How to fight pertussis?

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Nicole Guiso

Abstract: Universal pertussis vaccination has successfully decreased pertussis mortality and morbidity in childhood. However, despite intensive vaccination of young children, pertussis remains a major public health problem in both developing and industrialized regions. Recent epidemics in California and Australia demonstrated that the agent of the disease is still circulating. They also revealed several aspects that must not be neglected concerning vaccine-preventable diseases. Indeed, pertussis is one of the oldest vaccine-preventable bacterial diseases, so can provide a good illustration of all of the aspects associated with the need for surveillance after the introduction of vaccination. (i) The type of vaccine: two types of pertussis vaccine, whole cell and acellular, inducing different types of immunity are now used around the world. (ii) The vaccine strategy, the vaccine coverage and the duration of vaccine immunity: pertussis epidemics provide evidence that 90% of the infants must be vaccinated, vaccination must be sufficiently early and both vaccine-induced immunity and natural infection-induced immunity to pertussis wane with time indicating that pertussis is not only a pediatric disease. (iii) The agents of the disease, *Bordetella pertussis* and *Bordetella parapertussis*: the intensive vaccination of young infants modified the herd immunity, controlled bacteria similar to the vaccine strains but not all, revealing polymorphism of the agents of the disease evidencing the importance of continuing their isolation and their surveillance as well as monitoring their antibiotic resistance. (iv) The diagnosis of the disease: the epidemics showed the importance of specific diagnostic techniques that are easy to use by medical laboratories and the availability of the reagents required. (v) Communication with the public, the health authorities and the health providers: any changes of vaccine type, vaccine strategy, characteristics of the disease, and biological diagnosis must be associated with appropriate communication with the public and training of healthcare workers. Currently, herd immunity needs to be increased by introducing vaccine boosters for adolescents and adults to protect the most vulnerable group: unvaccinated newborns.

Keywords: *Bordetella* species, diagnosis, pertussis vaccines, surveillance

Introduction

Pertussis is a respiratory disease with serious consequences for newborns and populations at risk, such as pregnant women and seniors. This disease has long been responsible for severe morbidity and mortality worldwide [World Health Organization, 2011]. In the middle of the last century, pertussis vaccination was introduced for young children [World Health Organization, 2011]. This led to a dramatic decrease in the mortality and morbidity due to this disease. However, 30 years after the widespread introduction of vaccination, the numbers of infants hospitalized for pertussis has been increasing, even in regions with high vaccine coverage [Baron *et al.* 1998; Bass and Stephenson, 1987]. In 2012, pertussis is still a significant problem in regions where there is a long history of vaccination [World Health Organization, 2011; Zepp *et al.* 2011]. Pertussis is still causing at least 200,000 deaths per year and there are at least 16 million cases [World Health Organization, 2011] worldwide annually, mostly in developing countries. As there is a lack of active surveillance in many countries, the true figures may be higher [Zepp *et al.* 2011].

Why is this vaccine-preventable disease still not under control? Before trying to answer this question, it is important to summarize the characteristics of the disease, of its agents and of the type of

Correspondence to:
Nicole Guiso, PhD
Institut Pasteur, unité Prévention et Thérapie Moléculaires des Maladies Humaines, 25 rue du Dr Roux, 75724 Paris Cedex 15, France
nicole.guiso@pasteur.fr

vaccines developed, all elements that are often insufficiently addressed in recent publications.

Pertussis or whooping cough

Pertussis is a highly communicable disease transmitted from infected to susceptible individuals through droplets. The classical course of the disease in a nonimmune individual can be divided in four phases.

(1) An incubation period of 7–14 days.
(2) A catarrhal phase of 7–10 days, which resembles a simple cold with mild cough and in most cases without fever.
(3) A paroxysmal phase of 4–6 weeks involving cough, particularly severe at night and frequently followed by vomiting. In young infants, pertussis may cause apnea and cyanosis without cough, whereas in adolescents and adults, uncharacteristic, persistent cough may be the only manifestation of the disease
(4) A convalescence phase of 6–7 weeks.

Secondary infections are frequent: pneumonia and also subconjunctival hemorrhage, hypoxia, seizures, and encephalopathy [Cherry *et al.* 2012]. However, actually, in regions where children were intensively vaccinated, atypical mild forms of pertussis are observed, particularly, in partially immune subjects. During these forms, the duration of cough can be shorter and vomiting or paroxysm can be absent. However, most of the time the increase of coughing at night is observed [Cherry *et al.* 2012; Heininger, 2010].

Untreated patients may be contagious for 3 weeks or more following the onset of typical coughing attacks, although communicability diminishes rapidly after the catarrhal stage.

