Persistent \textit{Bordetella bronchiseptica} Pneumonia in an Immunocompetent Infant and Genetic Comparison of Clinical Isolates with Kennel Cough Vaccine Strains

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An infant who experienced recurrent episodes of respiratory failure received a diagnosis of pertussis on the basis of immunofluorescence testing, but culture revealed macrolide-resistant \textit{Bordetella bronchiseptica}. Genetic analysis demonstrated that the child was not infected with a kennel cough vaccine strain, although the family’s dog had recently been vaccinated. The infection cleared with imipenem therapy.

\textit{Bordetella bronchiseptica} is a respiratory pathogen closely related to the etiologic pathogen of whooping cough, \textit{Bordetella pertussis}. Both share virulence factors facilitating prolonged carriage in the respiratory tract \cite{1, 2}. \textit{B. pertussis} infects only humans, but \textit{B. bronchiseptica} naturally infects a variety of mammalian species, causing tracheobronchitis (“kennel cough”) in dogs and cats and atrophic rhinitis in swine \cite{3}. Human infections \cite{4–6} occur mostly in immunocompromised individuals exposed to infected farm and companion animals \cite{5, 7, 8}.

Kennel cough vaccines containing live, attenuated \textit{B. bronchiseptica} are commonly used in veterinary clinics. Transmission of vaccine strains to humans is a theoretical possibility, but no culture-proven cases have been reported \cite{8}.

Ribotyping of \textit{B. bronchiseptica} using \textit{PvuII} is highly discriminatory \cite{9, 10}. In addition, extensive sequence heterogeneity occurs in 2 repeat regions of the gene encoding the adhesin pertactin, \textit{prn} \cite{11–13}, which may also provide information useful in epidemiologic investigations.

Here, we report a culture-proven case of severe \textit{B. bronchiseptica} pneumonia in an otherwise healthy infant residing in a household with a dog recently vaccinated with a live, attenuated vaccine for kennel cough. The course of illness highlights the clinical relevance of the differential diagnosis between \textit{B. pertussis} and \textit{B. bronchiseptica} infection. Comparison of the \textit{PvuII} ribotypes and \textit{prn} repeat region sequences of the clinical isolates with those of all available live kennel cough vaccine strains was performed to determine whether transmission of a vaccine strain to the infant could have occurred.

\textbf{Case report.} A 6-week-old, previously healthy, African American infant presented to the emergency department with acute tachypnea, hypoxemia, and apnea requiring intubation and admission to the pediatric intensive care unit. This episode occurred only days after intranasal vaccination of the household dog with an attenuated live vaccine for \textit{B. bronchiseptica}. The identity of the vaccine used was unavailable, and it was not possible to obtain a culture specimen from the animal.

During hospitalization in the pediatric intensive care unit, the patient began treatment with ticarcillin plus clavulanate and recovered gradually over 2 weeks. A respiratory culture, the results of which were available after the patient’s discharge from the hospital, yielded \textit{B. bronchiseptica}. The child was not available for follow-up. At the ages of 4 and 6 months, the patient presented to 2 different emergency departments with 2 additional, almost identical episodes of respiratory failure and again required hospital admission. The physicians treating the second and third episodes were unaware of the previous diagnosis. In both instances, results of direct fluorescent antibody (DFA) testing of nasopharyngeal aspirate specimens were positive. At each hospital admission, the patient was treated for presumed pertussis with a 2-week course of azithromycin. At 8 months of age, the child was admitted to the pediatric intensive care unit for a fourth time with moderate apnea and persistent cough but recovered quickly with symptomatic treatment. Culture of nasopharyngeal aspirate specimens yielded \textit{B. bronchiseptica}. Shortly after culture results became available, the infant was admitted to the hospital for a fifth time with severe pneumonia and high fever. The patient was initially treated with
months of age were identified as *B. bronchiseptica*. A respiratory culture at hospital admission again yielded *B. bronchiseptica*. The patient recovered well during a 2-week course of imipenem therapy, was discharged from the hospital, and received trimethoprim-sulfamethoxazole treatment for an additional 2 weeks. Subsequent respiratory cultures demonstrated clearance of *B. bronchiseptica* infection, and the patient remained symptom-free throughout the second year of life. According to the caregiver, the patient had received all recommended vaccinations, including 4 doses of the diphtheria, tetanus, and acellular pertussis vaccine. No abnormalities in the peripheral WBC count or distribution were noted. Immunologic evaluation, including quantitative analysis of T cell and immunoglobulin subsets, revealed no evidence of immunosuppression.

Methods. Nasopharyngeal aspirate and bronchoalveolar lavage specimens were obtained from the infant (from 6 weeks of age to 9 months of age) during 5 pediatric intensive care unit admissions at 2 hospitals in New Orleans, Louisiana, from July 2003 through February 2004. The isolate obtained at 6 weeks of age was identified as *B. bronchiseptica* with use of standard methods [14] and was evaluated for antimicrobial susceptibility by microdilution assay (MicroScan; Dade Behring) but was not preserved. Two aspirate specimens from subsequent admissions to a different hospital were tested by DFA for *B. pertussis* (Accu-Mab Plus; Altachem Pharma). Results of both tests were reported as positive, and no further testing was pursued. *B. bronchiseptica* was isolated from 3 additional aspirate specimens (obtained at 8 months of age and 9 months of age) and was tested for antimicrobial susceptibility. DFA testing was performed for 2 of these isolates, designated F4563 and W48661; the isolates were found to be DFA positive for *B. pertussis* and were cryopreserved for PCR at a reference laboratory (New Orleans, LA). The PCR method used is not known because of laboratory infrastructure failure after a natural disaster.

