Comparative Medicine
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Vol 53, No 1
February 2003
Pages 11-20

Overview
Bordetella bronchiseptica Infection of Rats and Mice

David A. Bemis, PhD,[1,*] William R. Shek, DVM, PhD,[2] and Charles B. Clifford, DVM, PhD[2]

***Bordetella bronchiseptica* has long been associated with respiratory tract infections in laboratory research, food-producing, companion, and wildlife animal species. Its range of distribution also may include humans and contaminated inanimate environmental sources. Natural diseases due to *B. bronchiseptica* infections in laboratory rats and mice were described before many of the major pathogens of these hosts were discovered. To our knowledge, there are no recent reports of natural disease due to *B. bronchiseptica* in these species; as a result, some have questioned its role as a natural pathogen in murine hosts. We reviewed occurrence of natural *B. bronchiseptica* infections and present information gained from recent experimental infection studies in murine hosts. We also discuss the potential impact of natural *B. bronchiseptica* infections on research and methods of control.**

Introduction

Laboratory mice and rats have critical roles in biomedical research. Demand for these species has far surpassed that for other research animals. Many valuable breeding lines have been established, and commercial breeders are committed to producing and maintaining animals that are best suited to advance medical science. In most instances, this means healthy, immunocompetent animals but there is also an increasing array of breeding lines that produce specific disease models and immunodeficient animals. Continuous animal health monitoring and testing protocols are used to document the absence of common murine pathogens. The profile of agents used to define animals as specific pathogen free (SPF) varies from one facility to another, depending on use requirements. In primary source facilities, it often includes more than a dozen species each of viruses, parasites, and bacteria, including *Bordetella bronchiseptica*. The rationale for keeping *B. bronchiseptica* on such lists stems mostly from its historical association with laboratory animal facilities (1-3).

Contemporary accounts of naturally acquired *Bordetella* infections in rats and mice are lacking. The National Research Council categorized *B. bronchiseptica* as among agents "not conclusively demonstrated to be natural pathogens of mice or rats" (4). A Japanese selection system classified *B. bronchiseptica* as a non-lethal, opportunistic pathogen that can affect physiologic functions and for which routine monitoring was recommended in rats, but not mice (5, 6). In a recent review of natural pathogens of laboratory mice, rats, and rabbits, *B. bronchiseptica* was noticeably absent from discussions of rats and mice (7). The consequences of identifying a "listed" pathogen in an SPF animal or in its environment, even when no disease is observed, are substantial. Entire colonies are depopulated, revenues are lost, and research is interrupted. Health and breach-of-status risks are clearly different for immunodeficient and axenic animals than for immunocompetent SPF animals; however, the uncertainty surrounding the status of *B. bronchiseptica* as a natural pathogen of laboratory rats and mice has raised questions regarding its control in cesarian-derived, barrier-maintained populations. For example: how does *B. bronchiseptica* differ from other non-listed opportunistic pathogens; how accurately can *B. bronchiseptica* be distinguished from similar non-pathogenic isolates; if immunodeficient animals are infected, are immunocompetent animals in the same facility at increased risk; and will sub-clinical infection or colonization by *B. bronchiseptica* interfere with research uses of these animals? We describe new and historical accounts of *B. bronchiseptica* and its interactions with laboratory rats and mice in the context of prevention and control of *B. bronchiseptica* infection.

Received: 6/07/02. Revision requested: 7/23/02, 9/11/02. Accepted: 10/09/02.
[1]Department of Comparative Medicine, The University of Tennessee, College of Veterinary Medicine, Knoxville, Tennessee 37996-4543, and [2]Pathology and Technical Services, Charles River Laboratories, Wilmington, Massachusetts 01887.
[*]Corresponding author.

Biology of *Bordetella bronchiseptica*

A fundamental understanding of the basic biology of *Bordetella bronchiseptica* is essential to deal with problems that it may present. Several recent reviews on *Bordetella* spp. (8-12) have been published; a summary of important features of *B. bronchiseptica* is provided here.

Bordetella spp. are non-fermentative, gram-negative rods. Currently eight species of *Bordetella* are classified in the family *Alcaligenaceae*, along with closely related genera *Achromobacter* and *Alcaligenes* (13). *Bordetella pertussis*, *B. parapertussis*, *B. bronchiseptica*, and *B. avium* are associated with respiratory tract disease. *Bordetella pertussis* causes whooping cough in humans. Host-specific strains of *B. parapertussis* cause pertussis-like disease in humans and non-progressive pneumonia in lambs. *Bordetella bronchiseptica* is associated with infectious tracheobronchitis (kennel cough) in dogs, atrophic rhinitis in pigs, and respiratory tract disease in several other mammalian species. *Bordetella avium* causes coryza in turkeys and infects several other avian species. *Bordetella hinzii* (14), *B. holmesii* (15), and *B. trematum* (16) have been isolated from opportunistic infections of various tissues in humans. *Bordetella hinzii* is a commensal in the respiratory tract of chickens, whereas *B. holmesii* and *B. trematum* have been isolated only from humans. The newest species, *B. petrii*, is unusual in that, unlike

the other animal-associated, strictly aerobic species, it is facultatively anaerobic and was isolated from the environment (17).

Bordetella pertussis, *B. parapertussis*, and *B. bronchiseptica* share many traits. They represent a single genomospecies, with subspecies differences in host specificity and virulence arising through independent evolution from a *B. bronchiseptica* progenitor (18). *Bordetella avium* and *B. petrii* are genetically more divergent species and represent the closest link with the genus *Achromobacter* (9). Close phylogenetic relationships within the family *Alcaligenaceae* make it difficult, if not impossible, to accurately identify Bordetella, at the genus level, solely on the basis of phenotypic characteristics (19).

Virulence factors

Major virulence factors of *B. bronchiseptica* can be classified in two groups: those that promote colonization, and those that enable the bacterium to escape destruction in the host. Colonization is achieved through attachment by fimbrial and non-fimbrial bacterial adhesions and by the ability to replicate in the restricted environment of the respiratory tract mucosa. There are at least five types of proteinaceous bacterial fimbriae (FIM) (20, 21) produced by *B. bronchiseptica*, and two non-fimbrial, outer membrane protein adhesins, filamentous hemagglutinin (FHA) (22, 23) and pertactin (PRN) (24, 25). Replication in the respiratory tract is aided by preference of *B. bronchiseptica* for readily available amino acids as growth and energy sources (26) and by production of hydroxamate siderophores and binding proteins that mobilize iron from transferrin, lactoferrin, and heme (27-29).

