordetella pertussis*: towards the rational design of an improved acellular pertussis vaccine. PLoS Pathog 2013;9(4):e1003264. doi: http://dx.doi.org/10.1371/journal.ppat.1003264.

[44] Mills KH, Barnard A, Watkins J, Redhead K. Cell-mediated immunity to *Bordetella pertussis*: role of Th1 cells in bacterial clearance in a murine respiratory infection model. Infect Immun 1993;61(2):399–410.

[45] Hendrikx LH, Berbers GA, Veenhoven RH, Sanders EA, Buisman AM. IgG responses after booster vaccination with different pertussis vaccines in Dutch children 4 years of age: effect of vaccine antigen content. Vaccine 2009;27(47):6530–6. doi: http://dx.doi.org/10.1016/j.vaccine.2009.08.052.

[46] Schure RM, Hendrikx LH, de Rond LG, Oztürk K, Sanders EA, Berbers GA, et al. Differential T- and B-cell responses to pertussis in acellular vaccine-primed versus whole-cell vaccine-primed children 2 years after preschool acellular booster vaccination. Clin Vaccine Immunol 2013;20(9):1388–95. doi: http://dx.doi.org/10.1128/CVI.00270-13.

[47] Maertens K, Caboré RN, Huygen K, Hens N, Van Damme P, Leuridan E. Pertussis vaccination during pregnancy in Belgium: results of a prospective controlled cohort study. Vaccine 2016;34(1):142–50. doi: http://dx.doi.org/10.1016/j.vaccine.2015.10.100.

[48] Hoang HT, Leuridan E, Maertens K, Nguyen TD, Hens N, Vu NH, et al. Pertussis vaccination during pregnancy in Vietnam: results of a randomized controlled trial Pertussis vaccination during pregnancy. Vaccine 2016;34(1):151–9. doi: http://dx.doi.org/10.1016/j.vaccine.2015.10.098.

[49] Ladhani SN, Andrews NJ, Southern J, Jones CE, Amirthalingam G, Waight PA, et al. Antibody responses after primary immunization in infants born to women receiving a pertussis-containing vaccine during pregnancy: single arm observational study with a historical comparator. Clin Infect Dis 2015;61(11):1637–44. doi: http://dx.doi.org/10.1093/cid/civ695.

[50] Munoz FM, Bond NH, Maccato M, Pinell P, Hammill HA, Swamy GK, et al. Safety and immunogenicity of tetanus diphtheria and acellular pertussis (Tdap) immunization during pregnancy in mothers and infants: a randomized clinical trial. JAMA 2014;311(17):1760–9. doi: http://dx.doi.org/10.1001/jama.2014.3633.

[51] The Australian immunization handbook. 10th ed.; 2015 [chapter 3]. <http://www.immunise.health.gov.au/internet/immunise/publishing.nsf/Content/Handbook10-home~handbook10part4~handbook10-4-12#4.12.8>.

[52] Health Service Executive (HSE) immunization, Ireland. Vaccines and pregnancy. <http://www.hse.ie/eng/health/immunisation/pubinfo/pregvaccs/>.

[53] New Zealand Ministry of Health (NZMoH). Immunization handbook. 2nd ed.; April 2016. <http://www.health.govt.nz/your-health/healthy-living/immunisation/immunisation-pregnant-women>.

[54] Centers for Disease Control and Prevention (CDC). Updated recommendations for use of tetanus toxoid, reduced diphtheria toxoid, and acellular pertussis vaccine (Tdap) in pregnant women – Advisory Committee on Immunization Practices (ACIP), 2012. MMWR Morb Mortal Wkly Rep 2013;62(7):131–5.

[55] Pertussis Green book [chapter 24]. <https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/514363/Pertussis_Green_Book_Chapter_24_Ap2016.pdf>.

[56] De Schutter S, Maertens K, Baerts L, De Meester I, Van Damme P, Leuridan E. Quantification of vaccine-induced antipertussis toxin secretory IgA antibodies in breast milk: comparison of different vaccination strategies in women. Pediatr Infect Dis J 2015;34(6):e149–52. doi: http://dx.doi.org/10.1097/INF.0000000000000675.

[57] Abu Raya B, Srugo I, Kessel A, Peterman M, Bader D, Peri R, et al. The induction of breast milk pertussis specific antibodies following gestational tetanus-diphtheria-acellular pertussis vaccination. Vaccine 2014;32(43):5632–7. doi: http://dx.doi.org/10.1016/j.vaccine.2014.08.006.

[58] Halasa NB, O'Shea A, Shi JR, LaFleur BJ, Edwards KM. Poor immune responses to a birth dose of diphtheria, tetanus, and acellular pertussis vaccine. J Pediatr 2008;153(3):327–32. doi: http://dx.doi.org/10.1016/j.jpeds.2008.03.011.

[59] Knuf M, Schmitt HJ, Jacquet JM, Collard A, Kieninger D, Meyer CU, et al. Booster vaccination after neonatal priming with acellular pertussis vaccine. J Pediatr 2010;156(4):675–8. doi: http://dx.doi.org/10.1016/j.jpeds.2009.12.019.

[60] Wood N, McIntyre P, Marshall J, Roberton D. Acellular pertussis vaccine at birth and one month induces antibody responses by two months of age. Pediatr Infect Dis J 2010;29:209–15.

