Patterns of *Bordetella parapertussis* Respiratory Illnesses: 2008–2010

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Clinical specimens from 9 states during 2008–2010 were tested by PCR for *Bordetella pertussis* and *Bordetella parapertussis*. Of the positive samples, 13.99% were identified as *B. parapertussis*. It was concluded that *B. parapertussis* infections are more common than previously realized and contribute to cases thought to be vaccine failures.

During the last 25 years, reported pertussis has been increasing in the United States [1–3]. In particular, major epidemics occurred in 2004–2005 and in 2010 [3–5]. Considerable attention has been paid to the reasons for these epidemics and the 2010 California epidemic received considerable media attention [6]. An issue of major concern was the potential contribution of vaccine failure to the epidemic. In this regard, one suggestion was that many cases of reported pertussis were due to *Bordetella parapertussis*, for which vaccines offer little or no protection [7–10].

Today, a significant number of *Bordetella* spp. infections are diagnosed by the use of polymerase chain reaction (PCR) studies of nasopharyngeal specimens, collected from persons with suspected pertussis [1, 11–13]. In this regard, primers from nucleic acid segments from insertion sequences have been widely used. Specifically, the presence of insertion sequence 481 (IS481) is indicative of *Bordetella pertussis* infection, and the presence of IS1001 indicates *B. parapertussis* infection.

In 2005, one of us (J.D.C.) reviewed the epidemiology of *B. parapertussis* illness. In contrast with findings in Europe, the isolation of *B. parapertussis* between 1975 and 2005 was uncommon in the United States [1]. To investigate the role of

*B. parapertussis* as the cause of cough illnesses suspected of being pertussis in California and other selected areas of the United States, we have examined the results of studies performed at Focus Diagnostics in California. Specifically, we have analyzed the IS481 and IS1001 positivity of nasopharyngeal samples submitted for PCR during the 3-year period 2008–2010.

**METHODS**

Nucleic acids were extracted from 200 μL of upper respiratory specimens (nasopharyngeal swab in viral transport media; nasopharyngeal aspirate) on the MagNA Pure LC Instrument (Roche Diagnostics) with a Total Nucleic Acid Isolation Kit. An elution volume of 50 μL was used for the extracted nucleic acids. Two real-time PCR protocols were used to identify specimens containing *B. pertussis* and *B. parapertussis*. Both protocols amplified and detected sequences in IS481 and IS1001.

The first protocol was used to test specimens prior to October 2010 and was performed on the ABI Prism 7900HT instrument (Applied Biosystems). Primers were designed to amplify a 151-bp region of IS481 and a 213-bp region of IS1001. Dual-labeled probes were used to detect the products of amplification. Each reaction also contained TaqMan Exogenous Internal Positive Control Reagents (Applied Biosystems) to detect PCR inhibition. Real-time PCR results with cycle threshold (C_T) values <38 were considered to be positive for the appropriate target (IS481 or IS1001). Specimens with C_T values >38 but <50 were repeated to confirm the presence of IS481 or IS1001.

The second protocol was implemented beginning October 2010. In this method, IS481 and IS1001 targets were amplified and detected with *B. pertussis* Primer Mix and *B. parapertussis* Primer Mix (Focus Diagnostics) on the 3M Integrated Cycler (Focus Diagnostics). In the second protocol, real-time PCR results with cycle threshold (C_T) values <35 were considered to be positive for the appropriate target (IS481 or IS1001). Specimens with C_T values >35 but <40 were repeated to confirm the presence of IS481 or IS1001. Method comparison studies showed 100% correlation between the 2 protocols.

**RESULTS**

During the 3-year period 2008–2010, the majority of specimens were submitted from 9 states (California, Florida, Illinois, Michigan, New Jersey, Ohio, Texas, Virginia, and Washington); our analyses will involve only data from these states. Sixty-three percent of the samples were from children.
Sixty-nine percent of specimens were from California. Of the total specimens submitted, 9.5% were positive for either IS481 or IS1001.

Of all the positive samples, 13.9% were IS1001 positive. The 9-state overall IS1001 positivity rates by year were 16.5% in 2010, 7.9% in 2009, and 8.9% in 2008. The IS1001 positivity rates by age group were as follows: <5 years, 66%; 5–10 years, 28.7%; 11–20 years, 3.8%; and ≥21 years, 0.4%. In contrast, the IS481 positivity rates for the same age groups were 38.5%, 28.3%, 22.3%, and 10.9%, respectively. There were 17 dual-positive samples (positive for both IS481 and IS1001) during the 3-year period, and 12 of them (70.6%) occurred in 2010.

