A cross-sectional study of *Bordetella pertussis* seroprevalence and estimated duration of vaccine protection against pertussis in St. Petersburg, Russia

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**Abstract**

**Background:** In Russia as in other countries introduction of infant vaccination against pertussis in 1950s led to dramatic decrease of whooping cough. The current vaccination schedule includes a 3-dose infant series and toddler booster; the pre-school booster was cancelled in 1980s and never reintroduced. Whole-cell vaccines, and in a smaller proportion acellular vaccines are used for all doses. However, pertussis incidence in urban settings is high with highest burden in school children. We conducted a study of seroprevalence of recent pertussis infection to estimate the duration of protection from the 4-dose series.

**Materials and methods:** Sera sample from 395 St Petersburg children aged \( \geq \) 3 years and <14 years were tested for pertussis toxin antibodies using a commercial PT ELISA test. Only children with completed 4-dose vaccination course were included in the study. Age-specific seroprevalence of recent pertussis infection was analyzed for trends.

**Results:** Children fully vaccinated against pertussis at 3 years old had significant delays in infant vaccination schedule: only 83.5% received at least one dose of pertussis vaccine at 6 months of age and 25.6% received their toddler booster before 24 months-old. Overall, 10.6% of children demonstrated the serological signs of the infection in the last 12 months. A clear trend (\( r^2 = 0.692 \)) of increasing proportion of infection in the last 12 months was observed in children who had received their last dose of vaccine 6 years and more prior to the study.

**Conclusion:** Our study demonstrates that Russian children become susceptible to infection at or soon after entering school. The results confirm the waning of vaccine-elicited immunity around school-age and support the need for a booster dose at that age.

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During the epidemic of the 1990s, the highest age-specific incidence was observed in the 3–6 year-olds. With restored infant coverage rates, children under 1 year-old represent the highest age-specific incidence, and it has remained relatively stable since the late 1990s. Instead, the increase in cases reported among 7–14 year-olds was the primary cause of the overall incidence observed in the 2000s [17,6] (Fig. 2).

Earlier seroepidemiological studies conducted in Saint Petersburg have previously documented the prevalence of antibodies against pertussis toxin (PT) among children and adolescents [8,9]. These studies were carried out either by agglutination or antigen-complex ELISA methods. As PT is the only antigen strictly specific to Bordetella pertussis, confirmation of these earlier findings was needed to precisely and specifically estimate the seroprevalence of pertussis infection in a Russian setting such as St Petersburg.

The objective of this study was therefore to describe the distribution of recent infection rates according to age, in order to estimate the duration of protection against B. pertussis infection after the last dose of vaccine.
2. Materials and methods

2.1. Study population

The study involved 395 children aged ≥3 years and <14 years, with documented full course of vaccination against pertussis (i.e., 4 doses), recruited from outpatient healthcare facilities upon consultation for other reasons than infectious disease. The study excluded children vaccinated in the last year or vaccinated with a heterogeneous wPV/aPV schedule, institutionalized children, and immunodeficient patients. Informed consent was obtained from parents, as well as from participants ≥11 years-old. Reflecting the limited use of aPV in Russia, we recruited equal proportions of aPV- and wPV-primed 3–6-year-olds, while in 7–13-year-olds all participants were primed with the Russian-manufactured wPV.

2.2. Data collection

Socio-demographic data and patient/parent recalled history of clinical symptoms were collected by the study physician in a structured questionnaire; participants without verifiable, complete vaccination records were excluded.

“Typical pertussis” in the history of symptoms was defined by the presence of ≥3 of following symptoms during the last year: cough lasting >2 weeks, paroxysmal cough, nocturnal worsening of paroxysms, post-tussive emesis, apnea or dyspnea, normal body temperature, contact with a diagnosed case of pertussis or a person with persistent cough. Participants were assigned to the category of “questionable pertussis” if they reported a cough without or with ≤2 typical symptoms of pertussis during the last year. The category “no cough” included the participants without history of cough symptoms during the last year.

2.3. Serology

Samples were stored at −20 °C and analyzed concurrently. Serum samples were tested for anti-PT antibodies by enzyme-linked immunoassay (ELISA) using the SAVYON SeroPertussis™ kits (Savyon Diagnostics Ltd, Israel), previously validated against NIBSC standard [10]. Adapting from the manufacturer’s instructions, for this study, we defined the less stringent threshold of [IgG] ≥ 12 IU/ml with any [IgA] for the categorization of recent infection during the last 12 months (m12); the more stringent thresholds of [IgG] ≥ 100 IU/ml with any [IgA] or [IgG] ≥ 40 IU/ml along with [IgA] ≥ 12 IU/ml for the categorization of recent infection during the last 6 months (m6).