Chronic carriers of *Bordetella pertussis* have not been described.

It is important to treat the patient suspected of whooping cough, this should prevent transmission [Heininger, 2010]. *B. pertussis* is sensitive to many antibiotics, but macrolides, for example clarithromycin and azithromycin, are recommended for treatment. Although antibiotic treatment may prevent transmission of the bacterium to other patients, it cannot modify the course of the disease because the clinical symptoms are due to the combined actions of bacterial virulence factors, adhesins and toxins [Matto and Cherry, 2005]

The agents of the disease: *B. pertussis* and *B. parapertussis*

B. pertussis, the agent of the disease, is a small Gram-negative bacterium of the genus *Bordetella*. It is a strict human pathogen. Other members of the genus *Bordetella*, in particular *B. parapertussis*, may cause pertussis-like disease. Unlike in the case of *Corynebacterium diphteriae*, it was difficult to identify the agent of the disease, and indeed, its isolation took 6 years [Bordet and Gengou, 1906] and the development of a complicated medium.

This is an important point that many scientists now, more than a century later, forget: it is very difficult to isolate and grow the bacterium reproducibly.

Again, unlike *C. diphtheriae*, it was not possible to characterize its toxin(s) rapidly and consequently the first vaccine developed was a pertussis whole-cell (Pw) vaccine, i.e. a vaccine composed of heat-killed bacteria. It took about 70 years to develop an acellular vaccine, that is a vaccine containing only detoxified bacterial proteins [Edwards and Decker, 2008].

The virulence factors of *B. pertussis* are classified into two types: adhesins and toxins. Adhesins facilitate the attachment of the bacterium to epithelial and phagocytic cells of the host, and toxins destroy tracheal and phagocytic cells helping the bacterium to evade the immune system of the host [Mattoo and Cherry, 2005]. The best characterized adhesins are the two fimbrial proteins (FIM2 and FIM3), filamentous hemagglutinin (FHA), pertactin (PRN) and toxins are tracheal cytotoxin [TCT, which acts in synergy with the lipopolysaccharide (LPS)], pertussis toxin (PT) and adenylate cyclase–hemolysin (AC-Hly). *B. parapertussis* expresses similar proteins, but not PT, and produces a different LPS [Mattoo and Cherry, 2005]. Following natural infection, antibodies to these virulence factors are detected. However, again unlike diphtheria, these antibodies are not associated with protection. In particular, the titer of anti-PT antibodies (PT is the only *B. pertussis*-specific antigen) decreases very rapidly after infection or vaccination. An important characteristic of the disease, often ignored in the recent literature, is that natural infection does not confer long-lasting protection against pertussis.

Individuals can have pertussis two or three times in their lives [Wendelboe *et al*. 2005]

Pertussis vaccines

Two types of vaccine have been developed since the discovery of the causal agents of the disease, and both are currently on the market: whole-cell pertussis vaccines (Pw), and pertussis subunit or acellular vaccines (Pa) composed of purified and detoxified components of the bacterium [Edwards and Decker, 2008].

The Pw vaccine was the first to be introduced because of the time it took before toxins were characterized, as had been done for the tetanus and diphtheria toxoids. One of the main reasons for this was that it was particularly difficult to culture the bacterium. The Pw vaccine was thus developed and used, and the bacteria included in the vaccine were regularly changed until the introduction of fermentors, after which the strain composition of the vaccine remained constant. The final strains chosen for this vaccine were those circulating during the development of Pw vaccines. The protection induced by effective Pw vaccines is between 88% and 90% [World Health Organization, 2011]. However, there are two major problems with Pw vaccines. First, they are difficult to produce in a reproducible manner, due to the difficulty of growing *B. pertussis*. Indeed, in trials carried out between 1990 and 1995, the efficacy of some Pw vaccines was found to be as low as 30% [Edwards and Decker, 2008]. The second problem is that Pw vaccines frequently induce secondary reactions, including local reactions, fever, seizures and hypotonic episodes. These major problems led to substantial efforts to identify and characterize the virulence determinants of *B. pertussis* and the pertussis antigens responsible for inducing protective immunity. It took several decades before there was sufficient progress in the characterization of these factors to allow the development of Pa vaccines.