[61] Belloni C, De Silvestri A, Tinelli C, Avanzini MA, Marconi M, Strano F, et al. Immunogenicity of a three-component acellular pertussis vaccine administered at birth. Pediatrics 2003;111(5 Pt 1):1042–5.

[62] Wood N, Siegrist CA. Neonatal immunization: where do we stand? Curr Opin Infect Dis 2011;24:190–5. doi: http://dx.doi.org/10.1097/QCO.0b013e328345d563.

[63] Dias WO, van der Ark AA, Sakauchi MA, Kubrusly FS, Prestes AF, Borges MM, et al. An improved whole cell pertussis vaccine with reduced content of endotoxin. Hum Vaccin Immunother 2013;9(2):339–48.

[64] Goodwin MS, Weiss AA. Adenylate cyclase toxin is critical for colonization and pertussis toxin is critical for lethal infection by *Bordetella pertussis* in infant mice. Infect Immun 1990;58(10):3445–7.

[65] Villarino Romero R, Bibova I, Cerny O, Vecerek B, Wald T, Benada O, et al. The *Bordetella pertussis* type III secretion system tip complex protein Bsp22 is not a protective antigen and fails to elicit serum antibody responses during infection of humans and mice. Infect Immun 2013;81(8):2761–7. doi: http://dx.doi.org/10.1128/IAI.00353-13.

[66] Cheung GY, Xing D, Prior S, Corbel MJ, Parton R, Coote JG. Effect of different forms of adenylate cyclase toxin of *Bordetella pertussis* on protection afforded by an acellular pertussis vaccine in a murine model. Infect Immun 2006;74(12):6797–805.

[67] Betsou F, Sebo P, Guiso N. CyaC-mediated activation is important not only for toxic but also for protective activities of *Bordetella pertussis* adenylate cyclase-hemolysin. Infect Immun 1993;61(9):3583–9.

[68] Sebo P, Osicka R, Masin J. Adenylate cyclase toxin-hemolysin relevance for pertussis vaccines. Expert Rev Vaccines 2014;13(10):1215–27. doi: http://dx.doi.org/10.1586/14760584.2014.944900.

[69] Seubert A, D'Oro U, Scarselli M, Pizza M. Genetically detoxified pertussis toxin (PT-9K/129G): implications for immunization and vaccines. Expert Rev Vaccines 2014;13(10):1191–204. doi: http://dx.doi.org/10.1586/14760584.2014.942641.

[70] Mielcarek N, Debrie AS, Raze D, Bertout J, Rouanet C, Younes AB, et al. Live attenuated *B. pertussis* as a single-dose nasal vaccine against whooping cough. PLoS Pathog 2006;2(7):e65.

[71] Skerry CM, Mahon BP. A live, attenuated *Bordetella pertussis* vaccine provides long-term protection against virulent challenge in a murine model. Clin Vaccine Immunol 2011;18(2):187–93. doi: http://dx.doi.org/10.1128/CVI.00371-10.

[72] Feunou PF, Bertout J, Locht C. T- and B-cell-mediated protection induced by novel, live attenuated pertussis vaccine in mice. Cross protection against parapertussis. PLoS ONE 2010;5(4):e10178. doi: http://dx.doi.org/10.1371/journal.pone.0010178.

[73] Li R, Lim A, Phoon MC, Narasaraju T, Ng JK, Poh WP, et al. Attenuated *Bordetella pertussis* protects against highly pathogenic influenza A viruses by dampening the cytokine storm. J Virol 2010;84(14):7105–13. doi: http://dx.doi.org/10.1128/JVI.02542-09.

[74] Schiavoni I, Fedele G, Quattrini A, Bianco M, Schnoeller C, Openshaw PJ, et al. Live attenuated *B. pertussis* BPZE1 rescues the immune functions of Respiratory Syncytial virus infected human dendritic cells by promoting Th1/Th17 responses. PLoS ONE 2014;9(6):e100166. doi: http://dx.doi.org/10.1371/journal.pone.0100166.

[75] Li R, Cheng C, Chong SZ, Lim AR, Goh YF, Locht C, et al. Attenuated *Bordetella pertussis* BPZE1 protects against allergic airway inflammation and contact dermatitis in mouse models. Allergy 2012;67(10):1250–8. doi: http://dx.doi.org/10.1111/j.1398-9995.2012.02884.x.

[76] Thorstensson R, Trollfors B, Al-Tawil N, Jahnmatz M, Bergström J, Ljungman M, et al. A phase I clinical study of a live attenuated *Bordetella pertussis* vaccine–BPZE1; a single centre, double-blind, placebo-controlled, dose-escalating study of BPZE1 given intranasally to healthy adult male volunteers. PLoS ONE 2014;9(1):e83449. doi: http://dx.doi.org/10.1371/journal.pone.0083449.

[77] DeAngelis H, Scarpino SV, Fitzpatrick MC, Galvani AP, Althouse BM. Epidemiological and economic effects of priming with the whole-cell *Bordetella pertussis* vaccine. JAMA Pediatr 2016 Mar 28. doi: http://dx.doi.org/10.1001/jamapediatrics.2016.0047.

[78] Sawyer MH. The pertussis problem and a possible solution: will parents go along? JAMA Pediatr 2016. doi: http://dx.doi.org/10.1001/jamapediatrics.2016.0157.