2.4. Statistics

The data were analyzed descriptively, with stratification by either age or time since last vaccine dose. Trends across strata were analyzed for coefficient of determination. A two-tailed P-value of ≤ 0.05 was considered for statistical significance. Analysis was performed using the statistical package SAS (SAS Institute Inc., 100 SAS Campus Drive, Cary, NC 27513-2414, USA).

3. Results

3.1. Study population

Age and vaccine distributions of the study population are described in Table 1. Gender distribution was 49.6% females (range: 36.7–60.0%) and 50.4% males (range: 40.0–63.3%).

### Table 1

<table>
<thead>
<tr>
<th>Age, years</th>
<th>Participants vaccinated by wPV, n (%)</th>
<th>Participants vaccinated by aPV, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>34 (57.6)</td>
<td>25 (42.4)</td>
</tr>
<tr>
<td>4</td>
<td>26 (56.5)</td>
<td>20 (43.5)</td>
</tr>
<tr>
<td>5</td>
<td>22 (48.9)</td>
<td>23 (51.1)</td>
</tr>
<tr>
<td>6</td>
<td>27 (56.2)</td>
<td>21 (43.8)</td>
</tr>
<tr>
<td>7</td>
<td>30 (100.0)</td>
<td>–</td>
</tr>
<tr>
<td>8</td>
<td>29 (100.0)</td>
<td>–</td>
</tr>
<tr>
<td>9</td>
<td>28 (100.0)</td>
<td>–</td>
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<tr>
<td>10</td>
<td>30 (100.0)</td>
<td>–</td>
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<tr>
<td>11</td>
<td>25 (100.0)</td>
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<tr>
<td>12</td>
<td>28 (100.0)</td>
<td>–</td>
</tr>
<tr>
<td>13</td>
<td>27 (100.0)</td>
<td>–</td>
</tr>
<tr>
<td>Total</td>
<td>306 (77.5)</td>
<td>89 (22.5)</td>
</tr>
</tbody>
</table>

3.2. Vaccination status

168 participants (42.5%) had started the primary immunization series in compliance with the national schedule (first dose of pertussis vaccine before the age of 100 days). By 180 days (6 months), 83.5% had initiated the primary series. Vaccines doses 2 and 3 had been given beyond the officially recommended interval of 60 days in 32.4% (128/395) and 42.2% (163/395) of participants, respectively. In particular, 102 participants (25.8%) had intervals longer than 90 days or start of primary series beyond 120 days of life. Comparatively, 102 (25.8%) participants received their toddler booster dose beyond the age of 2 years, and 36 (9.1%) beyond 2.5 years (i.e. up to 4.17 years).

3.3. Serological indication of recent infection

According to the pre-defined interpretation algorithm, serological signs of recent infection in the last 12 months were detected in 42 participants (10.6%) overall. Seven participants in this group were younger than 6 years-old (y.o.); all of them had been vaccinated in the last 3 years, all but one of them with aPV vaccine. However, the delayed vaccinations may render these results difficult to interpret given the proximity of blood sampling to last immunization. Nonetheless, a trend ($r^2 = 0.812$) of increased proportion of children with signs of infection in the last 12 months was observed from 7 years of age onward (Fig. 3).

Serological signs of pertussis infection in the last 6 months were identified in 17 participants (4.3%), all of them 6–13 years old. A trend ($r^2 = 0.786$) of increased proportion of children with signs of infection in the last 6 months was observed from 7 years of age onward (Fig. 3).

As many participants had received delayed toddler booster doses, and the heterogeneous timing of series completion may translate into diversity in the age at which protection wanes, we analyzed the association between serological markers of recent pertussis infection and the time interval between last vaccine dose and blood sampling (Fig. 4). Overall, the participants vaccinated with wPV demonstrating serological signs of infection in the last 6 months had last been vaccinated at a median time of 9.5 years prior to the study. The participants vaccinated with wPV demonstrating serological signs of recent infection in the last 12 months had received their last dose of vaccine at a median time of 9 years prior to the study. The median age in both groups was 11 years. There were no statistically significant differences in stratified proportions of infection during the last 6 months (p = 0.086) or during the last 12 months (p = 0.780). However, a clear trend ($r^2 = 0.692$) of increasing proportion of infection in the last 12 months was observed from the interval of 6 years since the last dose of vaccine onward.
The age-specific distribution by quartiles of anti-PT IgG concentrations (GMC) of the children vaccinated with wPV (Fig. 5), clearly exhibited Q3 values below 40 IU/ml in all age groups, i.e. at least 75% of anti-PT GMCs were evaluated as negative. However, in 10–12 years old children, there was a clear, though not statistically significant, trend towards higher antibody concentrations approaching a positive value. In fact the age-specific anti-PT GMCs appeared to increase towards these values in children aged between 6 and 9 years.

