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***Bordetella bronchiseptica* in a Pediatric Cystic Fibrosis Patient: Possible Transmission from a Household Cat**

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Summary

Bordetella bronchiseptica is a zoonotic respiratory pathogen commonly found in domesticated farm and companion animals, including dogs and cats. Here we report isolation of *B. bronchiseptica* from a sputum sample of a cystic fibrosis patient recently exposed to a kitten with an acute respiratory illness. Genetic characterization of the isolate and comparison with other isolates of human or feline origin strongly suggest the kitten was the source of infection.

Keywords

Bordetella bronchiseptica; zoonoses; cystic fibrosis; molecular typing

Introduction

Bordetella bronchiseptica is closely related to the etiologic agent of whooping cough, *Bordetella pertussis*. While *B. pertussis* is a strict human pathogen, *B. bronchiseptica* infects a variety of companion and farm animals to which humans are frequently exposed, including cats and dogs in which it is a cause of tracheobronchitis or “kennel cough.” Human infections occasionally occur, most frequently in immunocompromised individuals, but related illness in healthy adults and children has also been reported (Mattoo and Cherry, 2005).

*Pvu*II ribotyping (Register et al., 1997; Register and Magyar, 1999) and multilocus sequence typing (MLST), based on partial sequencing of a set of seven housekeeping genes (Diavatopoulos et al., 2005), have been used to distinguish among strains of *B. bronchiseptica*. Both methods are highly discriminatory and can be used to reliably infer

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relationships among isolates and identify likely sources of exposure. Comparison among isolates of two repeat regions of the surface-exposed adhesin pertactin can also be informative in epidemiologic investigations since polymorphisms occur at a relatively high frequency (Boursaux-Eude and Guiso, 2000; Register, 2001, 2004).

Cystic fibrosis (CF) is a hereditary disorder in which mutations in the *CFTR* gene disrupt chloride ion transport of the exocrine glands, leading to abnormalities in multiple organ systems. A prominent clinical feature of CF is chronic inflammation of the airways, resulting in damage that increases susceptibility to pulmonary infections. Several recent studies demonstrate that *B. bronchiseptica* may cause airway infection in CF patients (Moissenet et al., 2005; Ner et al., 2003; Spilker et al., 2008; Wallet et al., 2002). In some cases, zoonotic transmission from family pets exhibiting an acute respiratory illness was hypothesized. However, no molecular subtyping analysis has been undertaken with any *B. bronchiseptica* isolate from a CF patient. Here we detail the isolation and molecular characterization of a *B. bronchiseptica* isolate cultured from a sputum sample of a pediatric CF patient recently exposed to a kitten with a history of respiratory illness.

Material and Methods

Sputum for culture was collected by spontaneous expectoration during clinic visits. *B. bronchiseptica* was identified from a single sample using standard methods.

Ribotype analysis was based on hybridization of *Pvu*II digestion fragments with a probe derived from the *Escherichia coli* *rmB* operon as reported previously (Register and Magyar, 1999). The *Bordetella* MLST methodology has been described elsewhere (Diavatopoulos et al., 2005). Allele variants and MLST sequence type (ST) were assigned using the *Bordetella* MLST database sited at the University of Oxford (<http://pubmlst.org/bordetella>). For detection of the *cyaA* gene encoding the *Bordetella* adenylate cyclase toxin (ACT), a Southern blot containing 2 ug of genomic DNA digested with *Bam*HI was hybridized with a DIG-labeled probe covering the 5' 1241 bp of the gene, following the manufacturer's recommendations for PCR probe labeling and Southern blotting (Roche). *B. bronchiseptica* strains 253 (Buboltz et al., 2008) and RB50 (Parkhill et al., 2003) were used as negative and positive controls, respectively. Pertactin gene repeat regions were amplified by PCR and sequenced as reported (Register, 2004). Final MLST and pertactin gene sequences were derived from a minimum of three reads, with at least one read in each direction. Sequences were deposited in GenBank with accession numbers JF960404 -JF960412.

