

## Pertactin deficiency of *Bordetella pertussis*: Insights into epidemiology, and perspectives on surveillance and public health impact

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### ABSTRACT

Pertussis resurgence has been documented even in countries with high pediatric vaccine coverage. The proportion of *Bordetella pertussis* isolates not expressing pertactin (PRN) has increased in several countries where acellular pertussis (aP) vaccines are used. We systematically reviewed published literature up to July 2023 on PRN-negative *B. pertussis* isolates in MEDLINE and Embase with no geographical limitations, complemented with a gray literature search. An increase in the proportion of PRN-negative isolates was observed in countries where aP vaccines were used, while such isolates seem to be absent in countries using whole-cell pertussis vaccination. We reviewed the data supporting aP vaccine-driven evolution of *B. pertussis*, explored the effects of PRN deficiency on the clinical presentation of pertussis, summarized the evidence for preserved aP vaccine effectiveness, and proposed actions to further improve assessment of the clinical significance of PRN deficiency and its potential impact on pertussis prevention.

### PLAIN LANGUAGE SUMMARY

The burden of pertussis (whooping cough), once a common cause of morbidity and mortality among children, was greatly reduced by the introduction of pediatric pertussis vaccination. Two types of pertussis vaccines exist: whole-cell pertussis (wP) vaccines, containing inactivated *Bordetella pertussis* bacteria, and acellular pertussis (aP) vaccines, containing purified antigens. Having lower reactogenicity, aP vaccines have replaced wP vaccines in many developed countries. In other parts of the world, wP vaccines are still used. Over time, the proportion of *B. pertussis* isolates not expressing pertactin (PRN, a surface antigen involved in virulence and contained in most aP vaccines) increased in countries using aP vaccines, but not in those using only wP vaccines. We performed a systematic literature review to collect data on the epidemiology of PRN-negative *B. pertussis* isolates identified worldwide. We combined these data with information on the type of pertussis vaccine used in each country and on the year aP vaccines were introduced, to assess if aP vaccine use was associated with a higher proportion of PRN-negative *B. pertussis* strains. We then selected three countries (Belgium, France, and Canada), for which we present data on pertussis diagnosis, surveillance, epidemiology, pertussis vaccine recommendations, and vaccine coverage, in connection with the PRN-negative *B. pertussis* data retrieved by our search. We found that the symptoms and severity of pertussis cases appear to be similar regardless of whether they were caused by PRN-negative or PRN-positive strains. There is no evidence of impaired protection by aP vaccines in countries where most circulating *B. pertussis* strains are PRN-negative.

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## Introduction

The first effective pertussis vaccine, developed in the 1930s,<sup>1</sup> was obtained by culturing and inactivating *Bordetella pertussis*. This whole-cell pertussis (wP) vaccine represented a breakthrough in the prevention of pertussis: its widespread use as part of the diphtheria-tetanus-pertussis combination vaccines has led to a major reduction in pertussis incidence.<sup>2</sup> While wP vaccines are effective and relatively inexpensive, producing consistent lots is difficult. wP vaccines are also quite reactogenic, frequently causing adverse reactions such as fever as well as redness or swelling at the site of injection, which tend to increase with age

and the number of injections.<sup>3</sup> In addition, the use of wP vaccines had decreased in many countries in the 1970s and 1980s based on suspicions that these vaccines may be linked to neurological damage in children,<sup>2,4–6</sup> although this hypothesis was subsequently disproved.<sup>7–9</sup>

To address the concerns related to the adverse reactions observed following wP vaccination, acellular pertussis (aP) vaccines were developed<sup>3</sup> and progressively introduced in national immunization programs. These aP vaccines contain 1–5 purified components of *B. pertussis*, all of them including pertussis toxin (PT), and, optionally, filamentous hemagglutinin (FHA,

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in 2-, 3-, and 5-component aP vaccines), pertactin (PRN, in 3- and 5-component aP vaccines), and/or fimbrial proteins (FIM2 and FIM3, in 5-component aP vaccines).<sup>10</sup> Their introduction in many developed countries increased vaccine acceptance and further decreased the global burden of pertussis, by allowing introduction of boosters in older children and adolescents, in whom the use of wP vaccines is not recommended due to high reactogenicity.<sup>11,12</sup> The aP vaccines were demonstrated to protect against severe and typical symptomatic pertussis disease. They do not, however, seem to prevent nasopharyngeal colonization and transmission of *B. pertussis* from asymptomatic or oligosymptomatic vaccinated individuals based on evidence from non-human primates<sup>13</sup> and mathematical models<sup>14</sup> (reviewed in<sup>15</sup>). Following their introduction, aP vaccines have often been used in parallel with wP vaccines for some time, before completely replacing them in most developed countries. There are differences in the antigens contained by the aP vaccines used in different countries, as well in the number of recommended doses and the ages at which they are administered.<sup>16</sup> The World Health Organization recommends that countries currently using wP vaccines continue their use for primary vaccination series.<sup>12</sup>

Over the past decades, pertussis resurgences have been reported, even in countries with high pediatric vaccination coverage irrespective of the use of wP or aP vaccines.<sup>17,18</sup> Multiple factors are thought to contribute to these resurgences.<sup>19</sup> Increased disease awareness and improved diagnostics have resulted in a better detection of cases. Additionally, as aP vaccines seem less effective than wP vaccines at preventing infection and transmission of *B. pertussis* (as outlined above),<sup>13–15</sup> a greater circulation of *B. pertussis* could occur in populations vaccinated with aP vaccines compared to wP vaccines, leading to more infections in susceptible individuals. Furthermore, the immunity induced by aP vaccines wanes more rapidly than the immunity induced by wP vaccines.<sup>19–21</sup> Vaccine hesitancy could increase the risk of pertussis and further contribute to the pertussis resurgences as it leads to lower vaccine coverage.<sup>22</sup> Another factor could be represented by the genetic changes exhibited by *B. pertussis*, which are the result of vaccine-induced selective pressure.<sup>15,19</sup> In line with this hypothesis, several independent mutations have been observed in phylogenetically different *B. pertussis* strains circulating since pertussis vaccine introduction, some of which result in isolates not producing one or more antigenic proteins.<sup>11,15,23</sup> One of the most impressive changes is represented by the increase in *B. pertussis* strains not expressing PRN.<sup>24</sup> PRN is an autotransporter protein on the outer membrane of *B. pertussis*.<sup>25</sup> Although it has been proposed to play a role in host cell adhesion<sup>26–28</sup> and immune modulation,<sup>29</sup> its exact role in pathogenesis is not completely understood.<sup>15</sup> Antibodies against PRN play a major role in the protection against pertussis,<sup>15,30,31</sup> and therefore several aP vaccines feature the PRN antigen in their composition.

Multiple mechanisms for PRN deficiency have been identified, including insertions, deletions, inversions, and point mutations affecting the genes encoding the PRN protein.<sup>23</sup> Despite the proposed role of PRN as a virulence factor, PRN-negative strains showed higher fitness than PRN-positive strains in mice immunized with an aP vaccine in terms of their ability to

colonize the respiratory tract.<sup>32</sup> Another study showed that aP vaccines (containing PRN as antigen) generate bactericidal antibodies that are only directed against PRN, as opposed to natural infection, which induces bactericidal antibodies against PRN and other antigens.<sup>33</sup> This suggests that in the context of widespread aP vaccine use, the cost of decreased virulence due to PRN loss might be compensated by a gain in fitness, potentially allowing increased transmission among vaccinated individuals, thus representing a selective advantage for PRN-negative strains.<sup>11,15,24</sup> Although concerns have been raised regarding the potential decrease of pertussis vaccine effectiveness (VE) against disease caused by the increasing proportion of PRN-negative *B. pertussis* strains, no conclusive evidence confirming this hypothesis has been presented to date.<sup>11,15,34</sup> Moreover, additional information on the severity and clinical presentation of pertussis cases caused by PRN-negative strains (in comparison with those caused by PRN-positive strains) is needed to define the clinical significance of PRN deficiency.

In this review, we aimed to i) provide an overview of the published data on the proportion of PRN-negative *B. pertussis* isolates, without imposing any geographical limitation; ii) assess the significance of a higher circulation of PRN-negative *B. pertussis* strains in three countries (Belgium, France, and Canada), by considering contextual epidemiological data, the pertussis surveillance system used, diagnostic practices, and vaccination policy; iii) propose actions that would further improve assessment of the clinical significance of PRN deficiency and its potential impact on pertussis prevention.

## Materials & methods

### Systematic literature review

For the first part of this review, a systematic literature search was performed on 21 July 2023 in MEDLINE (via the PubMed interface) and Embase. No limits regarding the year of publication, language, geographical scope, or publication type were applied.

This systematic search was complemented by a gray literature search, which included a Google search (performed on 14 February 2022 and on 2 August 2023) and screening of more than 24 websites (performed on 2 August 2023), including national websites/registries for six countries known to have relevant data (Australia, France, Germany, the Netherlands, the United Kingdom [UK], and the United States of America [USA]). Details regarding the search strategies employed can be found in Supplementary file 1.

Publications that included relevant data on the incidence or prevalence of PRN-negative *B. pertussis* strains, regardless of the age group from which the strains were isolated, were selected for analysis. Vaccine efficacy/effectiveness studies, studies presenting diagnostic tools/methods for genotyping, animal studies, and modeling studies without original data were excluded. After the title and abstract screening by two independent reviewers in duplicate, the full-text articles were screened for relevance by two reviewers (20% in duplicate). At this stage, studies that did not perform relevant methods or did not contain data relevant for the review objective (i.e., not identifying

*B. pertussis* strains), studies that did not mention PRN-negative strains, narrative reviews, studies of insufficient methodological quality, studies that contained no relevant quantitative data, and studies not published in peer-reviewed journals were excluded. An effort was made to identify and eliminate duplicate data, i.e., similar results from identical samples originating from the same source and covering the same period. Data from the included full-text articles were then extracted using the systematic review software DistillerSR. The study selection, data extraction, and quality assessment processes, as well as the statistical methods used to analyze the extracted data are described in Supplementary file 1.