Conversely, although the age-specific distribution of anti-PT IgG concentrations among children vaccinated with aPV (Fig. 6) also shows that the majority of children have antibodies below the threshold indicative of recent infection (i.e., in all age groups Q3 was below 40 IU/ml), a non-statistically significant, declining trend in anti-PT IgG antibodies seems apparent from 3 through 6 years of age.

3.4. Cough symptoms association

Based on their reported history of symptoms, 20 participants (5.1%) were categorized as “typical pertussis”, among whom 15% had serological indicators of pertussis infection during the last 6 months. One hundred and eighty four participants (46.6%) were categorized as “questionable pertussis”. Among them, 10.3% exhibited indication of pertussis infection during the last 12 months, including 4.4% with recent infection during the last 6 months.
total of 191 participants (48.4%) were categorized as “no cough”, even though serological signs of infection during the last 12 months were detected in 10.5% of them, including 3.1% in the last 6 months. The differences in proportions of participants with serological signs of infection relative to history of cough during the last year were statistically significant at the 6-month threshold (p = 0.045) but not at the 12-month threshold (p = 0.732).

4. Discussion and conclusions

This study aimed at documenting age-specific distribution of antibodies against pertussis toxin (PT) following the last dose of pertussis vaccine in the immunization schedule of the Russian Federation. Earlier studies [11–13] had shown that antibodies against PT can be detected in children who have received either aPV or
wPV. However, vaccination with aPV is more often associated with high levels (>80 IU/ml) of antibodies to PT than wPV [14]. In the present study the IgG level for the majority of wP-vaccinated children aged 3–9 years was low (<10 IU/ml). Slightly higher anti-PT antibody levels were observed among a few, mainly aPV-primed children 3–6 years of age who received their last dose of vaccine 1 to 2 years prior to the study, thus most likely representing residual immune response from the last booster vaccination in the second year of life. Therefore, identification of anti-PT IgG at a high level or anti-PT IgG in combination with anti-PT IgA among children who were vaccinated more than 2 years prior can be interpreted as a sign of a recent infection with B. pertussis. At population level, an increased seroprevalence of recent infection can consequently be seen as a proxy for waning of protection from the vaccine.

Although our study was originally intended to stratify serological signs of recent infection by age, the heterogeneity observed in the compliance with the vaccination schedule led us to also stratify serological signs of recent infection by number of years since the last dose of vaccine received (i.e. the toddler booster dose in all cases). When analyzed by age, our study found a significant trend in the proportion of children showing sign of recent infection from the age of 7 years, i.e. approximately 10–25% in children 7–13 years-old, compared to approximately 4–7% in younger age groups. The analysis by time since the toddler booster dose confirmed these findings. In fact, children who showed serological signs of recent infection had received their last dose of vaccine at a median interval of 9 years, and their proportion in the study population showed a significant increasing trend from the interval of 6–7 years since the last dose of vaccine. We also considered that the age-specific geometric mean concentrations (GMCs) in anti-PT IgG further strengthened the argument of an increased circulation of B. pertussis in the early school years. While most children did not present antibody concentrations qualified as serological signs of infection in the last year, children aged 10–12 years clearly had more elevated anti-PT IgG GMCs than younger age groups. We hypothesized that this trend of higher GMCs may also represent signs of infection beyond the preceding year. Studies in the dynamics of anti-pertussis antibody concentrations have documented their fast decline after vaccination or infection, which would suggest that the elevated yet below-threshold antibody levels we observed may be the sign of infection more than a year before the study but likely not more than an additional year or two [15,16].

We sought to investigate through self- or parent-reported information whether the increased risk of infection we identified translated into clinical burden. Although the approach has limited precision, it was important to note that a significant association was found between reporting of pertussis-like symptoms in the last year and serological signs of recent infection; most participants reporting pertussis-like cough symptoms were 9 years-old and above.