Case History

An 11-year-old girl with moderately severe but stable CF lung disease presented for a routine clinic visit at Brenner Children's Hospital, Winston-Salem, North Carolina. The patient reported no increased cough or respiratory disease symptoms. No change in pulmonary function and no fever, increase in cough or sputum expectoration, or other evidence of acute respiratory illness was noted during examination. *B. bronchiseptica* was isolated from a sputum sample collected during the visit. The patient had received the recommended childhood vaccinations for whooping cough and was being maintained on a regimen of tobramycin solution for inhalation. She was intermittently on ciprofloxacin but treatment was not ongoing at the time of *B. bronchiseptica* isolation and she was not treated for the *B. bronchiseptica* infection. Approximately 3 weeks before the visit, the family acquired a kitten displaying clinical signs of an acute respiratory disease. The kitten had been removed from the home by the time of the clinic visit and was not available for culture. There was no further isolation of *Bordetella* spp from sputa collected during her routine quarterly visits over the next two years.

Results and Discussion

A nonhemolytic, gram-negative bacterium isolated from a quarterly sputum sample was identified as *B. bronchiseptica* and assigned the designation T44625. No *Bordetella* spp were isolated from subsequent samples.

T44625 was identified as *PvuII* ribotype (RT) 4 (Fig. 1A). Previous studies that included 214 *B. bronchiseptica* isolates representing 11 different hosts reported 31 RT4 isolates, 28 of which originated in dogs or cats (Register et al., 1997; Register and Magyar, 1999). Of the remaining 17 RTs described, RT10 is most closely related to RT4. Only one RT10 strain has been reported to date, isolated from a cat (Register and Magyar, 1999). The same study demonstrated that the 18 *B. bronchiseptica PvuII* RTs constitute 5 major clusters, each sharing ~70% similarity to other RTs within the cluster. All cat isolates characterized to date fall within Cluster I of the phylogeny. Other than T44625, only 3 additional human *B. bronchiseptica* isolates have been ribotyped and these were reported as RT14, RT15 and RT18 (Register et al., 1997; Rath et al., 2008).

Based on MLST analysis T44625 is ST 27 which is found in Clonal Complex 1 of an MLST-based *Bordetella* phylogeny (Diavatoupolos et al., 2005). In contrast to nearly all other STs in Complex 1, which are primarily associated with *B. bronchiseptica* isolates from animals, ST 27 isolates are largely associated with human infections. Excluding those of unknown origin, ~71% (10/14) of the remaining ST 27 isolates were cultured from humans, with the remainder isolated from dogs. A total of 6 *B. bronchiseptica* isolates from cats have been typed by MLST (Diavatoupolos et al., 2005) and these represent STs 5, 7 and 23, all also found within Complex 1.

The *Bordetella* ACT is a major virulence factor that possesses both adenylate cyclase and hemolysin activity. All ST 27 isolates evaluated to date lack the gene encoding ACT and have reduced virulence in a mouse model of infection (Buboltz et al., 2008). Southern blotting (Fig. 1B) demonstrated that T44625 similarly lacks the gene for ACT, consistent with its nonhemolytic phenotype on initial isolation.

Using the nomenclature proposed previously (Register, 2001), T44625 has the 1-3a/2-8a pertactin gene repeat region allele. This variant is found in 13 additional *B. bronchiseptica* strains for which the host of origin is known (Diavatoupolos et al., 2005; Boursaux-Eude and Guiso, 2000; Register, 2001, 2004): 6 from humans, 5 from dogs and 2 from pigs. However, in a study comparing the pertactin gene repeat regions of ~100 *B. bronchiseptica* isolates representing a wide variety of hosts, 5 strains obtained from cats in the United States were all found to share the 1-3a/2-8a allele (Register, K.B., manuscript in preparation).