Properties of *B. bronchiseptica* that enable it to escape destruction in the host include: dermonecrotic toxin (DNT), a vasoconstrictive, mitogenic cytotoxin that inhibits osteoblasts and other cells by altering the function of small GTP-binding proteins involved in cell signaling pathways (30); adenylate cyclase toxin (ACT), a bifunctional pore-forming hemolysin and adenylate cyclase toxin that inhibits phagocyte functions (31); tracheal cytotoxin (TCT), a secreted, muramyl dipeptide that stimulates nitric oxide production and interferes with mucociliary function (32); type-III secretion products, undefined products that require contact-dependent secretion to inactivate the transcription factor NF-κB and cause modulating effects on the host immune response (33); lipopolysaccharide (LPS), an endotoxin and O antigen with biological effects similar to those found in other gram-negative bacteria (34); and cellular invasion and intracellular survival, in vitro properties involving epithelial (35, 36), dendritic (37, 38), and phagocytic (39-41) cells that may play a role in persistence of infection.

Bordetella bronchiseptica is principally an extracellular pathogen. Cellular invasion and intracellular survival properties currently being investigated have not been observed in vivo and are not comparable to those of well-known facultative intracellular bacteria like *Mycobacterium* spp. or *Brucella* spp. Survival time has been short, 48 to 72 h in murine macrophage and dendritic cells and one week in human epithelial cells. There is no evidence that *B. bronchiseptica* replicates within cells or spreads from cell to cell. An interesting finding was that *B. bronchiseptica*-containing phagosomes became rapidly acidified to pH 4.5 to 5, and treatment with compounds that increased intracellular pH greatly reduced intracellular survival of *B. bronchiseptica* (42).

Genetic regulation of virulence

Bordetella bronchiseptica virulence traits can change easily at the level of transcription. Plasmids, phage, and transformation in *B. bronchiseptica* have been described (43-45), but horizontal acquisition of new virulence genes does not appear to be common. Regional clustering of some virulence gene sequences suggests that horizontal acquisition as some type of pathogenicity island may have occurred during evolution. Repeated cytosine sequences within the promoters of major fimbrial subunit genes are prone to mutations during replication that inhibit or restore fimbrial gene expression in an "on-off" manner. In addition, silent, promoter-less fimbrial genes can cause changes in fimbrial type by recombinational events that restore promoter function (21). Proline- and leucine-rich repeated sequences in the PRN structural gene sequence likewise are prone to rearrangements that cause extensive polymorphism in this protein (46). Similar to *B. pertussis* (47), *B. bronchiseptica* also contains random insertion sequences that can cause large chromosomal inversions and genomic rearrangements. Use of genomic polymorphisms as fingerprints for epidemiologic studies will be discussed.

The most distinctive feature of *B. bronchiseptica* virulence gene regulation is a two-component sensory transduction system, called BvgAS (formerly called vir) (11). The Bvg operon encodes a membrane spanning, sensor protein and a trans-acting transcriptional regulator that simultaneously activate one set of genes (vir-activated genes—Vag's) and represses another set (vir-repressed genes—Vrg's). Environmental signals, such as mammalian body temperature, physiologic concentrations of sodium and potassium cations, and low concentrations of nicotinic acid and sulfate anions, trigger a complex histidine kinase phosphorelay system that activates the trans-acting regulatory protein, BvgA (8, 12). Many of the major virulence genes of *B. bronchiseptica* and the Bvg operon, itself, are activated by BvgA. When environmental signals are reversed, Vrg's are expressed and Vag's are repressed. The resulting en masse, reversible change of *B. bronchiseptica*'s virulence phenotype is called modulation. The BvgAS locus is also prone to frame shift mutations due to a stretch of cytosine sequences in its promoter region.

Understanding of virulence gene regulation in *B. bronchiseptica* has grown with recent observations suggesting that the BvgAS operon is required for expression of virulence genes that are not expressed in the Bvg^+ (virulent) phase or in the Bvg^- (avirulent) phase; the new phenotype has been called Bvg_i (intermediate) phase, and the regulatory mechanisms that distinguish it from the other two phases are not known (48, 49). A second two-component sensory transduction system has also been located in *B. bronchiseptica* (50).

Strain variation

Antigenic variation in *B. bronchiseptica* has long been recognized for its potential impact on vaccine efficacy and immunologic detection assays (51). Serologic typing systems have not been widely applied (52, 53). Strain diversity has been detected by polymorphisms in specific virulence genes (25, 46, 54, 55) and whole genomic markers (56-59). The possible existence of host-specific markers was suggested in a few studies (54-56, 58) but, in general, it has not been possible to establish a consistent correlation between phenotype or molecular fingerprint of an isolate and its host of origin (57, 59). Pulse field gel electrophoresis (PFGE) macro restriction analysis was useful for rec-

ognizing *B. bronchiseptica* isolates from different housing facilities, but did not distinguish cat from dog isolates (59, 60). The single, unique ribotype profile observed in seal isolates of *B. bronchiseptica* may also be more reflective of temporal and geographic aspects of a disease outbreak than of host adaptation (61). Molecular fingerprinting methods can be useful in disease outbreaks to identify potential sources of infection. It was documented by use of PFGE that a human *B. bronchiseptica* infection was related to contact with infected rabbits (62).

Infection and disease

The most consistent feature of *B. bronchiseptica* infection is prolonged colonization of ciliated airway epithelium (63). Many infected animals do not develop clinical disease. Young animals are most susceptible, and disease often involves co-infection with other agents. Coughing is a common clinical sign of disease; however, signs reflecting neutrophilic inflammation at all levels of the conducting airway epithelium may be seen. Infection with DNT-producing strains of *B. bronchiseptica* may induce transient inhibition of nasal turbinate bone growth, which if accompanied by infection with toxigenic strains of *Pasteurella multocida* in pigs, results in clinical atrophic rhinitis (64, 65). Signs usually resolve in a few days in uncomplicated *B. bronchiseptica* infections. The caudal portion of the respiratory tract of infected animals becomes culture negative within several weeks and is refractory to reinfection for several months (66).

Disease terminology

Fundamental to this review is the two-part question "does *B. bronchiseptica* cause a specific disease in mice or rats, and, if so, what is that disease?" It is well-recognized that Koch's postulates for proving bacterial causation of disease are not uniformly sufficient in all cases (67). Molecular modifications of Koch's postulates have been used to prove the relationship of uncultured agents and particular genes with disease (68), and the contribution of host factors in disease causation has been recognized (69). By any criteria for cause, the disease must first be defined. In this context, the "disease" must be a specific and consistent clinicopathologic entity. Indeed, the relevant definition of disease in *Dorland's Illustrated Medical Dictionary*, 25th ed. is "a definite morbid process having a characteristic train of symptoms." The definition of disease is sometimes expanded to imply any deviation from the normal state of structure and function at the organismal, cellular, and molecular levels, but this may be far removed from clinical disease. Establishing that an organism causes disease is critical to the use of terms to describe the relationship of the organism to the host. "Pathogen," "infection," and "virulence" are each dependent on knowing that an organism has the capacity to cause disease. "Commensal" and "colonization" have been used to suggest that an organism does not cause disease. Unfortunately, there is little consistency in the application of these terms.