The percentage of PRN-negative *B. pertussis* strains and both numerator and denominator of this proportion were recorded. The denominator was the sample of confirmed *B. pertussis* isolates evaluated for PRN deficiency. Whenever possible (i.e., if data was available per isolate), data was extracted separately for each year covered by a particular study.

### Contextual analysis of PRN deficiency proportion in selected countries

Three countries (Belgium, France, and Canada) were selected for performing an in-depth, contextual analysis of PRN-negative *B. pertussis* strain prevalence. These

countries were chosen based on the availability of recent data regarding the national vaccination schedule (including recommendations for booster vaccination), the type(s) of vaccine administered, pertussis vaccine uptake, diagnostic practices, surveillance of pertussis disease and availability of *B. pertussis* strains (including data on PRN deficiency), and/or VE evaluations.

## Results

### Systematic literature review

#### Study characteristics

Overall, 47 records were included in this review (Figure 1). All 47 studies had a retrospective design and were specifically targeting *B. pertussis* isolates or pertussis-suspected cases.

Samples were mainly acquired from two types of sources: either already-confirmed *B. pertussis* isolates from a national reference institution or from suspected/confirmed cases of (a) specific hospital(s). Isolates were obtained either from a regular country surveillance program or from a specific outbreak. Studies covered time periods spanning from 1 to 88 years; the sample size had a mean of 72, a median of 22, and ranged from 1 to 1,428 (Supplementary table S1). Most studies were of sufficient quality, as described in Supplementary file 2.

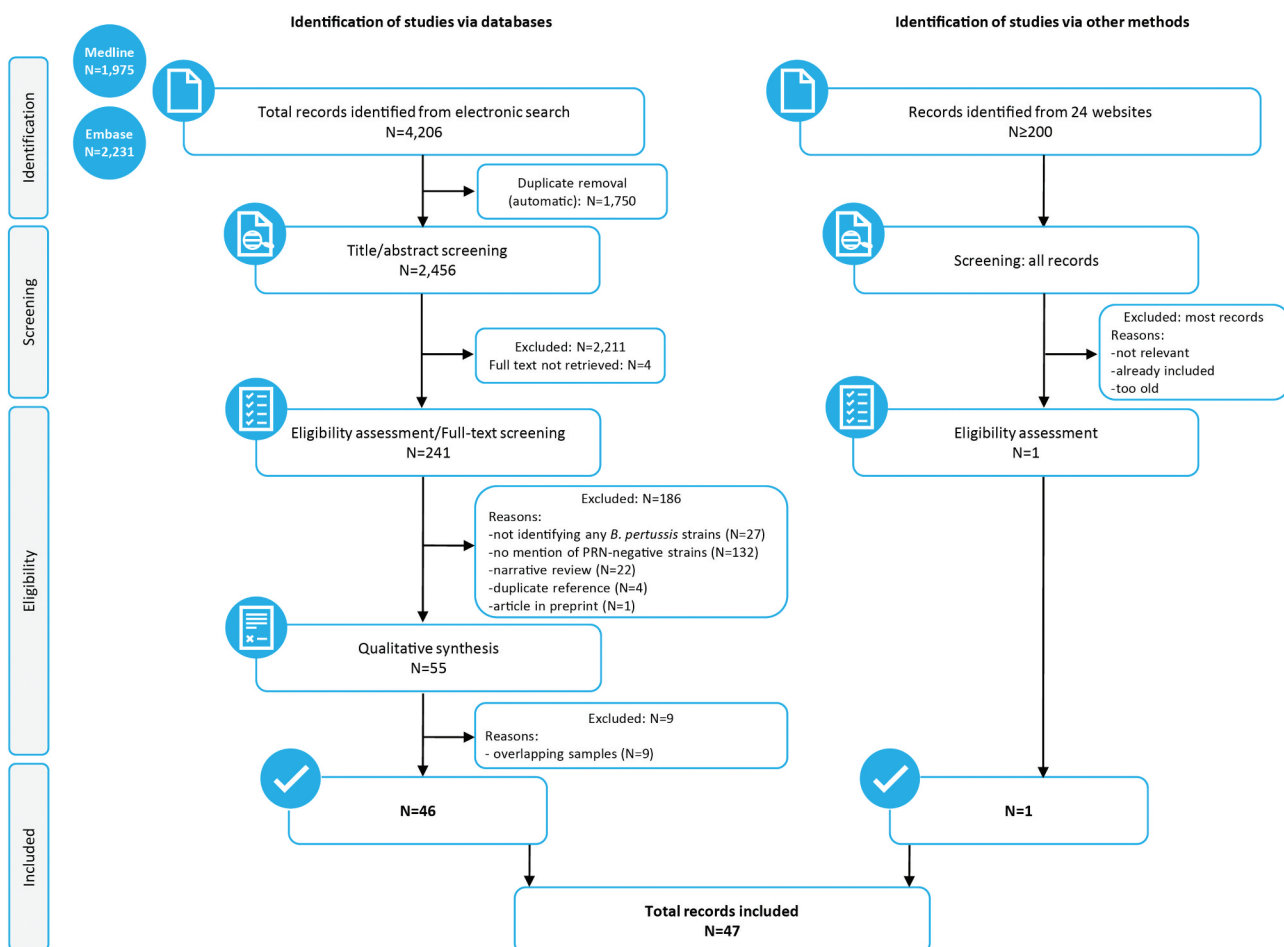


Figure 1. PRISMA flow diagram. N, number of records; *B. pertussis*, *Bordetella pertussis*; PRN, pertactin

### Epidemiological mapping of PRN-negative *B. pertussis* strains

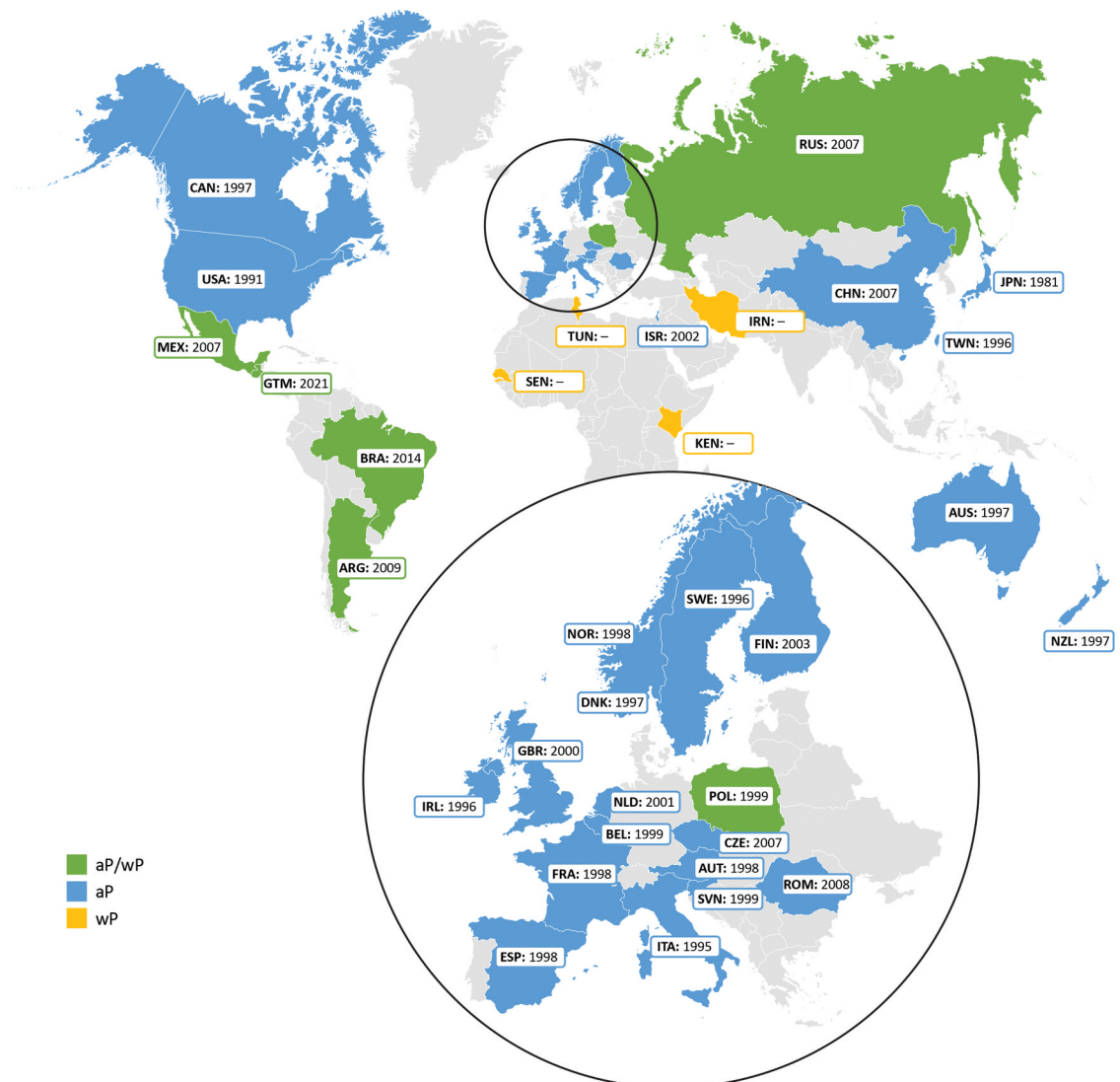
The 47 studies were conducted in 33 countries across all continents; most were from Europe. The countries with most publications were the USA (11) and France (8), followed by Canada and Italy (5 publications each), China, Finland, Japan, the Netherlands, Sweden, and the UK (4 publications each), Australia, Denmark, Norway, and Iran (3 publications each), Argentina, Belgium, Poland, and Spain (2 publications each). For all other countries with available data, these were extracted from one publication for each country (Supplementary table S1).