The genetic traits of T44625 and other human and cat isolates, combined with the clinical histories of the patient of origin and the kitten to which she was exposed, strongly suggest the kitten was the source of this patient's *B. bronchiseptica* infection. That T44625 did not appear to induce an acute respiratory exacerbation of CF is perhaps not surprising given the absence of ACT. ACT disables innate immunoprotective functions and is critical for virulence of *B. bronchiseptica* in humans and at least some animal model systems. In fact, a previous report similarly described isolation of ACT-negative *B. bronchiseptica* from a chronically infected patient without clinical evidence of respiratory disease (Gueirard et al., 1995).

Although the genetic traits of T44625 are consistent with a feline origin, we were unable to determine whether the sick kitten in contact with the patient was infected with *B. bronchiseptica*. However, if T44625 was responsible for the respiratory illness observed in the kitten, this would suggest that virulence factors other than ACT are sufficient to cause

clinically apparent disease in cats. *B. bronchiseptica* produces a variety of toxins, adhesins and other known or potential virulence factors but their relative contributions to the pathogenesis of disease in cats are unknown. Alternatively, the kitten's illness may have been due to other agents, perhaps including *B. bronchiseptica*, which is known to predispose to infection with other respiratory pathogens and to exacerbate resulting disease.

There is little definitive epidemiologic data related to transmission of *B. bronchiseptica* between domesticated animals and humans. Dogs and cats are common hosts for the bacterium and are frequently implicated as sources of human infection, but much of the evidence is circumstantial. Information related to potential human-to-human spread is similarly lacking. Acquisition of detailed clinical and epidemiologic data paired with discriminatory genetic comparisons of case isolates and contact isolates is needed to more firmly establish transmission patterns and identify likely contact risks.

Isolation of T44625 was not associated with increased symptoms or decreased pulmonary function in this CF patient. Nonetheless, our findings highlight the importance of considering *B. bronchiseptica* when evaluating samples from CF patients since chronic infection could predispose to viral or additional bacterial infections or otherwise contribute to exacerbation of respiratory disease. It was previously shown in a pig model of infection that a *B. bronchiseptica* mutant with reduced virulence retains the ability to predispose to secondary bacterial infection (Brockmeier and Register, 2007). The relevance of this finding to human infections is unclear but it may be prudent to eradicate *B. bronchiseptica* in asymptotically infected CF patients or other patient populations with underlying health disorders.

A retrospective literature review noted that the frequency in CF patients of *Bordetella* spp, including *B. bronchiseptica*, has not been adequately investigated and is likely to be underestimated (Bos et al., 2011). On the basis of our findings and several other recent reports (Moissenet et al., 2005; Ner et al., 2003; Spilker et al., 2008; Wallet et al., 2002), it seems advisable to counsel CF patients regarding adherence to practices that minimize opportunities for zoonotic transmission of *B. bronchiseptica* from family pets or other potentially infected animals (Hemsworth and Pizer, 2006; Reaser et al., 2008).

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Impacts

- *Bordetella bronchiseptica* infects a variety of companion and farm animals to which humans are frequently exposed, including cats. Although cats have been proposed as a source of zoonotic transmission, corroborating genetic evidence is lacking.
- The genetic traits of a *B. bronchiseptica* isolate cultured from a cystic fibrosis patient, combined with the patient's reported contact history, provide strong evidence for transmission from a household kitten with an acute respiratory illness.
- Veterinarians and physicians should provide appropriate counseling to pet owners and patients to minimize the opportunity for zoonotic transmission of *B. bronchiseptica* to cystic fibrosis patients or individuals with other underlying medical conditions.

Figure 1. Southern blots demonstrating the *PvuII* RT (A) or absence of *cyaA* (B) for *B. bronchiseptica* T44625. A, Lane 1, RT 10 standard; lane 2, isolate T44625; lane 3, RT 4 standard. B, Lane 1, *B. bronchiseptica* strain 253, lane 2, *B. bronchiseptica* strain RB50, lane 3, isolate T44625; relative positions of DNA marker fragments are indicated to the left of the panel.