Because *B. bronchiseptica* is frequently isolated from the cranial portion of the respiratory tract of healthy dogs (70), it is often referred to as a commensal. If it can cause disease in dogs, its presence should be referred to as causing an infection. In the complete absence of host defenses, there are circumstances in which all commensals can potentially cause disease. For this situation, the term "opportunistic pathogen" has been coined. *Bordetella bronchiseptica* is commonly isolated from animals that do not have clinical signs of disease, but it is not always clear whether its presence represents primary infection, recrudescence of infection, reinfection, or persistence as autochthonous flora.

Bordetella bronchiseptica in rats and mice

Infection and natural disease in rats. In the rat, naturally acquired disease associated with *B. bronchiseptica* was reported in an abstract by Borden and Kulp in 1939 (71), by Beer in 1959 (72), and by Winsser in 1960 (1). Borden and Kulp (71) isolated it from the lungs and heart blood of an unspecified number of rats in the "acute stage of pneumonia." They also claimed that intratracheal instillation of cultures of *B. bronchiseptica* reproduced the gross and histologic lesions of the original disease. However, details were not given, nor was the health status of the donor or recipient rats known with regard to viruses or other bacteria. Beer (72) concluded that *B. bronchiseptica* was not a primary pathogen. Winsser (1) mentioned that he isolated *B. bronchiseptica* from the respiratory tract and bulla of one of "several rats…that had come down with an often fatal pneumonia." Although he believed that *B. bronchiseptica* was responsible for the deaths, he was unable to reproduce the condition, nor did he provide a detailed description of the original lesions. Winsser cultured for pleuropneumonia-like organisms, but it is unknown whether he used media sufficient to support the growth of *Mycoplasma pulmonis*. Serologic testing for viruses was not reported. During that period, antibiotic treatments and vaccination had variable success in treatment of rats with pneumonias attributed to *B. bronchiseptica* (73, 74). Reports since 1960 of naturally acquired disease from *B. bronchiseptica* could not be found.

There also has been little information to confirm the continued presence of *B. bronchiseptica* infection in rats. Two authors reported isolating *B. bronchiseptica* from the respiratory tract of rats, either without lesions or without reporting whether gross or histologic examination was conducted. Switzer, in 1966 (75) reported isolating the organism from wild rats, but did not describe any lesions. Yoda, in 1976 (76) reported finding it in seven of 25 breeding colonies of rats in Japan, with a prevalence of 50% in some infected colonies. Similarly, a few authors have indirectly mentioned the existence of rat isolates without describing their isolation or role in disease (53, 77, 78).

Serologic tests have been used to monitor *B. bronchiseptica* antibodies in rodent colonies. Fujiwara and co-workers (79) found that 39% of rats from user colonies were positive for *B. bronchiseptica* antibodies in a bacterial agglutination test, whereas fewer than 6% of the mice tested from breeder colonies were positive for antibodies against *B. bronchiseptica*. A direct whole-cell ELISA had good sensitivity and specificity in rabbits and guinea pigs when multiple *B. bronchiseptica* strains, including a rat isolate, were used as antigens (77, 78). Using this test, seroconversion to *B. bronchiseptica* antigens was detected in two of 10 strains of euthymic rats after 15 strains of athymic rats from 11 breeding colonies were housed within an experimental facility for an immunologic study (80). Health records from the colonies of origin and sera obtained from the euthymic rats prior to the study gave no indication of *B. bronchiseptica* infection. Two rat strains housed in filter-top cages did not develop antibodies to *Bordetella* antigens. However, infection was not subsequently confirmed by culture; culturing was not re-

ported. Respiratory tract disease or gross or microscopic lesions were not reported in any of the rats. Considered together, these reports provide evidence that rats can serve as a host for *B. bronchiseptica*, but not that it is pathogenic.

Experimental infections and disease in rats. Experimental infection of the rat with *B. bronchiseptica* has been successful in creating lesions of the respiratory tract. Crude suspensions or broth culture dilutions killed young rats within 24 h after intraperitoneal injection (1, 81), but had no effect following subcutaneous injection (81). Similarly high doses of organisms induced bronchopneumonia in 100% of rats inoculated by the intratracheal route (71, 82). The pathologic effects that early investigators observed in rats are difficult to attribute entirely to *B. bronchiseptica* infection since other adventitious agents, especially mycoplasmas and viruses, were not well monitored and the high doses used may have contained overwhelming amounts of endotoxin. Nonetheless, Winsser (1) recognized that, although under normal conditions and after experimental intranasal exposure, rats had high resistance to clinical infection, but they developed a carrier state. Thus, *B. bronchiseptica* was isolated from the lungs of all and middle ears of most rats without clinical signs of disease that were killed two months after experimental intranasal exposure.

Experimental infection with *B. bronchiseptica* in rats has been used to investigate atrophic rhinitis in swine. For example, intranasal inoculation with > 10^5 colony-forming units (CFU) of a swine *B. bronchiseptica* isolate in a 20-µl volume resulted in significant reduction of ventral nasal turbinate length of 21-day-old, SPF Wistar rats (83). Inflammation of the nasal mucosa, consisting of infiltration of the lamina propria and epithelium by polymorphonuclear leukocytes and mononuclear cells and loss of ciliated cells, was present by day three and was greatest between nine and 16 days. By days 16 and 23, atrophy of osseous tissue of the nasal turbinates also was observed.

Rat models also have been used to study molecular aspects of *Bordetella* pathogenesis (23, 84-87). Intranasal instillation of a high concentration (> 10^6 CFU) of a wild-type rabbit *B. bronchiseptica* isolate, in a large volume (50 µl), resulted in reproducible deposition of 10% of the inoculum in the caudal portion of the respiratory tract. Doses of low concentration (100 to 500 CFU) and in low volume (5 µl), resulted in consistent colonization of the trachea within 10 days. Although it was emphasized that tracheal colonization can persist indefinitely in this low-dose, rat model (84), most animals, in similar studies, cleared the organism from the trachea by 60 days (23). At tenfold lower doses, translocation of colonization from the nasal cavity to the trachea did not occur. The median infective dose (ID_{50}) for low-volume (5 µl) intranasal inoculation was < 20 CFU (23). Comparative infections with gene-specific mutants of *B. bronchiseptica* indicated that filamentous hemagglutinin (FHA) was required for establishment of tracheal colonization and that a type-III secretion system and fimbriae were required for persistence of tracheal colonization (23, 84, 88). Direct intratracheal deposition of 10^5 CFU of a virulence factor-lacking, Bvg^- mutant was not sufficient to establish tracheal colonization in young Wistar or Lewis rats (23, 84). However, a *B. bronchiseptica* strain multiply deficient in FHA, FIM, PRN, and ACT persisted in the nasal cavities of rats for at least 60 days (8). An intermediate phase mutant, that expressed its own unique phase proteins, had reduced virulence in the rat intranasal infection model (86).