The weekly submission of specimens for PCR study for the 3-year observation period is presented in Figure 1. The 2008 and 2010 patterns are similar, with an increasing slope of submissions during the second half of the year. In contrast, there were more submissions during the first half of 2009.

The weekly positive rates for IS481 for the 3-year study period are presented as the percentages of total specimens in Figure 2. As can be seen, prolonged IS481 peaks are noted each year. In 2010 the peak period extended from mid-April to mid-August, whereas in the 2 preceding years the peaks started in June and extended until the end of September.

Similar data for IS1001 are presented in Figure 3. IS1001 positivity had no clear-cut peak or trough periods except that the pattern changed in 2010. Beginning in the 14th week of 2010, there was an increased percentage of IS1001-positive specimens. Of interest, this 2010 pattern for IS1001 also mirrors the increased specimen submission that occurred in 2010 (Figure 1). In contrast, the percentage of IS481 positivity decreased with increased sample submission in 2008 and in 2010.
DISCUSSION

*B. pertussis* and *B. parapertussis* have limited genetic diversity; nevertheless, the antibody generated against *B. pertussis* proteins that offers protection against *B. pertussis* offers little protection against *B. parapertussis* infection [1, 7–10]. Another notable difference between the 2 subspecies is that *B. pertussis* secretes pertussis toxin and *B. parapertussis* does not. *B. parapertussis* infections can cause unrecognized infection, mild pertussis, or classic pertussis [1, 14–17]. In general, pertussis due to *B. parapertussis* is generally milder and of shorter duration. However, classic pertussis with paroxysmal cough, whooping, and posttussive vomiting occurs.

In the prevaccine era, study findings suggested that *B. parapertussis* illnesses were much less common than *B. pertussis* illnesses [1]. Similar to those for *B. pertussis*, the epidemic cycles occurred at 3–4 year intervals. In conjunction with the pertussis vaccine efficacy trials in the 1990s, *B. parapertussis* illnesses were noted in Italy, Germany, and Sweden [8, 9, 14–17]. In 6 of the efficacy trials, *B. parapertussis* accounted for between 2.1% and 25% of the culture-positive cases. In contrast with the observations in Europe, documented *B. parapertussis* illnesses in the United States during the last 35 years have been uncommon [1].

Because of its increased sensitivity, the routine use of PCR in the diagnosis of pertussis has contributed to the finding of more cases of pertussis in recent years [1, 4, 13, 18]. *B. pertussis* contains ~238 copies of IS481 and no copies of IS1001, whereas *B. parapertussis* has ~22 copies of IS1001 and no copies of IS481 [19]. The multiple copies of IS481 are responsible for the high sensitivity of PCR, which uses this primer in the diagnosis of pertussis. However, this high level of sensitivity is associated with an increased risk of false-positive results [1, 11–13, 18, 20, 21]. False-positive diagnoses of pertussis caused by *B. pertussis* can be the result of DNA contamination in the clinic due to recent vaccine use or previous patients with pertussis [21]. False-positive identification of IS481 may have occurred in this study, but we have no way to address this possibility. However, false-positive identification of IS1001 seems unlikely, because IS1001 is not present in vaccines and its copy numbers are low.

*Bordetella holmesii* also may contain IS481 (8–10 copies) and IS1001 (unknown number of copies) so that if PCR assay is positive for both, this organism is thought to be the causative agent [18, 20]. However, dual positivity could also be the result of concomitant infection with both *B. pertussis* and *B. parapertussis* [8, 22, 23]. Because no cultures were performed, the cause of illness associated with our 17 dual-positive specimens is not known.

Recent experiences as well as the present study suggest that *B. parapertussis* infections are common but have been generally overlooked [24]. In this study we have shown that *B. pertussis*...
illness peaks in the summer, whereas *B. parapertussis* was endemically prevalent throughout the first 2½ years of the study. During the last 6 months of 2010, *B. parapertussis* infections became more prevalent and accounted for a much greater percentage of the positive samples. Our findings also suggest that the age spectrum of *B. parapertussis* illness is different from that of *B. pertussis* illness. Only 4.2% of the IS1001-positive specimens occurred in persons >10 years of age, compared with 32.2% for IS481-positive specimens. Our data indicate that *B. parapertussis* infections contribute significantly to the overall pertussis burden and contribute to the pool of children thought to have vaccine failure. This was particularly notable in the summer, fall, and early winter of 2010.

**Notes**

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