Among the studied countries, PRN-negative *B. pertussis* strains were identified in Canada, the USA, Argentina, Brazil, all 16 European countries, China, Israel, Japan, Australia, and New Zealand. Overall, the proportion of PRN-negative strains increased in most countries where assessments were performed over several consecutive time periods. In some countries, such as the USA or New Zealand, PRN-negative strains have risen to dominance, representing > 80% of identified strains.<sup>23,35–40</sup> In countries that primarily or exclusively use

wP vaccines, no or few PRN-negative *B. pertussis* strains have been reported, as observed in Tunisia,<sup>41</sup> Argentina,<sup>42,43</sup> and Brazil.<sup>44</sup>

### Vaccine types




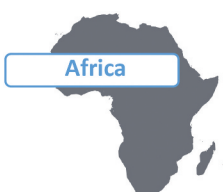
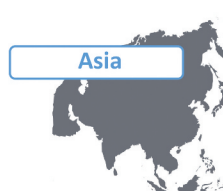

Both wP and aP vaccines were used in the studied countries. Most countries included in this review have introduced the aP vaccine between 1997 and 2007 for primary and/or booster vaccination, often completely replacing wP vaccines with aP vaccines in the immunization schedules; 10 of the studied countries still use wP vaccines in combination with aP vaccines, and four countries never introduced aP vaccines in their national immunization programs (Iran, Tunisia, Kenya, and Senegal) (Figure 2). Most of the studies included in this review did not report the type of the vaccine used in the population from which the sample originated from, but rather the vaccine introduction year, an overall strategy of the country and/or the national vaccination schedule (for primary and booster vaccination). Comprehensive information on these parameters is presented in Supplementary table S2.



**Figure 2.** Current use of whole-cell pertussis (wP) and acellular pertussis (aP) vaccines and year of aP vaccine introduction (primary and/or booster) for countries included in the systematic literature review. In countries using only wP vaccines, there might be aP vaccines available on the private market; however, the proportion of this private market may differ greatly from one country to another.

The major types of pertussis vaccines currently used worldwide for primary immunization are presented for each continent in Figure 3.<sup>45–58</sup> In North America, both Canada and the USA currently use only aP vaccines that include the PRN

antigen. In several regions of the world, wP and aP vaccines are both used.<sup>59</sup> In Argentina, a wP vaccine is generally used for primary vaccination, while the aP vaccine is used in the private sector and for the recommended boosters in

	wP vaccines	aP vaccines	PRN	Ref.
 North America	NA	<ul style="list-style-type: none"> <li>DT3aP-HepB-IPV</li> <li>DT3aP-IPV/Hib</li> <li>DT3aP-IPV-HepB/Hib</li> <li>DT5aP-IPV/Hib</li> <li>DT5aP-IPV-HepB-Hib</li> </ul>	Yes Yes Yes Yes Yes	Centers for Disease Control and Prevention [44] Government of Canada [45]
 South America	DTwP-HepB-Hib	Argentina (for high-risk infants/private market): <ul style="list-style-type: none"> <li>DT2aP-IPV-HepB-Hib</li> <li>DT3aP-IPV-HepB/Hib</li> </ul> El Salvador: <ul style="list-style-type: none"> <li>DT3aP-IPV-HepB/Hib (4<sup>th</sup> dose, at 18 months of age)</li> </ul> Costa Rica: <ul style="list-style-type: none"> <li>DT5aP-IPV/Hib</li> <li>DT2aP-Hib-HepB-IPV</li> </ul> Chile: <ul style="list-style-type: none"> <li>DT2aP-Hib-HepB-IPV</li> </ul>	No Yes Yes No No	Olivera et al., 2023 [46] Avila-Agüero et al., 2022 [47] Aguirre-Boza et al., 2021 [48] V Berlaimont, personal communication
 Europe	Poland: DTwP (except for risk groups, in which DTaP-Hib-IPV is administered)	<ul style="list-style-type: none"> <li>DT2aP-IPV-HepB-Hib</li> <li>DT3aP-IPV-HepB/Hib</li> <li>DT5aP-IPV-HepB-Hib</li> </ul>	No Yes Yes	Knuf et al., 2021 [49] World Health Organization [50]
 Africa	DTwP-Hib-HepB Mauritius: DTwP-Hib-HepB-IPV	<ul style="list-style-type: none"> <li>South Africa: DT2aP-Hib-HepB-IPV</li> </ul>	No	Muloiwa et al., 2018 [51] World Health Organization [52] Davis, 2019 [53]
 Asia	LMICs: penta- and hexavalent wP vaccines from local or Indian manufacturers	HIC: <ul style="list-style-type: none"> <li>Singapore: DTaP, DT3aP-IPV-HepB/Hib and DT3aP-IPV/Hib</li> <li>Hong Kong: DTaP-IPV, DT3aP-HepB-IPV/Hib, DT2aP-HepB-IPV-Hib, DT2aP-IPV-Hib</li> <li>South Korea: DTaP, DTaP-IPV, DTaP-Hib-IPV (DT2aP and DT3aP)</li> <li>Taiwan: DT3aP-IPV-HepB/Hib, DT3aP-IPV/Hib, DT2aP-IPV-HepB-Hib, DT2aP-IPV-Hib</li> <li>China, Japan: local aP vaccines</li> </ul>	Yes Yes/No Yes/No Yes/No ?	Jog et al., 2022 [54] Hong Kong Department of Health [55] V Berlaimont, personal communication
 Australia & New Zealand	NA	<ul style="list-style-type: none"> <li>DT3aP-IPV-HepB/Hib</li> <li>DT5aP-IPV-HepB-Hib</li> </ul>	Yes Yes	Australian Government, 2023 [56] New Zealand Ministry of health, 2020 [57]

**Figure 3.** Major pertussis vaccine types for primary immunization currently in use worldwide. aP, acellular pertussis (the number before aP indicates the number of pertussis antigens included in the vaccine); D, diphtheria toxoid; HepB, hepatitis B; Hib, *Haemophilus influenzae* type b; HIC, high-income country; IPV, inactivated poliovirus; LMIC, low- and middle-income country; NA, not applicable; T, tetanus toxoid; wP, whole-cell pertussis

adolescents, healthcare workers in contact with infants <12 months of age, household contacts of low-birthweight infants, and pregnant women<sup>47</sup> (Supplementary table S2). In Brazil, aP vaccines, which include the PRN antigen,<sup>44</sup> are used for infants in special groups and booster doses for adults in certain categories (Supplementary table S2). Currently, all European countries exclusively use aP vaccines, except for Poland, where both wP and aP vaccines are used (Supplementary table S2). Of the countries using aP vaccines, only Denmark has solely been using a vaccine that does not contain the PRN antigen during the study period.<sup>60</sup> Other countries have been using a mix of PRN-containing and non-PRN-containing aP vaccines, or only vaccines that contain PRN.<sup>60</sup>

### Emergence of PRN-negative strains and the use of aP vaccines

Although a formal comparison between countries is methodologically challenging, almost all countries showed a consistent increase in PRN-negative strains approximately a decade after the introduction of aP vaccines in their immunization schedules. The proportion of PRN-negative isolates over time according to the sample size and ordered by the year of aP vaccine introduction is shown for the USA, Italy, Sweden, Australia, France, the Netherlands, and Finland isolates in Figure 4. While in countries like the USA, Italy, Sweden, or Australia, the percentage of PRN-negative *B. pertussis* isolates has increased following aP vaccine introduction, this pattern was not consistent across countries. This might be due to differences in vaccination schedule, vaccine coverage, and the marked heterogeneity of the aP vaccine types used with regards to PRN content. In most countries analyzed, PRN-containing aP vaccines have been used for primary vaccination and boosting alternatively or in parallel with non-PRN-containing aP vaccines. However, publicly available data for each vaccine type are not sufficiently granular to allow delimitating periods of predominant or exclusive PRN-containing aP vaccine use from periods when immunization with non-PRN-containing aP vaccines was more common. Therefore, no definitive conclusion regarding the effect of PRN-containing aP vaccines on the proportion of PRN-negative *B. pertussis* isolates can be drawn based on the available data.

Japan was the first country to introduce aP vaccination in 1981, using both vaccines with and without PRN. Since the late 1990s, the proportion of PRN-negative *B. pertussis* has increased gradually, reaching a maximum of 41–100% (depending on the study) between 2005 and 2010.<sup>61,62</sup> However, a subsequent decrease in PRN-negative isolates has been recorded, with only 8% of the isolates collected between 2014 and 2016 exhibiting this characteristic; this trend emerged a few years after PRN-containing vaccines were replaced with vaccines that did not contain PRN.<sup>61</sup> Among European countries using aP vaccines, the proportion of PRN-negative *B. pertussis* isolates is lowest in Denmark: only four PRN-negative strains were detected since 2012 (4/27, 14.8%) (Supplementary table S1). These strains could have been isolated from cases potentially originating from neighboring countries, where the frequency of PRN-negative isolates is high.<sup>60</sup> Denmark is the only European country where a monocomponent (PT only) aP vaccine was used until 2019.<sup>60,63</sup>

In countries where aP vaccines have not been introduced in the national immunization program (Iran, Senegal, Kenya, and Tunisia), no PRN-negative strains have been identified (Supplementary table S1).