Infection and natural disease in mice

In mice, the only published report of naturally acquired disease due to *B. bronchiseptica* infection is from Keegan in 1920 (89). Lesions included bronchopneumonia, with marked bronchiectasis, bronchial epithelial hyperplasia with long prominent cilia or epithelial desquamation, and striking peribronchiolar and perivascular lymphocyte infiltration. These lesions are, however, also consistent with mycoplasmosis and/or cilia-associated respiratory bacillus infection. *Bordetella bronchiseptica* was isolated from the lesions, but must now be considered, at most, a secondary invader.

Similar to rats, mice also have been reported to harbor subclinical *B. bronchiseptica* infection, although they appear more resistant than are rats to infection. For example, Griffen, in 1955 (2), stated that *B. bronchiseptica* infection may spread to mice in close proximity to guinea pigs, rats, and other animals in which infection with *B. bronchiseptica* occurs, "even though mice are relatively insusceptible to the natural infection." A Japanese report in 1976 did not indicate detection of *B. bronchiseptica* among 1,031 individual mice sampled from 57 breeding colonies (76). Anecdotal results suggest that *B. bronchiseptica* is occasionally isolated from mice. A mouse *B. bronchiseptica* isolate was indirectly mentioned as the source of an antigen used in a recent experimental study (78). A message posted in 1994 on an electronic discussion list reported spontaneous *B. bronchiseptica* pneumonia in SCID and BALB/c nude mice and isolation of the organism from the trachea of healthy euthymic mice at a commercial production facility (90). It appears, on balance and from lack of more recent reports, that the prevalence of *B. bronchiseptica* is low in laboratory mouse colonies. However, formal documentation of the current prevalence of *B. bronchiseptica* and its role in natural disease in laboratory mice is insufficient.

Experimental infection and induced disease in mice

Mice have been used extensively for experimental *B. bronchiseptica* infections. Routes of inoculation have included intravenous (91-93), intraperitoneal (1, 20, 39, 81, 94-104), intracerebral (94, 96, 105, 106), subcutaneous (1, 93), intranasal (1, 31, 72, 96, 107-117), intratracheal (118), intrapleural (119), and aerosol exposure (120-122). The results of parenteral inoculations in mice were similar to those seen in rats. Lethality was probably due to toxin overload in the heavy inocula which, if diluted would not initiate disease. Winsser (1) noted that only undiluted cultures caused sickness and death after subcutaneous injection in mice, and he believed that *B. bronchiseptica* was unlikely to cause true septicemia. Mouse infection models created by parenteral administration were used extensively in early protection studies to evaluate *B. bronchiseptica* vaccines. Opsonophagocytic serum antibodies and perhaps other circulating immunologic effectors induce demonstrable beneficial effects in these models (20, 91, 99). However, they bear little resemblance to natural *B. bronchiseptica* infections.

Intranasal- and aerosol-induced infection with undiluted cultures or high doses (> 10^6 CFU) of *B. bronchiseptica* caused lethal bronchopneumonia in mice (1, 96, 107, 122). Bacteria also were occasionally recovered from blood, spleen, or liver (1). Lower doses (< 7×10^5 CFU) of *B. bronchiseptica*, given by the same routes, did not cause clinical illnesses (31, 109-111, 113, 114, 120). Bacteria colonized the nasal cavity, and then spread to the

trachea and lungs following low-dose intranasal infection. Infection was cleared from the lungs by 50 days, but persisted in the nasal cavity for > 270 days; the ID_{50} was < 20 CFU (109). Older mice were slightly more resistant (1). Young (2 to 14 days old) mice given sublethal doses of B. *bronchiseptica* by aerosol or intranasal routes developed inflammation of nasal mucosa, and nasal turbinate atrophy (112, 116, 121-123).

At least eight strains of immunocompetent mice, including NIH-3, CFLP, BALB/C, ddN, MFL, ICR, C57Bl/6, and CFW, and 11 wild-type strains of *B. bronchiseptica* were used in the studies described in the preceding paragraph to produce experimental intranasal- or aerosol-induced infections. The results cannot be compared directly, but the general properties of infection (e.g., mortality, time to death, bacterial clearance) were remarkably consistent.

Differences in virulence of *B. bronchiseptica* isolates in mouse respiratory tract infection models also have been observed (1, 92, 97, 112, 118). For example, investigators have recently used mouse intranasal *B. bronchiseptica* infection models to compare the virulence of isogenic mutants (107, 109-111, 113, 114, 124-126). Full virulence of *B. bronchiseptica* in experimental infections of immunocompetent mice requires the expression of DNT (124), ACT (110), lipopolysaccharide (LPS) (111, 126), and a type-III secretion system (114), as well as regulatory genes, *Bvg*AS (109, 114) and *ris* (45).

Immunodeficient mice with combined T- and B-cell deficiencies (SCID, SCID-beige, RAG-1-/-) succumbed within 40 to 70 days to low intranasal doses (500 CFU) of wild-type *B. bronchiseptica* (109-111, 114). *Bordetella bronchiseptica* was consistently isolated from the liver, spleen, heart, and blood of moribund mice following infection at a higher dose (5×10^5 CFU) (109). Mice rendered neutropenic, either as a result of a genetic knockout ($G\text{-}CSF^{-/-}$) or cyclophosphamide treatment, succumbed in one to four days to intranasal inoculation with 5×10^3 CFU of *B. bronchiseptica*.

Mouse models have also been used extensively to study *B. pertussis* infection. However, *B. pertussis* does not attach to tracheal epithelium or colonize the respiratory tract as well as does *B. bronchiseptica* (109, 127). *Bordetella pertussis* did not kill, but remained in the respiratory tract of immunodeficient mice for 200 days, much the same as did an ACT-negative *B. bronchiseptica* mutant (110). In contrast, type-III secretion mutants of *B. bronchiseptica* had enhanced lethality for SCID/beige mice, suggesting that type-III secretion products may suppress inflammation (114).

Pathologic features of experimental infection in rats and mice

Reports describing the gross and microscopic pathologic lesions associated with experimental infections in rats and mice have been few. Non-lethal infections in immunocompetent rats and mice are characterized by infiltration of neutrophils and, to a lesser extent, mononuclear cells in the mucosa of the respiratory tract. Severity has varied depending on dose and virulence of the infecting *B. bronchiseptica* strain. In more severe cases, intraepithelial microabscesses and intraluminal accumulations of purulent exudates have been accompanied by loss of cilia and epithelial hyperplasia (112, 117, 124). In young animals, dorsal and/or ventral nasal turbinates were shortened and the bone trabeculae of the osseous cores were rarified, with increased presence of osteoclasts (83, 117). Inoculation procedures that deposited virulent strains in the lungs resulted in extensive perivascular and peribronchiolar inflammation, with large numbers of infiltrating cells, principally neutrophils, throughout the lungs, with some areas of consolidation and necrosis by day 3 (109, 110). Apoptosis of infiltrating inflammatory cells also was observed (114). Minor inflammation with little tissue damage occurred when trachea and lungs were exposed to *B. bronchiseptica* strains defective in one or more virulence factors (109-111). The histopathological response following acute *B. bronchiseptica* exposure diminishes as bacterial numbers decrease. Since organisms are usually cleared from the lungs and trachea, a lingering inflammatory response in the caudal portion of the respiratory tract would not be expected. To the authors' knowledge, the histopathological response to subclinical persistence in the nasal cavity has not been systematically examined nor have lesions in natural infection been reported.