Our results indicate that 6 years after the toddler booster dose of pertussis vaccine, children are increasingly susceptible to infection with B. pertussis and subject to clinical pertussis disease. These data show great similarity with previous pertussis seroprevalence studies. As measured by Grimprel et al. in French children in the 1990s as well as Pebody et al. in several wPV-using European countries, a clear upward trend in the prevalence of anti-PT antibodies suggesting recent infection is visible in the early years after traditional start of school [13,17]. In the context of extensive infant vaccination, the circulation of B. pertussis is generally reduced compared to the pre-vaccine era, thereby reducing the exposure to infection and the risk of disease among the population as a whole. Consequently, in the face of waning vaccine immunity, seropositivity marking recent infection is expected to increase only progressively as a result of cumulative waning and exposure to infection. Our findings were consistent with this hypothesis, as after the first signs of re-increased rate of infection at about 6 years of age, significant increases in antibody levels and increase in the number of participants with serological markers of recent infection were found from age 10 onwards. Children whose immunity from a 4-dose series has waned are susceptible not only to infection but also to clinical disease, though it may be milder than in infants. Data collected in the French ACTIV surveillance network repeatedly demonstrated that wPV- and aPV-induced protection against disease wanes among school-aged children [18,19]. Furthermore, recent data collected in China shown the waning of vaccine-elicted protection at school-age and the under-recognized yet significant clinical burden suffered among school-children, adolescents and adults [20].

The primary limitation of our study was the sample size which limited the statistical power to precisely estimate seroprevalence, and any significant association with reported clinical symptoms. As was done by Grimprel et al., this evenly age-distributed sample still allowed us to analyze trends of seroprevalence by age in a statistically significant manner [17]. While changes in magnitude and age-distribution of disease in earlier years may have affected the risk of infection in our population, we considered that the worst it would have overestimated the duration of protection. We did not have public information on the effectiveness of the wPV used in our study population, and its possible impact on duration of protection. However, we considered that the trends of pertussis in Russia in recent years do not suggest any reduction in vaccine effectiveness. Our evaluation of waning in aPV-primed children compared with wPV-primed children was hindered by the recent introduction of the aPVs which limited the number and age distribution of aPV-primed children. The serologic cut-offs used in our study were somewhat arbitrary. We considered that thresholds validated against NIBSC standard for diagnosis of acute disease represented our most conservative option for a threshold defining recent infection, thus optimizing specificity at the cost of sensitivity. Finally, our study measured signs of infection but did not formally document disease burden. While we tried to introduce some measure of clinical burden, the self-reporting method of measurement is subject to individual recall bias and age-associated bias as symptoms experience may differ across the age groups. The symptomatic definition used for this analysis, while differing from international standards of pertussis clinical case definition, was intended to optimize sensitivity in detection of possible pertussis disease in recent history, possibly at the cost of specificity.

The significant reduction in incidence observed in Russia and in St Petersburg after high vaccine coverage (i.e. ≥95%) was restored is a clear indication of the effectiveness of the vaccine. However, vaccine-induced immunity wanes in the years following the last dose, and specifically around school-entry age if the schedule comprises only a primary series followed by a toddler booster. Our study demonstrates that Russian children entering school are susceptible to infection, and to pertussis clinical burden. The position school-aged children and adolescents have in social interaction networks also makes them important contributors to the spread of infectious diseases, and specifically potential direct and indirect sources of pertussis infection for young unvaccinated infants who are at high risk of severe disease and mortality from this pathogen [21]. The extended protection afforded by a booster against pertussis at this age has been shown to provide direct and indirect benefits in the control of pertussis [22,23]. Our data confirms the waning of wPV-elicted immunity around school-age and supports the need for a booster dose at that age, as recommended in the most recent WHO position paper on pertussis vaccines [2]. Since the current national immunization schedule includes a
school-entry booster at 7 years-old against diphtheria and tetanus, it seems logical to take the opportunity to supplement this booster dose with added protection against pertussis using a combination vaccine also containing acellular pertussis components. Importantly, our study also highlighted that primary and booster vaccinations are not always given on time according to public health recommendations. This also likely contributes to increase the proportion of susceptible toddlers.

Contributions

Study concept, design and methodology developed by NK, NG and DM. Field activities & data collection conducted by NK and ET. Data analysis conducted by NK, with support from DM and advice from NG. Interpretation of data, writing and reviewing of this manuscript by NK, ET, NG and DM.

Conflicts of interest

DM is currently employed by Sanofi Pasteur SA and also reports holding of shares in the Sanofi group of companies as part of his employee remuneration. All other authors declare no competing interests.

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