### Contextual analysis of PRN deficiency proportion in selected countries

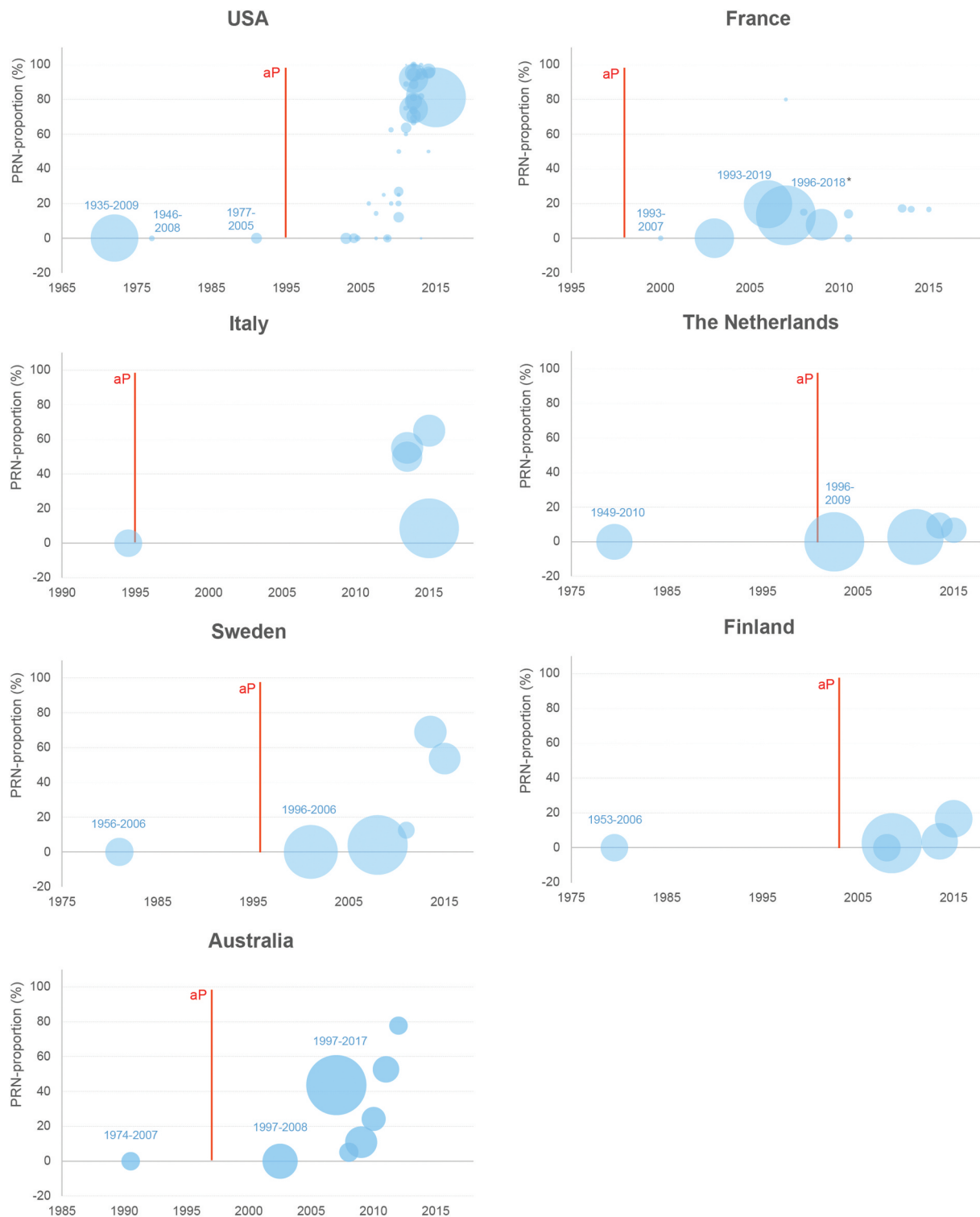
#### Belgium

Pertussis is a notifiable disease in all three Belgian regions (Brussels, Flanders, and Wallonia). Until 2019, it was mandatory to report all possible, probable, and confirmed pertussis cases. In 2020, compulsory notification underwent major adaptations in Wallonia and Brussels. In Wallonia, only confirmed cases in children <3 years of age are required to be reported, while in Brussels, only confirmed cases (regardless of age) are to be declared. In Flanders, no changes to the notification rules have been made.<sup>64</sup> In Belgium, pertussis is diagnosed by polymerase chain reaction (PCR) and serology;<sup>64,65</sup> as of April 2019, PCR diagnosis is only reimbursed for suspected pediatric cases, under certain conditions (Table 1).

Pertussis surveillance relies on three different sources, with information being obtained from notified cases and a laboratory network (Table 2). Data from all sources combined showed an increase in the number of pertussis cases between 2011 and 2014. From 2015 to 2017, this trend was less clear, with variations observed according to region and the source of the data (Figure 5A, Supplementary table S3). In 2018, the number of pertussis cases reported by all sources decreased. This drop continued to be observed in 2019 in all data sources, except for mandatory reporting data in the Brussels region, where cases increased. In 2020, cases again dropped sharply, likely due to the non-pharmaceutical interventions adopted during the coronavirus disease 19 (COVID-19) pandemic.

Currently available data on the proportion of PRN deficiency among strains isolated in Belgium comes from two published studies. Barkoff et al. (2019) found 10.5% (4/38) PRN-negative strains among *B. pertussis* isolates collected as part of the EUpertstrain IV study (2012 to 2015),<sup>60</sup> while Lefrancq et al. (2021) reported 20% (6/30) PRN-negative strains among *B. pertussis* isolates collected between 2014 and 2016.<sup>72</sup>

Childhood immunization against pertussis starts with a 3 + 1 primary vaccination series (at 2, 3, 4, and 15 months, using a hexavalent vaccine against diphtheria, tetanus, pertussis, *Haemophilus influenzae* type b, hepatitis B, and poliomyelitis [DTaP-Hib-HepB-IPV]). Until 2014, all hexavalent vaccine formulations used in the national immunization program contained PRN; a hexavalent formulation that does not include PRN has replaced the PRN-containing aP vaccines in Flanders since July 2014, and in the entire country from September 2015.<sup>73</sup> Starting from 2023, in Brussels and Wallonia a PRN-containing hexavalent vaccination is provided again as part of the national immunization schedule for primary vaccination; the hexavalent formulation not containing PRN is available in pharmacies for purchase.<sup>74</sup> Following primary vaccination, two booster



**Figure 4.** Proportion of PRN-negative strains over time in selected countries that use aP vaccines (regardless of PRN content). aP, acellular pertussis; PRN, pertactin \*During the aP vaccine-only period, the proportion of PRN-negative *B. pertussis* strains increased from 5.6% in 2007 to 48.4% in 2018.<sup>10</sup> Each bubble corresponds to a result in a study as presented in Supplementary table S1 (when a study is reporting results per year or several shorter periods, multiple results/bubbles will be presented for one study). Bubble size represents the sample size of the corresponding period (the denominator in the proportion), but it is not comparable across the charts for the different countries. For studies that report data over a time period, for the purpose of the plot, the midterm year (i.e., the average year between start and end years) was used. For all studies with a period longer than 10 years, the label is added (start year-end year). The red line represents the year of the first introduction of the aP vaccine for a given country.

doses are administered in all three regions. The first booster, scheduled at 5–6 years of age is represented by a PRN-containing vaccine, DTaP-IPV, which is provided free of charge;<sup>74,75</sup> a DTaP-IPV vaccine not containing PRN is

also available for purchase in pharmacies.<sup>74,75</sup> The second booster, recommended at 14–16 years of age, consists of a PRN-containing vaccine, the adult/adolescent formulation of tetanus toxoid, reduced diphtheria toxoid, and aP vaccine

**Table 1.** Recommended/reimbursed pertussis diagnosis methods in Belgium, France, and Canada.

Country	Ref.	Diagnosis of pertussis
Belgium	64,65	Reimbursed tests: serology and PCR (under certain conditions, since 1 April 2019): prescription by a pediatrician adequately completed request form sample type: nasopharyngeal wash/aspirate/swab, bronchoalveolar lavage or bronchial aspirate (no nasal/throat swabs) no macrolide/trimethoprim-sulfamethoxazole therapy of $\geq 5$ days only reimbursed once in a diagnostic investigation phase only for the following patients: children <16 years with pertussis-compatible symptoms for >6 days and <22 days, not vaccinated in the last 3 years OR children <1 year, not vaccinated/partially vaccinated (<3 doses) with catarrhal symptoms followed by nausea, bradycardia, or apnea(s).
France	66	Reimbursed tests: PCR and culture (aspiration or nasopharyngeal swab) Serology is not recommended (reimbursement has ceased in 2011)
Canada	67	Laboratory confirmation of pertussis cases is based on culture or PCR

PCR, polymerase chain reaction.

[Tdap]); some differences exist among the three regions regarding the age at which the pediatric booster doses are administered (Supplementary table S2). For adults, Tdap vaccination is recommended during each pregnancy and as part of the cocooning strategy for protecting newborns. In addition, decennial booster doses are also recommended (but not reimbursed) for adults (Table 3).<sup>16</sup>

Based on regional vaccination coverage studies, carried out between 2012 and 2021, pertussis vaccination coverage is  $\geq 94\%$  among children aged 18–24 months and varies between 31% and 85% in pregnant women (Table 4). We found no publicly available data regarding pertussis vaccine coverage in the general adult population.

### France

In France, pertussis is not a notifiable disease since 1986; however, outbreaks have to be declared.<sup>66,68,70,78</sup> Accepted (and reimbursed) diagnostic methods include PCR and culture from aspiration samples or nasopharyngeal swabs (Table 1).

Pertussis surveillance is performed by combining information from several sources (Table 2). These include two nationwide outpatient laboratories (Cerba and Biomnis, which carry out >90% of the ambulatory testing for pertussis in mainland France), a nationwide network of >40 hospitals (Réseau national de la coqueluche [RENACOQ], covering  $\sim 30\%$  of hospitalized pediatric pertussis cases), and a French pediatric ambulatory surveillance system (the Sentinelles network, including  $\sim 60$  pediatricians working in private practices).<sup>10,68,70,89</sup> As of 2016, only pertussis cases diagnosed in infants <12 months of age are reported by RENACOQ.<sup>70</sup> Within the RENACOQ, data on pertussis culture and PCR results, as well as on the clinical and epidemiological characteristics of pertussis cases, are collected in hospitalized infants <6 months of age.<sup>68</sup> Clinical isolates collected within the RENACOQ network and at other French hospitals are referred to the National Reference Center (Centre National de Référence de la coqueluche et autres Bordetelles);<sup>10,68</sup> most of these isolates are collected from children, but some originate from their close contacts, i.e., older siblings, parents, or grandparents.<sup>10</sup>

The most recent epidemiological peak of pertussis cases was recorded in 2017 (Figure 5B); over the following years, the number of pertussis cases detected in infants <12 months of

age has decreased, with historically low levels attained in 2021, in great part due to measures adopted during the COVID-19 pandemic (Supplementary table S3).