Detection of *B. bronchiseptica*

Detection of *B. bronchiseptica* infection in rats and mice has relied principally on bacterial culture of swab, aspirate, or wash specimens from the nasal cavity, tracheobronchial tree, or lungs that have been exposed by dissection or removed at necropsy. *Bordetella bronchiseptica* grows readily on simple nutrient media, such as *Brucella* agar, trypticase soy agar, or tryptose phosphate, with or without 5% defibrinated sheep blood. Bordet-Gengou agar with 15% horse or sheep blood is often used to distinguish different colony morphology. Selective-differential media, such as MacConkey-glucose agar or Smith-Baskerville agar, each containing the antibiotic furaltadone, and tergitol 7 agar containing tetrazolium chloride have been used for isolation from contaminated specimens (128). It may be difficult to identify *B. bronchiseptica* by use of traditional biochemical tests because of its similarity to other *Bordetella*-like environmental organisms. The latter are not well represented in most routine bacterial identification databases, and may require reference level testing methods. Accurate identification would be especially critical when disease is absent or when the number recovered is low and when culturing from environmental sources. Sensitivity of the culture method to detect infected rats and mice is probably low in animals without clinical signs of disease. The expected low prevalence of *B. bronchiseptica* in an infected colony would require that a large sample size be tested in a health-monitoring program to ensure, with confidence, that the colony is test negative.

Serologic tests have been used in experimental surveys to detect evidence of *B. bronchiseptica* infection (76-79), but have not been widely applied in routine animal health-monitoring programs. These tests are of value for determining the overall status of a colony, but are not as useful for diagnosis in individual animals. The antigens used in agglutination test and ELISAs were whole bacterial cells; it is likely that greater specificity and sensitivity will be achieved when purified antigens are used.

Genetic detection methods, using RNA/DNA and DNA/DNA hybridization probes, were used to identify *B. bronchiseptica*-specific rRNA and siderophore gene sequences, respectively, in cultures and nasal swab specimens from pigs (129, 130). Polymerase chain reaction (PCR) assays coupled with restriction fragment pattern analysis or specific probe detection steps have been developed for detecting *Bordetella* species in samples from humans (131-133). Targeted gene sequences have included a novel outer membrane porin gene, promoter regions of the

flagellin and pertussis toxin genes, and unique insertion sequences. To our knowledge, application of PCR and gene probe technologies for *B. bronchiseptica* surveillance in laboratory animals has not been reported.

Epidemiology of *B. bronchiseptica* infection

Transmission of *B. bronchiseptica* is thought to occur principally by direct contact or droplet aerosol from infected animals. Little is known about the carrier state. It has been stated that experimentally infected rats may become life-long carriers (8, 84). With low sensitivity of detection, infection rates within a colony could theoretically, become high before being detected. It is not known what proportion of animals become carriers, what exposure level is required to establish a carrier state, what the host response is to persistent infection, or what bacterial properties are required to cause persistent infections.

It is thought that the respiratory tracts of infected mammalian hosts are the primary sources for new infections. Whether murine infections are acquired mostly from non-murine hosts has yet to be determined. However, the potential for interspecies transmission is great. *Bordetella bronchiseptica* has the widest host distribution among the bordetellae (8, 134). Dogs, pigs, and guinea pigs are most frequently infected, but *B. bronchiseptica* is also commonly isolated from cats, horses, and rabbits. Rare isolation has been reported from opossums, raccoons, ferrets, skunks, koalas, lesser bushbabies, European hedgehogs, bears, foxes, seals, sea otters, Dall sheep, llamas, leopards, chickens, ostriches, and humans. Risk for transmission from and to humans is low. In an extensive review, Woolfrey and Moody (135) concluded that "… *B. bronchiseptica* has rarely been isolated from humans despite the considerable exposure of humans to animal sources of the organism."

Indirect transmission among animals (i.e., animal to animal, animal to human, and human to animal) also is thought to be possible, but has not been well documented (134). *Bordetella bronchiseptica* can survive on inanimate materials, such as bedding, litter, and in water, for short periods, and may potentially do the same on animal surfaces, such as hair and skin. Although there is no evidence that humans have introduced *B. bronchiseptica* into barrier rodent facilities, it seems prudent to advise animal handlers of potential sources of *B. bronchiseptica* and mandate that they be avoided. Likewise, maintenance of barriers against potentially contaminated air, water, and pests is important.

Elimination of *B. bronchiseptica* from a rodent colony is only ensured by elimination of infected animals and disinfection of all materials, equipment, and structural surfaces within the facility. At present, it is not feasible to identify all infected animals due to low prevalence of carriers in the population, low level of shedding in carriers, and inherent low sensitivity of the testing methods. Consequently, complete elimination and repopulation is favored over the test-and-cull approach. Preliminary attempts to eliminate carriers by vaccination have not been successful in large colonies of guinea pigs, and are unlikely to be successful in rats or mice (136). Although antibiotic treatment may seem an attractive alternative for small groups of animals, successful treatment regimens have not been reported. Assessing the outcome of treatment or preventative measures would also be difficult due to the aforementioned issues. Persistence of infection in a single animal could result in reinfection of the colony, and logistics of removing or disinfecting contaminated materials could be difficult.

The potential existence of natural *B. bronchiseptica* reservoirs other than infected animals has been questioned. The need for complex, environmentally controlled regulatory systems, like BvgAS, for survival of *B. bronchiseptica* is not apparent. Such loci may be vestiges of evolution from an environmental organism that existed prior to adaptation to animal infection. Alternatively, the regulatory systems may play a role in environmental survival and inter-host transmission. Flagella, which could provide an environmental survival advantage, are expressed in the Bvg^- avirulent phase (93, 137). *Bordetella bronchiseptica* can grow for 24 weeks from a small inoculum in natural fresh and salt water, with little loss in viability (138, 139). *Bordetella bronchiseptica* has been isolated from marine mammals (60, 61) but not from natural water sources.

Effects on research

Greatest emphasis should be placed on monitoring subclinically infected, immunocompetent animals to determine what effects infection may have on research and spread to other animals. One might expect that, during the height of acute infection, pathophysiologic effects of the bacterium could have adverse effects on research unrelated to *B. bronchiseptica*. In one such instance, unexpected inhibition of rabbit alveolar macrophage function led to the incidental discovery of *B. bronchiseptica* ACT (140, 141). These side effects were only observed when large numbers of *B. bronchiseptica* were present in lavage effluents and were reversible after prolonged recovery (140). Rats used by the same investigators had normal macrophage functions. There are no similar reports on the impact of natural *B. bronchiseptica* infections in rats and mice on research and it is not known whether any adverse effects would linger after infection subsides or in the carrier state. In 1913, Theobald Smith (3) stated, "The whole problem of bacteria carriers is becoming of such overshadowing practical importance that an accurate determination of the fate of strains in a community to which no new strains are admitted from outside is urgently demanded." This challenge is still relevant. Perhaps investigators should be surveyed systematically to help document the impact of *B. bronchiseptica* carriers on research.

