Pertactin-Negative Bordetella pertussis Strains: Evidence for a Possible Selective Advantage

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Background. A recent increase in Bordetella pertussis without the pertactin protein, an acellular vaccine immunogen, has been reported in the United States. Determining whether pertactin-deficient (PRN−) B. pertussis is evading vaccine-induced immunity or altering the severity of illness is needed.

Methods. We retrospectively assessed for associations between pertactin production and both clinical presentation and vaccine history. Cases with isolates collected between May 2011 and February 2013 from 8 states were included. We calculated unadjusted and adjusted odds ratios (ORs) using multivariable logistic regression analysis.

Results. Among 753 isolates, 640 (85%) were PRN−. The age distribution differed between cases caused by PRN−B. pertussis and cases caused by B. pertussis producing pertactin (PRN+) (P = .01). The proportion reporting individual pertussis symptoms was similar between the 2 groups, except a higher proportion of PRN+ case-patients reported apnea (P = .005). Twenty-two case-patients were hospitalized; 6% in the PRN− group compared to 3% in the PRN+ group (P = .11). Case-patients having received at least 1 pertussis vaccine dose had a higher odds of having PRN− B. pertussis compared with unvaccinated case-patients (adjusted OR = 2.2; 95% confidence interval [CI], 1.3–4.0). When restricted to case-patients at least 1 year of age and those age-appropriately vaccinated, the adjusted OR increased to 2.7 (95% CI, 1.2–6.1).

Conclusions. The significant association between vaccination and isolate pertactin production suggests that the likelihood of having reported disease caused by PRN− compared with PRN+ strains is greater in vaccinated persons. Additional studies are needed to assess whether vaccine effectiveness is diminished against PRN− strains.

Keywords. Bordetella pertussis; pertactin; acellular vaccine; waning immunity; mutations.

In the United States, pertussis is currently the least well-controlled vaccine-preventable disease despite excellent vaccination coverage and 6 vaccine doses recommended between 2 months of age and adolescence. In 2012, several states reported epidemic levels of disease, with >48,000 cases reported nationwide, the highest number since 1955 [1]. Increased rates were seen across all ages, with the greatest increases reported in older children and teens [2]. Waning immunity from acellular pertussis vaccines appears to be a significant factor leading to the increasing incidence [3–5]. Additionally, circulating Bordetella pertussis strains are undergoing genetic changes that may allow the organism to evade vaccine-induced immunity or be more virulent [6–10], which may be contributing to the increasing rates of pertussis. Notably, molecular analysis has identified a range of mutations resulting in B. pertussis not producing pertactin, a pertussis acellular vaccine immunogen thought to play a role in adherence to the upper respiratory epithelium [11–20]. All acellular pertussis vaccines currently used in the United States contain pertactin.

Bordetella pertussis isolates lacking pertactin production have been reported at low frequencies from Italy [19], France [20], Japan [13], and Finland [14] and at
a high frequency from Australia [21]. A study of 1300 US sur-
veillance and outbreak-related isolates from 1935 to 2012 doc-
umented a recent increase in pertactin-deficient (PRN⁻) B. pertussis isolates [18]. There have been no large studies in the
United States assessing for differences in clinical presentation
or case-patient vaccine receipt by whether or not B. pertussis
is producing pertactin. Understanding the epidemiologic and
clinical relevance of pertactin deficiency is necessary to fully
elucidate the possible reasons for the current increase in pertus-
sis in the United States.

MATERIALS AND METHODS

Isolates from 753 case-patients collected during May 2011 to
February 2013 from 8 states were included in the analyses
(Table 1). Six of the states participate as Enhanced Pertussis
Surveillance/Emerging Infections Program Network sites (Col-
orado, Connecticut, Minnesota, New Mexico, New York, and
Oregon) that routinely collect isolates on cases of all ages.
The other 2 states, Washington and Vermont, experienced ep-
emic levels of pertussis during 2012 and had a large propor-
tion of culture-confirmed cases and stored isolates available for
molecular testing. The availability of isolates from each state was
dependent on the routine pertussis diagnostic practices used
by healthcare providers in the individual states; however, all
available isolates from case-patients were sent to the Centers
for Disease Control and Prevention for analysis.