In the published literature, PRN deficiency of *B. pertussis* strains was assessed in multiple studies (Supplementary table S1), including a large study in which 2,280 isolates collected continuously between 1996 and 2018 were characterized.<sup>10</sup> Of the 1,428 tested isolates, 188 (13.2%) were found to be PRN-negative: six of these were collected between 2004 and 2006, corresponding to the later part of the period when wP and aP vaccines were used in parallel (1996–2006), while 182 were collected after aP vaccines were exclusively used for both primary immunization and boosting (2007 to 2018). During this aP vaccine-only period, the proportion of PRN-negative *B. pertussis* isolates increased from 5.6% in 2007 to 48.4% in 2018.<sup>10</sup> However, this documented increase in PRN deficiency proportion over the recent years does not seem to be temporally associated with a surge of pertussis cases in infants based on RENACOQ data.<sup>70</sup>

The current childhood immunization schedule in France comprises three doses of a hexavalent vaccine (DTaP-Hib-HepB-IPV) administered at 2, 4, and 11 months, one dose of DTaP-IPV at 6 years, and one dose of Tdap-IPV at 11–13 years.<sup>16</sup> Vaccination with aP vaccines started in 1996; currently, nine aP-containing vaccines are used, of which three do not include PRN.<sup>60,90</sup> Six are used for primary immunization, four for the pre-school booster, and two for the adult/maternal booster.<sup>90</sup> For primary vaccination of infants, only one hexavalent vaccine (which contained PRN) was available between 2005 and 2015/2016.<sup>79,80</sup> Among the three hexavalent vaccines currently on the market in France, one does not contain PRN. Since 2018, vaccination of children against pertussis is compulsory from the age of 2 months.<sup>68</sup> In adults, aP vaccination is recommended at 25 years of age (unless a pertussis-containing vaccine has been administered in the past 5 years), and during each pregnancy (Table 3, Supplementary table S2).

Vaccination coverage tends to be high in infants and toddlers, reaching 98.8% in children 24 months of age in 2019; data available for older age groups indicates lower coverage (61.2% at 11–13 years of age, 36.0% at 29 years of age, and 21.7% at 69 years of age in 2017; Table 4).



**Table 2.** Pertussis surveillance in Belgium, France, and Canada.

Country	Ref.	Pertussis surveillance
Belgium	64	<p>Sources:</p> <p>Laboratory network (Sciensano): serological and PCR results National Reference Center for <i>B. pertussis</i>: Universitair Ziekenhuis Brussel performs PCR, cultures, confirmation, and strain typing, while Sciensano performs the determination of anti-PT IgG antibodies Compulsory notification data:</p> <ul style="list-style-type: none"> <li>- only confirmed cases in children &lt;3 years of age in Wallonia</li> <li>- only confirmed cases, regardless of age, in Brussels</li> <li>- all possible, probable, and confirmed cases in Flanders</li> </ul> <p>Case classification:</p> <p>Possible case: fulfilling clinical criteria Probable case: fulfilling clinical and epidemiological criteria Confirmed case: fulfilling clinical and laboratory criteria</p>
France	66,68	<p>Sources:</p> <p>Two nationwide outpatient laboratories (general population surveillance) A nationwide network of 42 hospitals (RENACQ) collecting hospitalized pertussis cases in infants &lt;1 year of age (monitoring of severe pediatric cases) Clinical isolates collected within the RENACQ network and at other French hospitals can be referred to the National Reference Center French pediatric ambulatory surveillance system, including around 60 pediatricians working in private practices: all suspected cases are invited for biological confirmation of pertussis</p> <p>Case classification:</p> <p>Confirmed case: positive in culture and/or PCR Epidemiological case: acute cough lasting <math>\geq 14</math> days in a child or adult who was in contact with a confirmed case</p>
Canada	67,69	<p>Sources:</p> <p>Cases reported to the Public Health Agency of Canada through the Canadian Notifiable Disease Surveillance System by public health professionals (passive surveillance) The Immunization Monitoring Program, ACTive, an active hospital-based pediatric surveillance system supported by the Public Health Agency of Canada (active surveillance)</p> <p>Case classification:</p> <p>Suspect case: suggestive clinical symptoms with no other known cause Probable case: cough lasting &gt;2 weeks and other suggestive clinical symptoms, no laboratory confirmation or epidemiological link to a confirmed case Confirmed case:</p> <ul style="list-style-type: none"> <li>- Between 2000–2008: a case was confirmed based on laboratory confirmation of infection OR an epidemiological link to a laboratory-confirmed case and the presentation of <math>\geq 1</math> clinical symptom from a predefined list</li> <li>- Since 2009: PCR-positive cases are only confirmed if clinically compatible symptoms are also present</li> </ul>

IgG, immunoglobulin G; PCR, polymerase chain reaction; PT, pertussis toxin; RENACQ, Réseau national de la coqueluche (National pertussis network).

### Canada

Pertussis is a notifiable disease in Canada,<sup>67</sup> only confirmed cases (defined as cases with laboratory confirmation or an epidemiological link to a confirmed case, and specific symptomatology) are to be reported (Table 1).

Pertussis surveillance in Canada includes a passive component, based on the confirmed cases reported by public health professionals through the Canadian Notifiable Disease Surveillance System. In addition, active surveillance data is also collected through the Immunization Monitoring Program, ACTive (IMPACT), a hospital-based pediatric surveillance system supported by the Public Health Agency of Canada (Table 2). At each hospital enrolled in IMPACT, a designated nurse monitor actively searches for children hospitalized with pertussis, under the supervision of a volunteer pediatrician/infectious diseases specialist.<sup>91</sup>

In a study that assessed pertussis epidemiology from 2005 to 2019 (after the recommendation for the aP vaccine adolescent booster was made), an average annual incidence rate per 100,000 population of 6.4 (ranging from 2.0 in 2011 to 13.4 in 2012) was reported.<sup>69</sup> During this period, epidemiological peaks in pertussis cases were observed in 2012 and from 2015 to 2017 (Figure 5C). Most of the disease burden was seen in infants <1 year of age (Supplementary table S3).

Several studies reported data on PRN-negative *B. pertussis* strains isolated in Canada (Supplementary table S1). Tsang et al. (2019) identified PRN deficiency in 34.6% (143/413) of

*B. pertussis* isolates cultured at the Public Health Ontario Laboratory.<sup>92</sup> Over time, the proportion of PRN-negative *B. pertussis* isolates increased from 16.7% in 2011 to 70.8% in 2016; however, among isolates from 2017, only 46.2% were PRN-negative. This decrease in PRN-negative isolates might be linked to a decrease in the circulation of sequence type 2 of *B. pertussis*.<sup>92</sup>

The current childhood immunization program against pertussis varies across the different Canadian provinces and territories (Supplementary table S2). According to the Canadian Immunization Guide, a DTaP-IPV-Hib-containing vaccine should be used for primary vaccination, followed by a booster dose at 4–6 years of age (using DTaP-IPV or Tdap-IPV), and another booster dose at 14–16 years of age (using Tdap); the latter booster dose was introduced between 1999 and 2004.<sup>46,69</sup> Pertussis vaccination is also recommended for adults and during pregnancy (Table 3).<sup>69</sup> All aP vaccines currently used in Canada contain the PRN antigen.<sup>46</sup>

Based on 2018–2019 estimates, pertussis vaccine coverage in children is ~80%, and even lower in adults (Table 4), although the pertussis vaccine coverage during pregnancy has increased between 2019 and 2021.<sup>69,84</sup>

### Discussion

In the first part of this review, we provided a comprehensive insight into the proportion of PRN deficiency in *B. pertussis*

isolates, using a large body of systematically collected evidence. PRN deficiency arises as a result of mutations in the promoter or coding regions of the *prn* gene. However, it is important to note here that selection pressure is not exerted exclusively on the PRN antigen, but also on others. These include PT, the main factor responsible for overt symptomatology (through mutations in the PT A gene [*ptxA*], and the PT promoter region [*ptxP*]), and FIM2/3 (through mutations in the *fim2* and *fim3* genes). The fitness of genotypes with different combinations of mutations or alleles varies: in the aP vaccine era, PRN-negative genotypes appear to demonstrate a higher fitness than PRN-positive genotypes, but only when PRN deficiency is combined with a *ptxP3* allele.<sup>72</sup> The seemingly conserved pathogenicity of PRN-negative *B. pertussis* strains could contribute to the high number of isolates with this characteristic being identified in comparison to strains exhibiting other mutations. In comparison, PT-negative *B. pertussis* strains have been associated with a milder or even asymptomatic clinical course, meaning that such strains are less likely to be isolated in the context of the current surveillance systems.<sup>11,15</sup>

A study among infants <6 months old conducted in France<sup>93</sup> and a study among different age groups conducted in the USA<sup>24</sup> found no differences between the clinical presentation of pertussis cases in patients infected with PRN-negative strains and those infected with PRN-positive strains, except for a higher frequency of apnea in the latter category.<sup>24</sup> This is in line with findings of other studies, in which PRN-negative strains were shown to cause similar clinical signs and symptoms as PRN-positive strains.<sup>38,94,95</sup> Moreover, a recently published study from France among 361 symptomatic infants <6 months of age showed that PRN-positive strains collected between 2008 and 2019 were associated with an increased risk of fulminant pertussis.<sup>96</sup>

In the same study conducted in the USA, individuals vaccinated with PRN-containing aP vaccines appeared to have greater susceptibility to PRN-negative strains compared to PRN-positive strains.<sup>24</sup> By contrast, the EUpertstrain IV study found no significant difference in the proportion of PRN-negative isolates between vaccinated and unvaccinated patients from the studied countries, except for the UK, where (in line with the study conducted in the USA)<sup>24</sup> these isolates were found more in vaccinated patients.<sup>60</sup>

Clinical data is often not captured in studies evaluating PRN-negative *B. pertussis* prevalence, thus limiting the ability of formulating a comprehensive evaluation of the clinical significance of such strains. As a general practice, isolates are primarily obtained from infants and children, with a predominant focus on the hospitalized population subset; therefore, the representativeness of the characterized strains remains unknown.