It is expected that immunodeficient animals are more susceptible to *B. bronchiseptica* than are immunocompetent animals, but there is no published statistical information on isolates recovered from immunodeficient animals to indicate that they are more susceptible to *B. bronchiseptica* than to other less-regarded pathogens. Burgeoning numbers of genetically modified rodents, which may have intended or unintended decrements in host defense mechanisms, represent another population where bacterial infection may have different consequences than those in fully immunocompetent hosts. However, as with mutant immunodeficient mice and rats, genetically modified rodents are frequently and appropriately housed in systems designed to exclude common environmental and anthropozoonotic agents, such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Pneumocystis carinii*. These housing systems, often individually ventilated cages, static filter-top cages or isolators, are also sufficient to exclude *B. bronchiseptica*. For whatever reason (successful exclusion, low prevalence of the bacterium, or low pathogenicity), *B. bronchiseptica* does not seem to be a substantial problem in ge-

netically modified or immunodeficient mice and rats. For example, one major rodent diagnostic laboratory with an international clientele has never isolated *B. bronchiseptica* from immunodeficient or genetically modified mice or rats, despite processing hundreds of these animals each week, using appropriate media and techniques. In contrast, *Pasteurella pneumotropica*, *Pneumocystis carinii*, *Helicobacter hepaticus*, and *Staphylococcus aureus* are often isolated from lesions in immunodeficient and genetically modified mice in this sample stream (142), supporting the conclusion that *B. bronchiseptica* poses less contemporary risk than do these common opportunistic infections.

Approaches taken to control or prevent *B. bronchiseptica* infection in laboratory animal facilities must be realistic and cost effective. Minor improvements in hygiene, barrier, and health-monitoring practices can be helpful; however, the overall risks that *B. bronchiseptica* presents must be evaluated for each facility and use. At present, in taking action, one should consider the lack of convincing evidence of natural disease in rats or mice, although both species are susceptible to persistent colonization.

Conclusions

Reports of naturally acquired disease attributable to *B. bronchiseptica* in mice or rats have been few since 1960. It also is unclear if mice and rats are natural hosts for *B. bronchiseptica*. The apparent low prevalence of *B. bronchiseptica* in laboratory rats and mice may reflect current hygiene, barrier, and health-monitoring practices; however, diseases previously attributed to *B. bronchiseptica* may also have been due to more recently discovered pathogens. Therefore, more information is needed to determine the true contemporary risk for natural infection. Isolation frequencies should be recorded (even if low), isolates should be compared (within and between labs) by use of molecular fingerprinting and virulence testing methods, and should an outbreak occur, it should be thoroughly investigated to determine the extent of spread, pathologic changes, and presence of other agents. The role of non-murine hosts, especially humans, as sources of the organism in animal facilities, also should be examined further.

Acknowledgments

In accordance with AALAS policy, all animal activities conducted at the authors' institutions have been approved by their respective institutional animal care and use committees. We thank William J. White and Anne T. Morin for their advice and input in the preparation of the manuscript.

References

1. **Winsser, J.** 1960. A study of *Bordetella bronchiseptica*. Proc. Anim. Care Panel **10:**87-104.
2. **Griffen, C. A.** 1955. Bacterial diseases of some common laboratory animals. Proc. Anim. Care Panel **6:**92-112.
3. **Smith, T.** 1913. Some bacteriological and environmental factors in the pneumonias of lower animals with special reference to the guinea pig. J. Med. Res. **24:**291-324.
4. **National Research Council.** 1991. Infectious diseases of mice and rats, p. 35. National Academy Press, Washington, D.C.
5. **Waggie, K., N. Kagiyama, A.M. Allen, and T. Nomura (ed.).** 1994. Manual of microbiologic monitoring of laboratory animals, 2nd ed. USDHHS, PHS, NIH, NCRR, NIH Publication No. 94-2498, Washington, D.C.
6. **Takimoto, K., Y. K. Yamada, A. Yasushi, Y. Suzaki, Y. Mikiko, and A. Toshihiko.** 1999. Experiences of microbial contamination of animal colonies maintained in the national institute of infectious diseases, Japan (NIID). Jpn. J. Infect. Dis. **52:**255-256.
7. **Baker, D. G.** 1998. Natural pathogens of laboratory mice, rats and rabbits and their effects on research. Clin. Microbiol. Rev. **11:**231-266.
8. **Cotter, P. A. and J. F. Miller.** 2001. *Bordetella*, p. 621-671. *In* E. A. Groisman (ed.), Principles of bacterial pathogenesis. Academic Press, San Diego, Calif.
9. **von Wintzingerode, F., G. Gerlach, B. Schneider, and R. Gross.** 2002. Phylogenetic relationships and virulence evolution in the Genus *Bordetella*. Curr. Topics Microbiol. Immunol. **264:**177-199.
10. **Mattoo, S., A. K. Foreman-Wykert, P. A. Cotter, and J. F. Miller.** 2001. Mechanisms of *Bordetella* pathogenesis. Front. Biosci. **6:**E168-E186.
11. **Bock, A. and R. Gross.** 2001. The BvgAS two-component system of *Bordetella* spp.: a versatile modulator of virulence gene expression. Int. J. Med. Microbiol. **291:**119-130.
12. **Coote, J. G.** 2001. Environmental sensing mechanisms in *Bordetella*. Adv. Microb. Physiol. **44:**141-181.
13. **DeLey, J., P. Segers, K. Kersters, W. Mannheim, and A. Lievens.** 1986. Intra- and intergeneric similarities of the *Bordetella* ribosomal ribonucleic acid cistrons: proposal for a new family, *Alcaligenaceae*. Int. J. Syst. Bacteriol. **36:**405-414.
14. **Vandamme, P., J. Hommez, M. Vancanneyt, M. Monsieurs, B. Hoste, B. Cookson, C. H. Wirsing Von Konig, K. Kersters, and P. J. Blackall.** 1995. *Bordetella hinzii sp. nov.*, isolated from poultry and humans. Int. J. Syst. Bacteriol. **45:**37-45.
15. **Weyant, R. S., D. G. Hollis, R. E. Weaver, M. F. M. Amin, A. G. Steigerwalt, S. P. O'Connor, A. M. Whitney, M. I. Daneshvar, C. W. Moss, and D. J. Brenner.** 1995. *Bordetella holmesii sp. nov.*, a new Gram-negative species associated with septicemia. J. Clin. Microbiol. **33:**1-7.
16. **Vandamme, P., M. Heyndrickx, M. Vancanneyt, B. Hoste, P. DeVos, E. Falsen, K. Kersters, and K-H. Hintz.** 1996. *Bordetella trematum sp. nov.*, isolated from wounds and ear infections in humans and reassessment of *Alcaligenes denitrificans* (Ruger and Tan 1983). Int. J. Syst. Bacteriol. **46:**849-858.
17. **Von Wintzingerode, F., A. Schattke, R. A. Siddiqui, U. Rosick, U. B. Gobel, and R. Gross.** 2001. *Bordetella petrii sp. nov.*, isolated from an anaerobic bioreactor and emended description of the genus *Bordetella*. Int. J. Syst. Evol. Microbiol. **51:**1257-1265.
18. **van der Zee, A., F. Mooi, J. van Embden, and J. Musser.** 1997. Molecular evolution and host adaptation of *Bordetella spp.*: phylogenetic analysis using multilocus enzyme electrophoresis and typing with three insertion sequences. J. Bacteriol. **179:**6609-6617.
19. **Yabuuchi, E., Y. Kawamura, Y. Kosako, and T. Ezaki.** 1998. Emendation of genus *Achromobacter* and *Achromobacter xylosoxidans* (Yabuuchi and Yano) and proposal of *Achromobacter ruhlandii* (Packer and Vishniac) *comb. nov.*, *Achromobacter piechaudii* (Kiredjian et al.) *comb. nov.*, and *Achromobacter xylosoxidans subsp. denitrificans* (Ruger and Tan) *comb. nov*. Microbiol. Immunol. **42:**429-438.
20. **Lee, S., A. Way, and E. Osen.** 1986. Purification and subunit heterogeneity of pili of *Bordetella bronchiseptica*. Infect. Immun. **51:**586-593.
21. **Savelkoul, P. H., D. P. de Kerf, R. J. Willems, F. R. Mooi, B. A. van der Zeijst, and W. Gaastra.** 1996. Characterization of the fim 2 and fim 3 fimbrial subunit genes of *Bordetella bronchiseptica*: roles of Fim 2 and Fim 3 fimbriae and flagella in adhesion. Infect. Immun. **64:**5098-5105.
22. **Jacob-Dubuisson, F. J., B. Kehoe, E. Willery, N. Reveneau, C. Locht, and D. Relman.** 2000. Molecular characterization of *Bordetella bronchiseptica* filamentous hemagglutinin and its secretion machinery. Microbiology **146:**1211-1221.
23. **Cotter, P. A., M. H. Yuk, S. Mattoo, B. J. Akerley, J. Boschwitz, D. A. Relman, and J. F. Miller.** 1998. Filamentous hemagglutinin of *Bordetella bronchiseptica* is required for efficient establishment of tracheal colonization. Infect. Immun. **66:**5921-5929.