Pa vaccines are safer than Pw vaccines. All Pa vaccines contain purified and detoxified PT, either alone or together with FHA, FHA plus PRN, or FHA plus PRN plus FIM. The efficacy of Pa vaccines with at least two components (e.g. PT+FHA) is about 75–85% [Edwards and Decker, 2008]. Vaccines for children (Pa vaccines) include more antigen than those for adolescents and adults (pa vaccines). All Pa and pa vaccines are combined with tetanus and diphtheria anatoxins and, sometimes, also with poliomyelitis, *Haemophilus influenza* b and hepatitis B vaccines. These Pa vaccines can be used for primary vaccination but, unlike Pw vaccines, Pa and pa vaccines can also be used for booster vaccinations.

The immunity induced by Pw and Pa vaccines is different. Pw vaccine induces an immunity directed against a wide array of antigens whereas Pa vaccine induces immunity against only a few antigens.

The efficacies of Pa and Pw vaccines vary depending upon the case definition used for pertussis. However, the best Pa vaccines are more effective than low-efficacy Pw vaccines but they may be less effective than the highest-efficacy Pw vaccines in preventing whooping cough [World Health Organization, 2011].

Furthermore, the duration of immunity induced by Pw and Pa vaccines might be different. However, comparisons between the duration of immunity induced by the two types of vaccines are often unreliable, because values for duration depend mostly on the biological diagnosis used for the disease and in the last 10 years the sensitivity and specificity have changed considerably [Riffelman *et al.* 2005]. In the last study of Sheridan and colleagues [Sheridan *et al.* 2012], in which control for diagnostic method was taking into account, it was suggested that the duration of immunity induced by Pa was shorter than that induced by Pw vaccine.

Epidemiology of the disease

Once Pw vaccines had been developed and became available, the vaccination strategy recommended in developed countries was primary vaccination at 3, 4 and 5 months of age, with a booster injection at 2 years of age. This strategy led to a considerable decrease in mortality and morbidity in regions in which vaccination coverage was high [World Health Organization, 2011]. However, the Pw vaccines used were produced by different manufacturers and differed considerably both in efficacy and in the induction of secondary effects. Some countries decided to stop vaccination. In others, vaccination was still recommended but the coverage decreased and in a third group of countries vaccination coverage remained very high. This led to differences emerging in the epidemiology of the disease between regions.

A few years later, the incidence of the disease increased substantially in those countries that had stopped their vaccination programs, resulting in high levels of morbidity and mortality [Zepp *et al.* 2011]. The decrease in vaccination coverage in some regions had major consequences and was the origin of huge differences in the epidemiological characteristics between regions [Grimprel *et al.* 1999].

Another epidemiological change was associated with a modification of the transmission of the disease which was observed 30 years after the introduction of vaccination of young children. There was an increase in the number of infants admitted to hospital due to contact with infected older sibling or infected parents. Thus, child-to-child transmission was replaced by adolescent/adult-to-infant transmission [Zepp *et al.* 2011]. These observations led to several transmission studies in developed countries, which showed that neither vaccine-induced nor natural immunity is lifelong [Zepp *et al.* 2011] and that whooping cough can affect individuals of all ages. This disease is not an exclusively pediatric disease.

There was therefore a need to reinforce herd immunity. Pw vaccines were not appropriate for this purpose, due to their secondary effects. Booster vaccines only became available with the release onto the market of Pa vaccines. In France, adolescent boosters were first introduced in 1998 [Conseil Supérieur d'hygiène publique de France, 1998], the cocooning strategy in 2004 [Conseil Supérieur d'hygiène publique de France, 2004] and adult boosters in 2008 [Haut conseil de la santé publique, 2008]. Several other European and North American countries have now introduced adolescent and adult boosters [Advisory Committee on Immunization Practices (ACIP), 2011; Zepp *et al.* 2011].

However, there is little awareness of this problem among either healthcare workers or the public. Whooping cough is still thought of as a childhood disease and public health authorities need to understand that the protection of infants involves not only vaccination of the mother and the father: vaccination is required for healthcare workers, daycare workers, grandparents and others coming into contact with infants, who may also transmit the disease.

To analyze the consequences of the introduction of boosters for adolescents and adults, surveillance of the disease is required in the countries where these boosters were introduced, with similar definitions of cases and where specific and sensitive biological diagnostic methods are used and available. This is not currently the case and is a major problem.

Surveillance of the disease

Whatever surveillance system is implemented, it is important for each country to have a National Reference Center, a clinical case definition for all age groups, and appropriate biological diagnostic procedures with availability of the reference reagents required. Although whooping cough is a bacterial disease of the respiratory tract, its diagnosis is not straightforward.

The major problems are as follows.