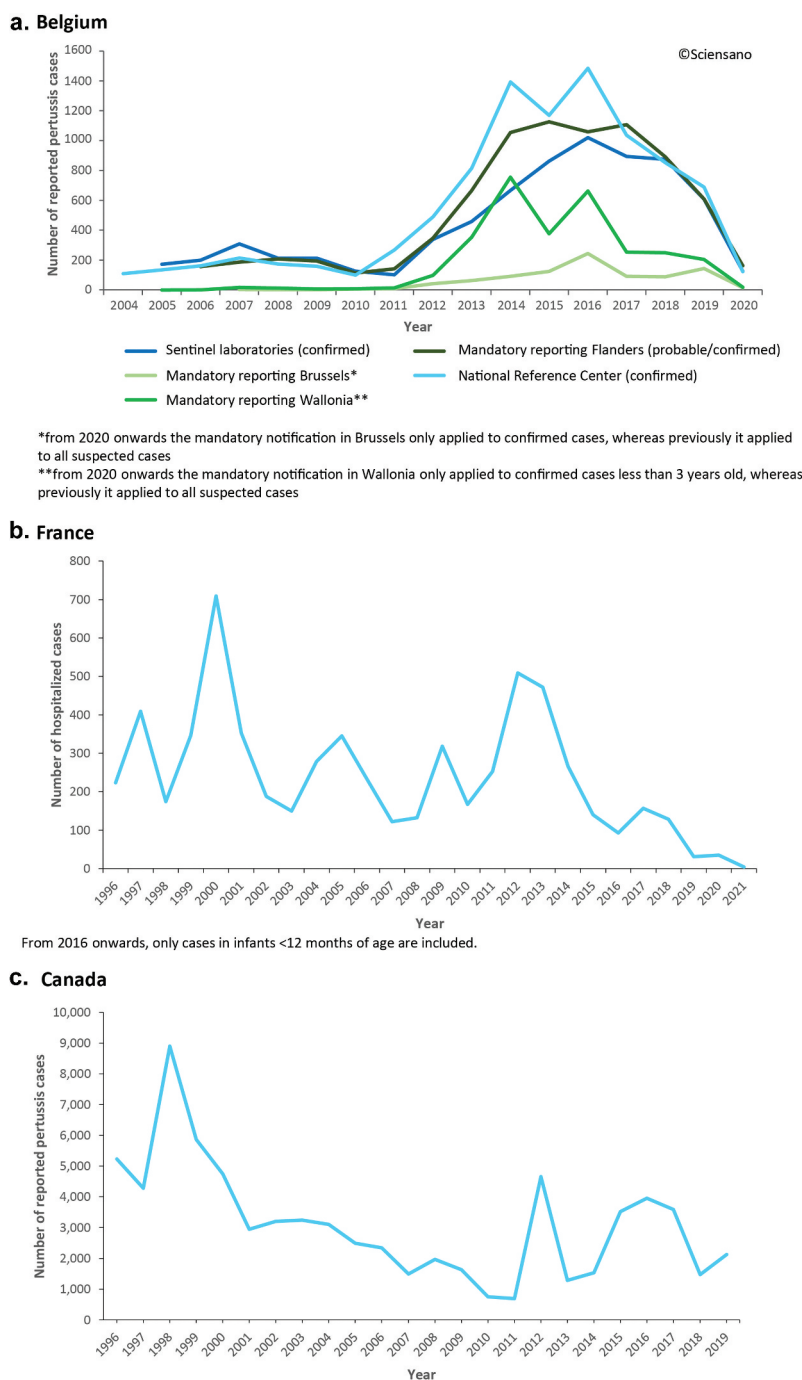
We aimed to illustrate that the proportion of PRN-negative *B. pertussis* isolates exhibited a steady increase over the recent years in countries using aP vaccines; in most cases, these were PRN-containing aP vaccines, albeit they were often used in parallel with aP vaccines which do not include the PRN antigen. Although a correlation between the widespread use of (mostly PRN-containing) aP vaccines and an increase in the proportion of PRN-negative *B. pertussis* strains has been

shown previously<sup>10,15,43,97</sup> and appears to also be supported by the results of our systematic literature review, a causal relationship between these two findings cannot be automatically implied. Nevertheless, as very few PRN-negative *B. pertussis* isolates have been identified before the use of aP vaccines,<sup>10,97,98</sup> evidence for the increase in PRN-negative strains as a result of vaccine pressure is strong.<sup>11,15,23</sup> Moreover, the appearance and increase of PRN-negative strains does not seem to occur in countries that use aP vaccines without PRN, as suggested by the examples from Japan<sup>61</sup> and Denmark.<sup>60</sup>

In some countries, >80% of *B. pertussis* isolates are PRN-negative.<sup>24,35–38,40,72</sup> In contrast, no PRN-negative strains were identified in the countries included in this review in which only wP vaccines are being used.<sup>41,43,99,100</sup> However, these results should be interpreted in the light of the overall low number of isolates and the limitations in surveillance, as well as strain isolation and their molecular characterization in developing countries. Interest in PRN deficiency (along with other mutations in circulating *B. pertussis* strains) has been increasing in the scientific community, as manifested by the growing number and scale of the studies exploring data related to *B. pertussis* strains exhibiting various mutations.

The PRN antigen is not specific to *B. pertussis*, as it can also be found in *B. parapertussis* and *B. bronchiseptica*.<sup>15</sup> The emergence of PRN-negative *B. parapertussis* isolates has also been recorded in France from 2007 onwards.<sup>11,97,101,102</sup> The absence of notable differences observed in the pathogenicity of PRN-negative and PRN-positive *B. parapertussis* strains (similar to what is seen in *B. pertussis*) supports the hypothesis that loss of PRN does not result in a significant change in virulence.<sup>101</sup> Furthermore, the simultaneous increase in the proportion of PRN-negative *B. pertussis* and *B. parapertussis* strains following the introduction of aP vaccines, including those that contain PRN, could be interpreted as evidence supporting a potential impact (and subsequent selection pressure) of PRN-containing aP vaccines on *B. parapertussis*, although studies assessing the effectiveness of aP vaccines (including those that contain PRN) against *B. parapertussis* are scarce.<sup>11,103</sup> To date, this increase in PRN-negative *B. parapertussis* strain proportion has only been reported in France; in Iran, where wP vaccines are used, none of the seven *B. parapertussis* isolates collected between 2012 and 2015 were PRN-negative.<sup>104</sup>

In the second part of the review, we gathered in-depth information on pertussis surveillance, epidemiology, and prevention in Belgium, France, and Canada; this allowed interpretation of the findings related to the proportion of PRN-negative *B. pertussis* isolates in a broader context. Pertussis is a notifiable disease in Belgium and Canada, but not in France.<sup>64,67,105</sup> In all three countries, pertussis surveillance generally relies on different data sources, such as hospital surveillance networks or laboratory networks. Another common aspect is represented by the strong focus on pediatric cases across all three countries analyzed.<sup>64,68–70,91</sup> In the two federal countries assessed (Belgium and Canada), regional differences have been identified in terms of reporting requirements, vaccine types used, or ages at which the various vaccine doses are recommended. For example, in Belgium, all possible,



**Figure 5.** Trends in pertussis epidemiology in Belgium (a), France (b), and Canada (c). a. Number of reported pertussis cases in Belgium between 2004 and 2020, by source;<sup>64</sup> b. Number of hospitalized cases of pertussis in children under 17 years of age in France between 1996 and 2021, data reported by the RENACOQ network;<sup>70</sup> c. Number of reported pertussis cases in Canada between 1996 and 2019.<sup>71</sup>

probable, and confirmed pertussis cases must be reported in Flanders, while only laboratory-confirmed cases are to be reported in Wallonia (in children under 3 years of age) and Brussels (regardless of age).<sup>64</sup> Nevertheless, PCR (the more frequently used laboratory confirmation method), is only reimbursed in unvaccinated/partially vaccinated children,<sup>64</sup> meaning that breakthrough cases could be more easily missed in Wallonia and Brussels, while the probability of detecting them would be higher in Flanders.

All three countries are currently using aP vaccines that contain two (i.e., without PRN), three and/or five pertussis

antigens.<sup>16,46,60,76</sup> In France, six of the nine pertussis-containing vaccines used include the PRN antigen;<sup>90</sup> the proportion in which these vaccines were used has evolved across time following their successive introduction, with an aP vaccine that does not contain PRN being currently used to vaccinate ~70% of infants. The uptake of pertussis-containing vaccines is monitored in young children in all three countries; vaccination coverage among 2-year-old children was high in Belgium and France ( $\geq 93\%$ ), and somewhat lower in Canada ( $< 80\%$ ).<sup>69,86–88</sup> However, vaccine coverage data in adolescents and adults is scarce.