Isolates were screened for an array of mutations that have
been documented to cause pertactin deficiency by previously
described polymerase chain reaction amplification and molecu-
lar sequencing methods [18]. Isolates not found to have a pre-
viously identified mutation by molecular methods were assessed
for pertactin production by Western blots (previously de-
scribed) [18] and/or enzyme-linked immunosorbent assay
(ELISA). For the ELISA, microtiter plates were coated with
B. pertussis cell preparations and incubated overnight at 37°C;

<table>
<thead>
<tr>
<th>Table 1. Number of Bordetella pertussis Isolates Collected Between May 2011 and February 2013</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>State Submitting Isolate</strong></td>
</tr>
<tr>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>Colorado</td>
</tr>
<tr>
<td>Connecticut</td>
</tr>
<tr>
<td>Minnesota</td>
</tr>
<tr>
<td>New Mexico</td>
</tr>
<tr>
<td>New York</td>
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<tr>
<td>Oregon</td>
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<tr>
<td>Vermont</td>
</tr>
<tr>
<td>Washington</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

washed and incubated at 37°C for 1 hour each with 1:40,000 di-
luted sheep anti-PRN sera 97/558 (NIBSC, Hertfordshire, En-
land) and then 1:2000 diluted peroxidase-labeled antisheep
immunoglobulin G antibody (KPL, Gaithersburg, Maryland);
and washed and incubated for 10 minutes with tetramethylben-
zidine color substrate. Optical densities were read at 450 nm.

Routine collected case-investigation data included demo-
graphics, pertussis symptoms, and vaccination history. Pertussis
symptoms and vaccine histories were linked to isolates by
unique identifiers. Case-patients aged <13 years of age were
considered to be fully vaccinated if they received diphtheria
and tetanus toxoids and acellular pertussis vaccine doses 1–3
by 1 year of age, dose 4 between ages 1 and 2 years, and dose
5 between ages 4 and 6 years. Those older than 13 years also
needed to have received a dose of tetanus toxoid, reduced diph-
theria toxoid and acellular pertussis (Tdap) vaccine to be con-
sidered fully vaccinated. Only case-patients confirmed during
case-investigation interviews as unvaccinated were classified as
unvaccinated to distinguish them from those with missing dose
information.

We calculated unadjusted odds ratios (ORs) as well as adjust-
ed ORs using multivariable logistic regression analysis using
SAS software, version 9.3. When modeling the association be-
 tween pertactin production and vaccination receipt, we first
compared all case-patients with at least 1 documented dose of
pertussis vaccine to those documented to be unvaccinated. Sec-
don, vaccinated case-patients were restricted to those who were
up-to-date according to schedule, and we only included vacci-
nated and unvaccinated case-patients at least 1 year of age to
limit the population to those who were eligible to receive at
least 3 doses of pertussis vaccine.

RESULTS

Overall, 85% (640/753) of isolates were PRN⁻. The proportion
of PRN⁻ isolates ranged from 67% in Colorado to 100% in New
Mexico; however, the number of isolates available for testing
from each state varied widely (range, 4–255) (Table 1). Nine
previously recognized mutations that result in pertactin de-
icency were identified among the isolates, and 2 isolates with
uncharacterized mutations were also found to be PRN⁻ by
ELISA [18].