Three live kennel cough vaccines (designated A, B, and C) were licensed for use in the United States at the time that the infant was infected. DNA from vaccine strains and patient isolates was used for ribotyping with *Pvu* II, as described elsewhere [10]. Pertactin gene repeat regions were amplified by PCR and were sequenced as previously detailed [13]. GenBank accession numbers for sequences in the F4563 and W48661 isolates are EU275368–EU275371.

Results. Isolates derived from the patient at 1.5, 8, and 9 months of age were identified as *B. bronchiseptica* [14]. Susceptibility testing confirmed identical patterns in all isolates, as follows: resistance to aztreonam, ceftriaxone, cefepime, and macrolides and susceptibility to ciprofloxacin, gentamicin, trimethoprim-sulfamethoxazole, imipenem, levofloxacin, and piperacillin-tazobactam. Isolates that were DFA positive for *B. pertussis* were PCR negative for *B. pertussis*.

Comparison with previously defined patterns [10] revealed that the F4563 and W48661 isolates were *B. bronchiseptica* ribotype 18. Kennel cough vaccines A and B are ribotype 9, and vaccine C is ribotype 3 (figure 1). Both clinical isolates contain an identical, novel prn repeat region variant, designated 1–5b/2–8b according to the proposed nomenclature [12]. This variant was not found in any kennel cough vaccine evaluated (figure 1B and 1C). These data indicate that the source of the infant’s infection was not the kennel cough vaccine recently administered to the family dog.

Discussion. We report, to our knowledge, the first culture-proven case of persistent *B. bronchiseptica* infection in an immunocompetent infant exposed to a vaccine for kennel cough. Genetic comparison of clinical isolates and vaccine strains eliminates the vaccine as a source of the infant’s exposure, although transmission of a naturally occurring strain from the dog to the infant cannot be ruled out. Alternatively, infection may have resulted from exposure to some other animal (although the family denied any other animal exposure). Ribotype analysis of >400 isolates of *B. bronchiseptica*, obtained from 15 host species (including humans and dogs), revealed only 2 other ribotype 18 strains, 1 of which was from a swine and 1 of which was from a human (K.B.R., unpublished data) [9, 10]. Interestingly, the prn repeat sequences from the human isolate, obtained in Louisiana in 1984, are identical to those of the F4563 and W48661 isolates (K.B.R., unpublished data). To date, ~35 pertactin repeat region sequences from other human isolates are available (either in GenBank or our laboratory [K.B.R., unpublished data]), none of which are identical to F4563 and W48661.

The number of reports of human *B. bronchiseptica* infection in immunocompromised individuals is increasing, but little is known about the prevalence and persistence of *B. bronchiseptica* infection in immunocompetent humans. Underreporting in healthy individuals is likely because of a low index of suspicion among clinicians. Also unclear is the potential for concurrent infections due to *B. pertussis* and *B. bronchiseptica* in humans. In the reported case, not all of the positive DFA test results were followed up further, but coinfection seems highly unlikely because of the clinical course, the vaccination history, the absence of lymphocytosis, and negative results of culture and PCR for *B. pertussis*.

In routine clinical practice, a diagnosis of pertussis is often made on the basis of a positive DFA test result alone, without consideration of culture results. Information provided by the manufacturer and expert reviews agree that positive DFA test results alone are inconclusive, because, as demonstrated here, *B. bronchiseptica* may cross-react with the antibody used [7]. Reliance on DFA test results alone resulted in inadequate treat-
Figure 1. Comparison of Pvu II ribotype (A) or pertactin repeat region 1 (B) and 2 (C) of clinical Bordetella bronchiseptica isolates and kennel cough vaccine strains. A, Lane 1, ribotype 18 standard; lane 2, isolate F4563; lane 3, isolate W48661; lane 4, ribotype 9 standard; lane 5, kennel cough vaccine A; lane 6, kennel cough vaccine B; lane 7, ribotype standard 3; lane 8, kennel cough vaccine C. B and C, Multiple alignments of the predicted amino acid sequences indicated. Dashes in the alignment represent gaps in the sequence; asterisks below indicate amino acids that distinguish the clinical isolates from the vaccine strains.

ment of the patient with azithromycin. Clearance was achieved only after treatment based on valid identification and susceptibility data.

B. bronchiseptica rarely responds to macrolide antibiotics [5, 7], which are first-line agents for B. pertussis, and macrolide sensitivities are not routinely reported in panels for gram-negative bacteria. B. bronchiseptica may survive intracellularly for several days after uptake by phagocytes, whereas B. pertussis is killed readily, [15] which may, in part, explain the persistence of infection in the immunocompetent patient described here. Thus, early initiation of appropriate treatment is crucial and is dependent on differentiation between B. pertussis and B. bronchiseptica. PCR targeting the insertion element IS481 is often used for identification of B. pertussis but also may provide false-positive results when used for some B. bronchiseptica isolates [16] and for Bordetella holmesii [14].

Little is known about the route of transmission of B. bronchiseptica infection. Airborne human-to-human transmission has been reported in a hospital setting [17]. The route of infection in the case reported here, which occurred outside the hospital setting, remains unknown. Furthermore, it is unclear whether diphtheria, tetanus, and pertussis vaccination offers protection against B. bronchiseptica infection and whether vaccinated infants and unvaccinated individuals may be particularly susceptible, not only to B. pertussis, but also to B. bronchiseptica. Additional investigations and prospective analyses are needed to assess the role of B. bronchiseptica as a pathogen in otherwise healthy individuals.

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References