24. **Kobisch, M. and P. Novotny.** 1990. Identification of a 68 kilodalton outer membrane protein as the major protective antigen of *Bordetella bronchiseptica* by using specific pathogen free piglets. Infect. Immun. **58:**352-357.
25. **Register, K. B.** 2001. Novel genetic and phenotypic heterogeneity in *Bordetella bronchiseptica* pertactin. Infect. Immun. **69:**1917-1921.
26. **Plotkin, B. J. and D. A. Bemis.** 1998. Carbon source utilization by *Bordetella bronchiseptica*. J. Med. Microbiol. **47:**761-765.
27. **Menozzi, F. D., C. Gantiez, and C. Locht.** 1991. Identification and purification of transferring- and lactoferrin-binding proteins of *Bordetella pertussis* and *Bordetella bronchiseptica*. Infect. Immun. **59:**3982-3988.
28. **Giardina, P. C., L. A. Foster, J. M. Musser, B. J. Akerley, J. F. Miller, and D. W. Dyer.** 1995. Bvg repression of alcaligin synthesis in *Bordetella bronchiseptica* is associated with phylogenic lineage. J. Bacteriol. **177:**6058-6063.
29. **Pradel E., N. Guiso, F. D. Menozzi, and C. Locht.** 2000. *Bordetella pertussis* TonB, a Bvg- independent virulence determinant. Infect. Immun. **68:**1919-1927.
30. **Horiguchi, Y., N. Inoue, M. Masuda, T. Kashimoto, J. Katahira, N. Sugimoto, and M. Matsuda.** 1997. *Bordetella bronchiseptica* dermonecrotizing toxin induces reorganization of actin stress fibers through deamidation of Gln 63 of the GTP-binding protein Rho. Proc. Nat. Acad. Sci. USA **14:**11623-11626.
31. **Gueirard, P. and N. Guiso.** 1993. Virulence of *Bordetella bronchiseptica*: role of adenylate cyclase-hemolysin. Infect. Immun. **61:**4072-4078.
32. **Flak, T. A. and W. E. Goldman.** 1996. Autotoxicity of nitric oxide in airway disease. Am. J. Respir. Crit. Care Med. **154:**S202-S206.
33. **Yuk, M. H., E. T. Harvill, P. C. Cotter, and J. F. Miller.** 2000. Modulation of host immune responses, induction of apoptosis and inhibition of NF-KB activation of the *Bordetella* type III secretion system. Mol. Microbiol. **35:**991-1004.
34. **Di Fabio, J. L., M. Caroff, D. Karibian, J. C. Richards, and M. B. Perry.** 1992. Characterization of the common antigenic lipopolysaccharide O-chains produced by *Bordetella bronchiseptica* and *Bordetella parapertussis*. FEMS Microbiol Lett. **76:**275-281.
35. **Savelkoul, P. H. M., B. Kremer, J. G. Kusters, B. A. M. van der Zeijst and W. Gaastra.** 1993. Invasion of HeLa cells by *Bordetella bronchiseptica*. Microb. Pathogen. **14:**161-168.
36. **Schipper, H., G. F. Krohne, and R. Gross.** 1994. Epithelial cell invasion and survival of *Bordetella bronchiseptica*. Infect. Immun. **62:**3008-3011.
37. **Guzman, C. A., M. Rohde, M. Bock, and K. N. Timmis.** 1994. Invasion and intracellular survival of *Bordetella bronchiseptica* in mouse dendritic cells. Infect. Immun. **62:**5528-5537.
38. **Guzman, C. A., M. Rohde, and K. N. Timmis.** 1994. Mechanisms involved in uptake of *Bordetella bronchiseptica* by mouse dendritic cells. Infect. Immun. **62:**5538-5544.
39. **Forde, C. B., R. Parton, and J. G. Coote.** 1998. Bioluminescence as a reporter of intracellular survival of *Bordetella bronchiseptica* in murine phagocytes. Infect. Immun. **66:**3198-3207.
40. **Forde, C. B., X. Shi, J. Li, and M. Roberts.** 1999. *Bordetella bronchiseptica*-mediated cytotoxicity to macrophages is dependent on bvg-regulated factors, including pertactin. Infect. Immun. **67:**5972-5978.
41. **Banemann, A. and R. Gross.** 1997. Phase variation affects long-term survival of *Bordetella bronchiseptica* in professional phagocytes. Infect. Immun. **65:**3469-3473.
42. **Schneider, B., R. Gross, and A. Hass.** 2000. Phagosome acidification has opposite effects on intracellular survival of *Bordetella pertussis* and *B. bronchiseptica*. Infect. Immun. **68:**7039-7048.
43. **Shimizu, M., K. Kuninori, M. Inoue, and S. Mitsuhashi.** 1981. Drug resistance and R plasmids in *Bordetella* isolates from pigs. Microbiol. Immunol. **25:**773-786.
44. **Zealey, G., M. Dion, S. Loosmore, R. Yacoob, and M. Klein.** 1988. High frequency transformation of *Bordetella* by electroporation. FEMS Microbiol. Lett. **56:**123-126.
45. **Rauch, H. C. and M. J. Pickett.** 1961. *Bordetella bronchiseptica* bacteriophage. Can. J. Microbiol. **7:**125-133.
46. **Boursaux-Eude, C. and N. Guiso.** 2000. Polymorphism of repeated regions of pertactin in *Bordetella pertussis*, *Bordetella parapertussis* and *Bordetella bronchiseptica*. **68:**7214.
47. **Stibitz, S. and M-S. Yang.** 1999. Genomic plasticity in natural populations of *Bordetella pertussis*. J. Bacteriol. **181:**5512-5515.