Table 2 provides a breakdown of case-patient demographic
and clinical variables by B. pertussis pertactin production. Al-
though the overall age distribution of case-patients with isolates
was largely similar to the national age distribution of reported
cases in 2012 (data not shown), we found a significant differ-
ence in the age distribution between the PRN⁻ and pertactin-
producing (PRN⁺) groups (unadjusted \( P = .01 \)). No significant
differences were found between the 2 groups for sex or race.
The proportion of case-patients reporting pertussis symptoms
was similar by PRN− and PRN+, with the exception that a higher proportion of case-patients infected with PRN+ B. pertussis reported apnea (unadjusted \( P = .005 \); Table 2). The results for apnea remained significant after controlling for age group and state (\( P = .01 \)). The proportion of case-patients reporting at least 2 or at least 3 weeks of cough did not differ by PRN− and PRN+ status (Table 2). A total of 22 case-patients were hospitalized, with 6% in those with PRN+ B. pertussis compared to 3% in those with PRN− B. pertussis (unadjusted \( P = .11 \)).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Pertactin Protein Deficient, No. (%)</th>
<th>Pertactin Protein Produced, No. (%)</th>
<th>( P ) Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;6 mo</td>
<td>31 (5)</td>
<td>13 (12)</td>
<td>.01</td>
</tr>
<tr>
<td>6 mo–7 y</td>
<td>104 (17)</td>
<td>23 (21)</td>
<td></td>
</tr>
<tr>
<td>7–11 y</td>
<td>152 (24)</td>
<td>22 (20)</td>
<td></td>
</tr>
<tr>
<td>11–13 y</td>
<td>84 (13)</td>
<td>14 (13)</td>
<td></td>
</tr>
<tr>
<td>13–18 y</td>
<td>172 (27)</td>
<td>17 (16)</td>
<td></td>
</tr>
<tr>
<td>≥18 y</td>
<td>87 (14)</td>
<td>20 (18)</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>308 (49)</td>
<td>47 (42)</td>
<td>.20</td>
</tr>
<tr>
<td>Female</td>
<td>322 (51)</td>
<td>67 (58)</td>
<td></td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>13 (3)</td>
<td>2 (2)</td>
<td>.71</td>
</tr>
<tr>
<td>White</td>
<td>364 (91)</td>
<td>74 (89)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>25 (6)</td>
<td>7 (8)</td>
<td></td>
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<tr>
<td>Apnea</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Yes</td>
<td>126 (23)</td>
<td>37 (37)</td>
<td>.005</td>
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<tr>
<td>No</td>
<td>415 (77)</td>
<td>64 (63)</td>
<td></td>
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<tr>
<td>Whoop</td>
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<tr>
<td>Yes</td>
<td>170 (32)</td>
<td>34 (33)</td>
<td>.74</td>
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<tr>
<td>No</td>
<td>367 (68)</td>
<td>68 (67)</td>
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<tr>
<td>Posttussive vomit</td>
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<tr>
<td>Yes</td>
<td>239 (44)</td>
<td>46 (45)</td>
<td>.83</td>
</tr>
<tr>
<td>No</td>
<td>310 (56)</td>
<td>57 (55)</td>
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<tr>
<td>Paroxysms</td>
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<tr>
<td>Yes</td>
<td>487 (87)</td>
<td>92 (90)</td>
<td>.37</td>
</tr>
<tr>
<td>No</td>
<td>72 (13)</td>
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<tr>
<td>Cyanosis</td>
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<tr>
<td>Yes</td>
<td>10 (6)</td>
<td>7 (12)</td>
<td>.14</td>
</tr>
<tr>
<td>No</td>
<td>160 (94)</td>
<td>51 (88)</td>
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<tr>
<td>Cough ≥2 wk</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>462 (87)</td>
<td>86 (88)</td>
<td>.91</td>
</tr>
<tr>
<td>No</td>
<td>67 (13)</td>
<td>12 (12)</td>
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<tr>
<td>Cough ≥3 wk</td>
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<td></td>
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<tr>
<td>Yes</td>
<td>182 (37)</td>
<td>33 (38)</td>
<td>.81</td>
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<tr>
<td>No</td>
<td>316 (63)</td>
<td>54 (62)</td>
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</tr>
<tr>
<td>Hospitalized</td>
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<td></td>
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<tr>
<td>Yes</td>
<td>16 (3)</td>
<td>6 (6)</td>
<td>.11</td>
</tr>
<tr>
<td>No</td>
<td>560 (97)</td>
<td>98 (94)</td>
<td></td>
</tr>
</tbody>
</table>

* \( P \) value calculation did not include missing data.

Vaccinated case-patients receiving at least 1 dose had a significantly higher odds of having PRN− B. pertussis compared with unvaccinated case-patients (unadjusted OR = 3.2; 95% confidence interval [CI], 1.9–5.3). When case-patients were restricted to those at least 1 year of age and vaccinated case-patients were further restricted to those according to schedule and fully up to date with pertussis vaccinations, the OR increased to 3.7 (95% CI, 1.9–7.1). When the analyses were adjusted for submitting state and age group, the ORs remained significant (Table 3).