**Table 3.** Pertussis vaccination recommendations and pertussis vaccine type(s) used in Belgium, France, and Canada.

Country	Ref.	Vaccination recommendations
Belgium	16,60, 73–77	<p>Current schedule:</p> <p>Pediatric population: at 2, 3, 4, and 15 months (DTaP-Hib-HepB-IPV), at 5–6 years of age (DTaP-IPV), and at 14–16 years of age (Tdap)</p> <p>Adult population:</p> <ul style="list-style-type: none"> <li>- During each pregnancy, between weeks 24 and 32 of pregnancy</li> <li>- In the general population, adult booster doses of a pertussis-containing vaccine are recommended at 10-year intervals in all three provinces; however, adult boosters are only reimbursed in Flanders</li> <li>- Adults in close contact with young infants are also advised to receive a booster aP vaccine</li> </ul> <p>Changes in the vaccination schedule:</p> <p>The 1950s: wP vaccine was introduced in the childhood immunization schedule at 2, 3, and 4 months</p> <p>1999 (in Flanders) and 2002 (in Wallonia): introduction of aP vaccine (as DTaP-IPV-Hib) with administration at 2, 3, 4, and 15 months, with booster doses at 4–6 years and at 14–16 years (vaccine containing the PRN antigen)</p> <p>2013: recommendation for vaccination during pregnancy</p> <p>2014–2015: a hexavalent formulation that does not include PRN has replaced the PRN-containing aP vaccines</p> <p>2023: PRN-containing hexavalent formulation used for primary vaccination in Brussels and Wallonia</p>
France	16,60,68, 78–83	<p>Current schedule:</p> <p>Pediatric population: at 2, 4, and 11 months (DTaP-Hib-HepB-IPV), at 6 years (DTaP-IPV), and at 11–13 years (Tdap-IPV)</p> <p>Adult population:</p> <ul style="list-style-type: none"> <li>- During each pregnancy (Tdap-IPV)</li> <li>- Cocooning strategy (Tdap-IPV): vaccination of all close contacts of infants during their first 6 months of life, if the mother was not vaccinated during pregnancy, or if she gave birth less than a month after her vaccination; as part of the cocooning strategy, a dose of pertussis-containing vaccine should be administered to individuals under the age of 25 years not vaccinated against pertussis in the past 5 years and to individuals over 25 years of age not vaccinated against pertussis in the past 10 years</li> <li>- General population: for those who did not receive a dose of pertussis-containing vaccine during the past 5 years, a booster with a quadrivalent vaccine (Tdap-IPV) is recommended at the time of the Td-IPV booster at 25 years of age; catch-up vaccination with Tdap-IPV vaccine can be proposed until 39 years of age</li> <li>- Healthcare personnel: a dose of pertussis-containing vaccine should be administered at the ages of 25, 45, and 65 years</li> </ul> <p>Changes in the vaccination schedule:</p> <p>aP vaccine was introduced as booster for the 11–13-year-old age group in 1998 and progressively replaced wP for primary vaccination from 2000</p> <p>1998: DTWP-IPV-Hib was recommended at 2, 3, 4 months, DTaP-IPV-Hib at 16–18 months, and DTaP-IPV at 11–13 years. Vaccines used as boosters contained the PRN antigen</p> <p>2004: DTaP-IPV-Hib was recommended at 2, 3, 4 months and at 16–18 months, DTaP-IPV at 11–13 years. A booster was recommended for all adults in close contact with a newborn (parents, grandparents, nannies, etc.) and healthcare personnel in contact with newborns. Two vaccines were used until 2005 (one that contained the PRN antigen and one that did not); afterward, only one vaccine (containing PRN) remained available</p> <p>2008: DTaP-Hib-HepB-IPV was reimbursed for pediatric vaccination; booster vaccination was recommended for all adults, in particular for healthcare workers, especially those in contact with newborns or elderly people. One vaccine (containing PRN) was used</p> <p>2013: the childhood immunization schedule was simplified, with a 2 + 1 primary vaccination being recommended at 2, 4, and 11 months. Boosters were recommended at the age of 6 years (DTaP-IPV), then at 11–13 years of age (Tdap-IPV) and in young adults on the occasion of the 10-year Td-IPV booster fixed at the age of 25, and in the absence of pertussis vaccination in the last 5 years</p> <p>Since 2016, two vaccines were used (one that contains the PRN antigen and one that does not). In 2018, a third (PRN-containing) hexavalent vaccine was introduced in the national immunization program</p> <p>2018: vaccination of children against pertussis becomes compulsory from the age of 2 months</p> <p>2022: recommendation to vaccinate during each pregnancy, starting from the 2<sup>nd</sup> trimester, between 20 and 36 weeks of amenorrhea. If no pertussis vaccine was administered during pregnancy, the cocooning strategy should be proposed</p>
Canada	16,46,69, 84,85	<p>Current schedule:</p> <p>Pediatric population: at 2, 4, 6, and 12–23 months of age (generally given at 18 months of age; DTaP-IPV-Hib), at 4–6 years of age (school entry; DTaP-IPV/Tdap-IPV), and at 14–16 years of age (Tdap)</p> <p>Adult population:</p> <ul style="list-style-type: none"> <li>- One dose of Tdap vaccine is recommended to all adults ≥18 years of age</li> <li>- One dose of Tdap vaccine is recommended in every pregnancy, ideally between 27 and 32 weeks of gestation</li> </ul> <p>Differences between some provinces and territories exist with regards to vaccine products administered and the recommended vaccination schedules</p> <p>Changes in the vaccination schedule:</p> <p>Between 1999 and 2004: introduction of a Tdap booster dose at 14–16 years of age</p> <p>Between July 1997 and April 1998: aP vaccines replaced wP vaccines in universal infant immunization schedules in all provinces and territories</p> <p>2018: recommendation for vaccination during pregnancy; free pertussis vaccination is offered to pregnant women in all jurisdictions since April 2022</p>

aP, acellular pertussis; D, diphtheria toxoid; HepB, hepatitis B; Hib, *Haemophilus influenzae* type b; IPV, inactivated poliovirus; PRN, pertactin; T, tetanus toxoid; Td, tetanus toxoid and reduced diphtheria toxoid; Tdap, tetanus toxoid, reduced diphtheria toxoid, and acellular pertussis; wP, whole-cell pertussis.

Coverage data regarding adult pertussis vaccination administered as part of the cocooning strategy or during pregnancy were available for two of the three countries included in the in-depth analysis. A recently published study used vaccination coverage rates among parents of newborns to evaluate the implementation of the cocooning strategy introduced 12 years earlier in France; in 2016–2017, 46–47% of both mothers and fathers of newborns

were up to date on their pertussis vaccination.<sup>88</sup> In Belgium, regional Tdap vaccination uptake in pregnant women varied greatly, ranging from 31% in Brussels to 85% in Flanders.<sup>86</sup> For Canada, only data on pertussis booster vaccination coverage in the general population were available, showing a coverage rate of 33–65%.<sup>69,84</sup> These data suggest that there is room for improvement when pertussis vaccine uptake in adults is concerned.

**Table 4.** Pertussis vaccination coverage in Belgium, France and Canada.

Country	Ref.	Pertussis vaccination coverage
<b>Belgium</b>	86	Children aged 18–24 months, based on regional vaccination coverage studies carried out between 2012 and 2021 (weighted average): DTP, 3rd dose: 97.5% (Flanders 2020, Brussels-Wallonia 2019) DTP, 4th dose: 93.9% (Flanders 2020, Brussels-Wallonia 2019) Tdap during pregnancy: 85% uptake in Flanders in 2020, 38.9% in Wallonia in 2019, and 31.1% in Brussels
<b>France</b>	68,87,88	Primary pertussis vaccination series: 98.3% at 9 months of age in 2015, 98.8% at 24 months in 2018 and 2019 Pertussis booster: 96.1% at 24 months in 2019, 96.4% in 2020 At 6 years of age: 63.8% in 2017 At 11–13 years of age: 61.2% in 2017 Adults up to date with pertussis vaccination, as recommended in the NIP: 36.0% at 29 years of age, 25.0% at 49 years of age, 21.7% at 69 years of age, 15.8% at 79 years of age, 10.9% at 89 years of age, and 4.4% at 99 years of age, in 2017 Parents of newborns: 47% in 2016, 46% in 2017 (mothers and fathers)
<b>Canada</b>	69,84	Estimates from the 2019 Canadian Childhood National Immunization Coverage Survey: ≥4 doses of DTP administered by 2 years of age: 78% ≥5 doses by 7 years of age: 78% One booster dose by 17 years: 95% Vaccination during pregnancy: 44% of the mothers who knew whether or not they had been vaccinated against pertussis during their pregnancy Estimates of the 2018–2019 Seasonal Influenza Vaccination Coverage Survey: Adults >18 years of age who received a pertussis booster in adulthood: 33% Estimates of the Survey on Vaccination during Pregnancy 2021: 65% of the mothers who knew whether or not they had been vaccinated against pertussis during their pregnancy

D, diphtheria toxoid; NIP, National Immunization Program; P, pertussis toxoid; T, tetanus toxoid; Tdap, tetanus toxoid, reduced diphtheria toxoid, and acellular pertussis.

Consistent with the overall findings of the systematic literature review, PRN-negative *B. pertussis* isolates were observed in all three countries evaluated in detail. Even though in some years the number of collected samples was small, the proportion of PRN-negative isolates followed an overall increasing trend, although yearly variations were also observed.<sup>92,106</sup> PRN-negative strains appear to be more prevalent in Canada, with recent estimates of up to 70%,<sup>92</sup> compared to less than 48% in France.<sup>10,60,72,106</sup> Differences in vaccine types used, as well as in vaccine coverage among the different age groups could explain some of the variability in the proportion of PRN-negative isolates observed in the three countries. However, these findings should be interpreted with caution, considering that the representativeness of the characterized isolates is unknown, and the significance of the apparent differences in proportion of PRN-negative *B. pertussis* strains has not been established. In most cases, limited information was available on the demographic profile of the patients and the clinical characteristics of the cases from which the *B. pertussis* isolates were obtained. When examining this increasing trend in the context of pertussis epidemiology, we found that the recent increase in PRN-negative strain proportion among isolates from France did not seem to coincide with an overall increase of pertussis cases in infants.<sup>10,70</sup> More recently (1 January 2024 to 31 May 2024), pertussis cases in France peaked, with 66 out of 67 isolates being PRN-positive. This is in contrast with the 228 isolates collected between 2016 and 2020, of which 51.3% were PRN-negative. The resurgence of PRN-positive strains may have been facilitated by the use of an aP vaccine that does not contain PRN, thereby enabling PRN-positive strains to spread more efficiently. Similar increases were seen in Czechia, Denmark, and Spain.<sup>107</sup>

As mentioned previously, the clinical significance of PRN-negative *B. pertussis* strains is not yet defined; nevertheless, these findings suggest that increases in PRN deficiency are not associated with increases in the number of pertussis cases.