(1) The heterogeneity of clinical expression. Clinical diagnosis was easy during the prevaccine era, but now, 60 years after the intensive use of P vaccines for young children, it is more complex. According to the vaccine type, the vaccine strategy and the vaccine coverage, the transmission of the disease varies among regions and the clinical diagnosis varies according to the age of the patients. Currently, the clinical signs of whooping cough depend strongly on the level of immunity of the individual concerned, i.e. on their previous contact with the agent of the disease (natural infection or vaccination). For this reason, the Global Pertussis Initiative recently proposed definitions according to the age of the suspected case [Cherry *et al.* 2012].

(2) The necessity for biological diagnosis. Biological diagnosis may be either direct, for example by culture to isolate the causal agent of the disease or by real-time polymerase chain reaction (PCR) to detect genetic material from the bacterium responsible. Alternatively, it may be indirect, for example by measuring anti-PT antibody titers in the serum of the patient with suspected *B. pertussis* infection. However, the accuracy of these diagnostics depends on the delay since the beginning of the catarrhal phase, how the samples were obtained and the availability of both the reagents and the expertise required for these diagnostic methods

[Guiso, 2011; Loeffelholz, 2012; Zepp *et al.* 2011].

- Culture should be used during the catarrhal phase and the first 2–3 weeks of the paroxysmal phase. It is the gold standard of diagnostic methods and the cheapest to confirm *Bordetella* infection [Guillot *et al.* 2012]. It has a high sensitivity in infants but a lower sensitivity in adults who often present later to their physician after the beginning of the cough.
- Real-time PCR is more sensitive than culture, in particular for adolescents and adults consulting their physician after 3–4 weeks of cough, and is also faster than culture. It is now the most widely used diagnostic method. However, it is technically demanding [Loeffelholz, 2012]. Furthermore, it is not specific for *B. pertussis* and a positive result may reflect the presence of other *Bordetella* species such as *B. holmesii* or *B. bronchiseptica* [Njamkepo *et al.* 2011; Riffelmann *et al.* 2005; Rodgers *et al.* 2012; Yih *et al.* 1999], mostly found in adolescents and adults. This lack of specificity can be a problem, in particular during outbreaks [Rodgers *et al.* 2012; Yih *et al.* 1999].
- After 3 weeks of cough, culture or PCR diagnostic tests can be used for secondary cases, because the disease is highly contagious. If no secondary case is detected, indirect diagnostic tests can be used: testing for anti-PT antibodies, the only antibodies specific for *B. pertussis*. This diagnosis must be performed using enzyme-linked immunosorbent assay (ELISA) and purified PT as the antigen [Guiso, 2011].

(3) The availability of the diagnostic reagents. Not all commercial PCR and ELISA kits have been validated and National Reference Centers need to be aware of this major problem when they establish protocols for surveillance in their country [Lanotte *et al.* 2011; Riffelmann *et al.* 2010].

The surveillance of the disease is of crucial importance for adapting vaccine strategies, for analyzing the effects of modifications to such strategies, and monitoring the consequences of changes in the populations of the agents of whooping cough. This surveillance can be developed in industrialized countries since consensus papers are published concerning clinical and biological diagnosis and reagents are available. However, reagents are far from available to all countries because of transportation and cost. When resources are very low, surveillance of infants hospitalized for pertussis can be developed with the gold standard which is also the cheapest tool for diagnosis: culture.

Surveillance of the causal agents of whooping cough

Whatever the surveillance method used, it is important to remember that eubacteria evolve and adapt rapidly to new ecosystems, and have been doing so for much longer than humans.

The routine use of PCR for biological diagnosis should not stop reference laboratories from isolating the bacterium for two reasons. First, isolation is valuable for monitoring antibiotic resistance: in 2012, we isolated a macrolide-resistant *B. pertussis* for the first time in Europe [Guillot *et al.* 2012]. Second, it allows analysis of the polymorphism of *B. pertussis* and *B. parapertussis* [Mooi *et al.* 2007]. We previously showed that the use of Pw vaccine for more than 25 years in France controlled the population of vaccine strain types but not all bacterial types [Guiso, 1997; Weber *et al.* 2001]. Despite the evolution of the *B. pertussis* species, data from a national transmission study over the same period in France [Baron *et al.* 1998] showed that vaccine efficacy was still as high as 94%. The influence of Pw vaccine-induced immunity was confirmed by the analysis of isolates circulating in 1991–1995 in Niakkhar, Senegal, a region with low vaccination coverage [Njamkepo *et al.* 2008]. The isolates circulating in this region were different from those collected in France over the same period, but were similar to those circulating before the introduction of the vaccine.