**DISCUSSION**

Our finding of a 2- to 4-fold greater odds of having PRN− B. pertussis when fully vaccinated according to schedule suggests that vaccinated persons have greater susceptibility to PRN− strains compared with PRN+ strains. Waning of immunity in children and adolescents primed with pertussis acellular vaccines is believed to be one of the primary drivers behind the changing epidemiology in the United States. All birth cohorts born since 2000 in the United States have received exclusively acellular vaccines, including increasing numbers of preteens receiving a Tdap booster following acellular priming. As these cohorts age, they are experiencing higher rates of pertussis, and recent studies suggest that the lifelong risk of pertussis among children primed with acellular vaccines is greater than in those primed with whole-cell vaccines [22, 23]. Additionally, data from a nonhuman primate model indicate that acellular vaccines may not prevent infection, although they can prevent disease symptoms [24]. We hypothesize that the increasing population-level susceptibility to pertussis among children and adolescents primed with a limited number of acellular vaccine antigens may have contributed to increasing transmission.
and allowed the rapid proliferation of PRN− B. pertussis in the United States once those strains appeared. Furthermore, the multiple different mutations and mechanisms of pertactin non-production found in our sample, rather than clonal expansion of a single PRN− strain, argues for a selective advantage to lacking the protein [18].

Pertactin was included in acellular vaccines due to its putative role in mediating adherence to the epithelium of the respiratory tract [25]. Acellular vaccines including pertactin generally had greater efficacy in licensure trials than those without [26–31], although the actual role of pertactin and antibodies directed against it remains unclear. Murine models provide evidence that pertactin may also play a functional role in resisting neutrophil-mediated clearance [32, 33], which could impact persistence of infection and, theoretically, transmission or severity of disease. Despite the 50-year record number of cases reported in 2012 and a high proportion caused by PRN− B. pertussis, other than for apnea, we note no differences between clinical presentation of case-patients with PRN− and PRN+ strains. Analysis from France similarly reported no major differences in assessed clinical outcomes in infants with PRN− or PRN+ B. pertussis [15]. With no indication of diminished capacity to infect or alterate in clinical presentation of severity, the full ramifications of the appearance and rapid proliferation of pertactin deficiency are unclear. If pertactin plays an important role in infection and persistence, compensatory changes may have occurred; however, the corresponding genetic and proteomic changes that have filled this functional niche are unknown but warrant investigation. Absent substantial compensatory changes, renewed investigation into the role of anti-pertactin antibody in protection against pertussis is needed.

Although Enhanced Pertussis Surveillance sites provide high-quality data with minimal missing data, vaccination history collected through case investigations may still be incomplete. We expect some misclassification in vaccination receipt status. The misclassification is likely nondifferential between case-patients with PRN− and PRN+ B. pertussis, meaning that the misclassification rates are likely similar between the groups and should bias the findings toward the null hypothesis of no association with vaccine receipt. Additionally, some case-patients included in our analysis could have received a 2-component acellular vaccine (discontinued in the United States in 2011) that does not include pertactin for all or some of the childhood doses. Because data on vaccine brand are often missing, we are unable to control for this in our results. The cases included in our analysis may not have represented the full spectrum of clinical presentation; milder cases may not have sought medical care, or clinicians may not have considered pertussis for atypical or mild illness.

Although our findings may be influenced by the predominant strains that are circulating in different geographic areas and possibly by other factors such as age, we attempted to control for these potential differences in exposure by controlling for submitting state and age group in the multivariable models. Our findings remained significant when controlling for these factors. This suggests that our findings are not solely due to differences in the strains to which vaccinated and unvaccinated case-patients are exposed.

Although recently conducted effectiveness studies provide evidence that acellular vaccines continue to provide good protection against pertussis in the short term [3, 5, 34], additional studies are needed to further assess whether effectiveness or durability of protection of acellular vaccine is diminished against PRN− strains. Ongoing surveillance for pertactin production in circulating B. pertussis is needed, as well as surveillance for other possible changes to the B. pertussis population including lack of expression of other immunogens included in acellular vaccines. Additional studies that bridge between the clinical and epidemiological findings and these novel molecular genomic and proteomic findings are necessary to continue to expand the evidence base for the development of more-effective vaccines against pertussis disease.

Notes


Potential conflicts of interest. C. K. received grants from Centers for Disease Control and Prevention during the conduct of the study. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References