Furthermore, an equally reassuring finding is that the efficacy of aP vaccines, including those that contain PRN, appears to be preserved in the first years following childhood vaccination even in settings with a high proportion of PRN-negative isolates, although a decline in VE is observed 4–8 years after the last dose.<sup>108,109</sup> To date, limited data on effectiveness of aP vaccines against PRN-negative *B. pertussis* strains are available, and concerns have been expressed that PRN-negative strains might have a higher potential to escape immunity induced by aP vaccines.<sup>11,15</sup> Studies evaluating the pertussis VE often use the case-control methodology or the screening method. They are generally conducted over several years and very rarely provide information on the molecular evolution of the strains during the studied period.

In contrast, during an outbreak, the high incidence makes it possible to assess VE over a shorter period, often in a restricted geographical area (school, region). We found only one example of a VE evaluation conducted in the USA following a major pertussis outbreak in 2012, which also reported information on the characteristics of the isolated strains.<sup>34</sup> Overall, the effectiveness of a five-dose primary vaccination schedule against pertussis disease in children 4–10 years of age was 84%, even though more than 90% of the *B. pertussis* isolates collected at the time of the study were PRN-negative. A VE case-control study conducted between 2009 and 2015 in Ontario, Canada, estimated that pertussis vaccination was ~90% effective during the first years after primary vaccination in children up to 8–9 years of age, ~50% in 12–13-year-olds, and ~80% after the adolescent booster in those 14–16 and 16–22 years of age.<sup>108</sup> While this study did not report data resulting from strain characterization, such data is available for Ontario for approximately the same period, showing a steady increase in the proportion of PRN-negative isolates over time, from 16.7% in 2011 to 61.3% in 2015.<sup>92</sup> In 2017, a year during which the most recent epidemiological peak of pertussis was recorded in Ontario, the proportion of PRN-negative isolates declined.<sup>92</sup> This could indicate a greater transmission potential of

*B. pertussis* strains expressing PRN and suggests that aP vaccines continue to be protective despite a high proportion of PRN-negative strains.

In the absence of studies directly evaluating VE on PRN-negative versus PRN-positive strains, circumstantial evidence can be inferred from other sources. For example, surveillance data collected in France, corroborated with data on PRN-negative strain circulation, do not suggest a decreased VE against these isolates, as the steady increase in the proportion of PRN-negative strains isolated was not reflected in a corresponding increase of pertussis cases.<sup>70,106</sup> In contrast, a recent increase in the number of pertussis cases in 2024 coincided with an increase in the proportion of PRN-positive strains.<sup>107</sup> Taken together, this suggests that the PRN-negative strains that are currently circulating may not be able to escape immunity induced by aP vaccines.

Future studies should assess vaccine coverage and dominant strains as well as the clinical significance of PRN deficiency and its potential impact on pertussis disease control. Guidelines on laboratory diagnosis and molecular surveillance of *B. pertussis*, issued by the European Centre for Disease Control and Prevention in 2022, mention that, although the use of culture has been decreasing from year to year, it is still highly recommended as the isolates can be used for molecular surveillance of the changes in bacterial populations.<sup>110</sup> Monitoring of the circulating isolate variants is important for evaluation of VE and for future vaccine development.

To fulfill this requirement, a public health surveillance system needs to incorporate a strong laboratory component to collect and characterize representative isolates. Ideally, isolates (*B. pertussis* and *B. parapertussis*) should be collected from patients of different ages (i.e., from both children and adults), various degrees of illness severity (i.e., from both hospitalized and outpatient cases), and a broad geographical distribution of samples within a given country should also be ensured. This is challenging, as the routine use of culture for diagnosis of pertussis has declined since the introduction of PCR methods.<sup>111</sup> Moreover, it is often not possible to isolate *B. pertussis* from cultures from adolescents and adults because they tend to seek medical advice only after several weeks of coughing.<sup>110</sup> In these patients, diagnosis by serology (based on anti-PT immunoglobulin G) remains the most commonly used method. Ideally, information to be collected should include patient demographics (age, geographical area) and vaccination status (including the type of vaccine received, and the administration dates), clinical characteristics of the illness episode, and information on whether the case belongs to an outbreak. Furthermore, sequencing of collected isolates should be performed whenever possible.

Pertussis vaccine uptake studies beyond early childhood are important to accurately estimate the coverage in the entire population. Obtaining a broad coverage in all age groups through regular booster vaccination is important to prevent pertussis resurgence, especially in the context of increasing prevalence of mutations observed in *B. pertussis* strains.

Future effectiveness studies should include collection of contextual information on PRN deficiency during the study period and within the covered geographical area. An

effectiveness study design in which strains would be characterized (e.g., case-control study) could also be considered.

Of note, steps are already being taken to better characterize the landscape of pertussis in the context of bacterial evolution under selection pressure. To study genetic changes in *B. pertussis* populations in Europe, panels of *B. pertussis* isolates have been serially collected during four periods, starting in 1999, in a total of 11 European countries.<sup>60</sup> The ongoing EUpertstrain V project aims to continue monitoring the proportion of PRN-negative isolates and to collect information on the vaccination status and clinical severity associated to pertussis cases across several countries. This study, as well as similar ones that will potentially be conducted in other parts of the world, could provide additional insights to help decipher the mechanisms, clinical relevance, and required actions in the face of vaccine-driven selection pressure exerted on *B. pertussis* strains.

The systematic literature review presented here has limitations. The main limitations of the data on PRN-negative *B. pertussis* isolates relate to the heterogeneity between the included studies, variable and sometimes very long study periods, different vaccination strategies, overlapping samples, and very limited data for countries and/or periods when wP vaccines were primarily used. Furthermore, the information available on the PRN-negative strains and the population in which they were isolated is often limited. Genetic characterization of the isolates is rarely performed, thus data on the genomic events that cause PRN deficiency is scarce. Data on the type, number, and timing of pertussis vaccines received is not usually recorded for individuals from whom *B. pertussis* strains were isolated, hampering the identification of breakthrough pertussis cases. On a population level, the proportion of the various types of pertussis vaccines administered is not readily available, further limiting the possibility of correlating the extent of PRN-containing aP vaccine use and the proportion of PRN-negative *B. pertussis* isolates. Finally, finding an association between the increase of PRN-negative strains following introduction of aP vaccines is not irrefutable proof of cause and effect. For this, additional studies are needed, especially in countries where more types of pertussis vaccine (wP and aP, aP vaccines with and without a PRN component) are used concurrently, to analyze isolates from individuals with breakthrough infection following vaccination with the different vaccine types.

In conclusion, the proportion of PRN-negative *B. pertussis* strains has been increasing in most countries that use aP vaccines. Although currently available data do not suggest a decreased protection against pertussis disease conferred by aP vaccines in this context of increasing PRN deficiency, further studies are needed to provide stronger evidence and more data regarding VE and the duration of protection. Ideally, these studies should link the clinical and epidemiological characteristics, as well as the vaccination status of the cases with the molecular characterization of the strains. Enhanced disease surveillance and effectiveness estimation can only be achieved by incorporating a strong laboratory component in pertussis surveillance, including selection of representative *B. pertussis* (and *B. parapertussis*) isolates for strain characterization. In the

context of waning immunity, and the increase in PRN-negative isolates (or other mutations driven by vaccine pressure), regular aP vaccine booster doses across life may be needed to limit pertussis resurgence.

## Highlights

- Pertussis childhood immunization programs using combination vaccines containing either whole-cell (wP) or acellular pertussis (aP) components remain crucial in reducing the burden of disease worldwide; however, pertussis resurgences have been documented, even in countries with high pediatric vaccine coverage.
- *Bordetella pertussis* strains not expressing pertactin (PRN) have been increasingly identified in countries where aP vaccines are used but remain low or absent in countries with predominant wP vaccination, suggesting selection pressure of aP vaccines, in particular those that contain PRN as an antigen, on bacterial strain evolution.
- In-depth analysis of contextual data regarding pertussis surveillance in three selected countries (Belgium, France, and Canada) revealed a strong focus on pediatric pertussis cases, although variations in surveillance systems among countries or regions within the same country were noted; data on pertussis cases in adults were not systematically collected. As PRN-containing and non-PRN-containing aP vaccines have been used alternatively or concomitantly in most countries, and the vaccination status of the patients from whom PRN-negative strains have been isolated is rarely reported, no definitive conclusion regarding the effect of PRN-containing aP vaccines on the proportion of PRN-negative *B. pertussis* isolates can be drawn based on the available data.
- Surveillance data do not show an increase of pertussis pediatric cases during the period in which the prevalence of PRN-negative strains increases, suggesting no critical impairment of the vaccine effectiveness against PRN-negative strains; although aP vaccines are very effective, immunity conferred by aP vaccines is waning significantly after 5–10 years following childhood vaccination, regardless of PRN expression.
- To accurately assess changes in the circulating *B. pertussis* strains as well as the impact of these changes, surveillance systems should include steps for adequate characterization of the isolates; to do so, nasopharyngeal specimens from patients with cough illnesses should not only be tested by polymerase chain reaction (PCR), but growth of *B. pertussis* in culture should also be encouraged. In addition, sampling should ideally target all age groups instead of predominantly focusing on the pediatric population. This is challenging, as the routine use of culture as a method for diagnosing pertussis has declined since the introduction of PCR and as it is often not possible to isolate *B. pertussis* from cultures in older children and adults because they tend to seek medical advice only after several weeks of coughing.

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**Valérie Berlaimont** is holder of a PhD in Pharmaceutical Sciences and has more than 20 years experience in local, regional (APAC) and global Medical Affairs, successively at Sanofi, Boehringer-Ingelheim and GSK.

## Author contributions statement

All authors made substantial contributions to the conception and design, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; approved the version to be published; and agree to be accountable for all aspects of the work.

## Data availability statement

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

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