Since 2000, vaccination coverage in France has been increasing in adolescent and adult populations. Pa vaccines, which target the virulence determinants of the bacterium rather than the whole bacterium, have been used. As predicted [Guiso, 2009], the circulation of *B. pertussis* isolates not expressing PRN has been observed since 2005, and their prevalence is increasing [Hegerle *et al.* 2012]. Similar observations have recently been reported in Finland, Japan and United States [Barkoff *et al.* 2012; Otsuka *et al.* 2012;

Queenan *et al*. 2013]. Our preliminary comparison of the clinical symptoms induced by isolates not expressing PRN and isolates expressing PRN in infants less than 6 months of age indicate no major clinical differences between the two groups [Bodilis and Guiso, 2013]

All of these observations suggest that vaccine-induced immunity is again influencing the *B. pertussis* population. Furthermore, we have observed that, as reported for *B. pertussis*, the *B. parapertussis* isolates currently circulating in France do not express PRN [Bouchez *et al.* 2011; Hegerle *et al.* 2012]. This observation suggests that Pa vaccine-induced immunity has also affected *B. parapertussis*.

How to fight pertussis?

The objective in industrialized countries has changed since the prevaccine era. The incidence of the disease is low in young children and adolescents in regions with extensive vaccination coverage of these populations. This indicates that the Pa vaccines are successfully inducing protective immunity. The immunity induced by the vaccine is not lifelong, but nor is the immunity induced by natural infection. There is still a lot of work to do before we can do better than natural infection.

Currently, it is important to recognize that *B. pertussis* is circulating in all age groups and, therefore, there is a need, universally, to vaccinate all age groups with Pa vaccines, as was the case for diphtheria and tetanus toxoids.

Pertussis infection among infants can be reduced by vaccinating as early as at 6 weeks of age. However, as highlighted by the WHO [World Health Organization, 2011] vaccine coverage needs to be high and each dose of vaccine must be administered at the appropriate time. Nevertheless, it is important to be aware that even one dose of vaccine can confer protection on infants under 6 months of age: the risks of hospitalization are reduced and the symptoms milder [Olin *et al.* 2003; Tozzi *et al.* 2003; World Health Organization, 2011].

Neonatal immunization with Pa vaccines may be a well-tolerated strategy which can protect early in life against pertussis [Wood and Siegrist, 2011]. However, there are still questions on vaccine interference, efficacy of the strategy and public acceptance.

The cocooning strategy is in theory the best and is recommended [Ullooa-Guttierrez *et al*. 2012]. Recently, it was shown in US that the reduction of infant hospitalizations would be greater if both parents are vaccinated compared with vaccination of just the mothers [Peters *et al.* 2012]. However, the success of this strategy has not yet been established and different models should be investigated in different situations Furthermore, it is still very difficult to implement.

Immunization of pregnant women is another strategy which was recently recommended in the United States [Advisory Committee on Immunization Practices (ACIP), 2011]. However, there are still several issues to resolve before the use of this strategy should be generalized. They include the timing of immunization, the duration of immunity, and the blunting of infant immune responses in the presence of maternal antibodies. Furthermore, immunization of pregnant women will also be difficult to implement in many countries.

The development of a new pertussis vaccine inducing a long-lasting immunity would obviously be of enormous value. Unfortunately, it is likely to take 10–20 years, and will undoubtedly require substantially more basic research on *Bordetella* species. Indeed, optimizing the adjuvants, genetic detoxification of current vaccine antigens and the inclusion of new antigens can all be envisaged to improve vaccine efficacy. However, new rules need to be established for licensing these vaccines, because new efficacy trials cannot be performed. In the short term our objectives should be to stop the transmission of the disease to infants. Pa and pa vaccines are safe and boosters may be administered at any age [Beytout *et al.* 2009; Halperin *et al.* 2012] as for tetanus and diphtheria vaccines.

In conclusion, vaccination strategies should be optimized on the basis of epidemiological data. Consequently, disease surveillance, with standardized and biological diagnostic methods, is required to generate accurate and exploitable epidemiological data. The diagnostic techniques used must be both sensitive and specific. The discovery that *B. holmesii* can also be detected in the respiratory tracts of adults and adolescents teaches us an important lesson [Njamkepo *et al.* 2011; Rodgers *et al.* 2012; Yih *et al.* 1999]. Surveillance systems must be comparable worldwide, based on a uniform case definition, but currently this is not the case. It is important to recall

that antibiotic prophylaxis (mainly macrolides such as clarithromycin and azithromycin) of contacts of confirmed cases will stop the spread of infection. There is also a need for continuous communication with public health authorities, healthcare workers and the public, to provide information about the reasons for changes in vaccination strategy.

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Conflict of interest statement

The author declares that there are no conflicts of interest.

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