48. **Register, K. B. and M. R. Ackermann.** 1997. A highly adherent phenotype associated with virulent Bvg^+-phase swine isolates of *Bordetella bronchiseptica* grown under modulating conditions. Infect. Immun. **65:**5295-5300.
49. **Cotter, P. A. and J. F. Miller.** 1997. A mutation in the *Bordetella bronchiseptica bvg*S gene results in reduced virulence and increased resistance to starvation, and identifies a new class of Bvg-regulated antigens. Mol. Microbiol. **24:**671-685.
50. **Jugnitz, H., N. P. West, M. J. Walker, G. S. Chhatwal, and C. A. Guzman.** 1998. A second two-component regulatory system of *Bordetella bronchiseptica* required for bacterial resistance to oxidative stress, production of acid phosphatase and in vivo persistence. Infect. Immun. **66:**4640-4650.
51. **Nakase, Y.** 1957. Studies on *Hemophilus bronchisepticus* I. The antigenic structures of *H. bronchisepticus* from guinea pig. Kitasato Arch. Exp. Med. **30:**57-72.
52. **Eldering, G., C. Hornbeck, and J. Baker.** 1957. Serological study of *Bordetella pertussis* and related species. J. Bacteriol. **74:**133-136.
53. **Pedersen, K. B.** 1975. The serology of *Bordetella bronchiseptica* isolated from pigs compared with strains from other animal species. Acta. Path. Microbiol. **5:**471-480.
54. **Burns, E. H., Jr., J. M. Norman, M. D. Hatcher, and D. A. Bemis.** 1993. Fimbriae and determination of host species specificity by *Bordetella bronchiseptica*. J. Clin. Microbiol. **31:**1838-1844.
55. **Shina, A., C. A. Hart, M. D. Stenton, S. Dawson, C. M. McCracken, S. H. Binns, R. M. Gaskell, and C. Winstanley.** 2002. Distribution of fim 3 and fla A TTGE sequence types amongst isolates of *Bordetella bronchiseptica* from different host animals. J. Med. Microbiol. **51:**557-563.
56. **Register, K. B., A. Boisvert, and M. R. Ackerman.** 1997. Use of ribotyping to distinguish *Bordetella bronchiseptica* isolates. Int. J. Syst. Bacteriol. **47:**678-683.
57. **Sacco, R. E., K. B. Register, and G. E. Nordholm.** 2000. Restriction endonuclease analysis discriminates *Bordetella bronchiseptica* isolates. J. Clin. Microbiol. **38:**4387-4393.
58. **Musser, J. M., D. A. Bemis, H. Ishikawa, and R. K. Selander.** 1987. Clonal diversity and host distribution in *Bordetella bronchiseptica*. J. Bacteriol. **169:**2793-2803.
59. **Binns, S. H., A. J. Speakman, S. Dawson, M. Bennett, R. M. Gaskell, and C.A. Hart.** 1998. The use of pulsed-field gel electrophoresis to examine the epidemiology of *Bordetella bronchiseptica* isolated from cats and other species. Epidemiol. Infect. **120:**201-208.
60. **Foley, J. E., C. Rand, M. J. Bannasch, C. R. Norris, and J. Milan.** 2002. Molecular epidemiology of feline bordetellosis in two animal shelters in California, USA. Prev. Vet. Med. **54:**141-156.
61. **Register, K. B., R. E. Sacco, and G. Foster.** 2000. Ribotyping and restriction endonuclease analysis reveal a novel clone of *Bordetella bronchiseptica* in seals. J. Vet. Diagn. Invest. **12:**535-540.
62. **Gueirard, P., C. Weber, A. LeCoustumier, and N. Guiso.** 1995. Human *Bordetella bronchiseptica* infection related to contact with infected animals: persistence of bacteria in host. J. Clin. Microbiol. **33:**2002-2006.
63. **Bemis, D. A. and J. R. Kennedy.** 1981. An improved system for studying the effect of *Bordetella bronchiseptica* on the ciliary activity of canine tracheal epithelial cells. J. Infect. Dis. **144:**349-357.
64. **Chanter, N., T. Magyar, and M. Rutter.** 1989. Interactions between *Bordetella bronchiseptica* and toxigenic *Pasteurella multocida* in atrophic rhinitis of pigs. Res. Vet. Sci. **47:**48-53.
65. **Brockmeier, S. L., K. B. Register, T. Magyar, A. J. Lax, G. D. Pullinger, and R. A. Kunkle.** 2002. Role of the dermonecrotic toxin of *Bordetella bronchiseptica* in the pathogenesis of respiratory disease in swine. Infect. Immun. **70:**481-490.

66. **Bemis, D. A., H. A. Greisen, and M. J. G. Appel.** 1977. Pathogenesis of canine bordetellosis. J. Infect. Dis. **135:**753-762.
67. **Evans, A. S.** 1976. Causation and disease: the Henle-Koch postulates revisited. Yale J. Biol. Sci. **49:**175-195.
68. **Falkow, S.** 1988. Molecular Koch's postulates applied to microbial pathogenicity. Rev. Infect. Dis. **10:**S274-S276.
69. **Casadevall, A. and L-.A. Pirofski.** 1999. Host-pathogen interactions: redefining the basic concepts of virulence and pathogenicity. Infect. Immun. **67:**3703-3713.
70. **Greene, C. E.** 1998. Respiratory infections, p. 582-594. *In* C. E. Greene (ed.), Infectious diseases of the dog and cat. W. B. Saunders Co., Philadelphia.
71. **Borden, K. and W. L. Kulp.** 1939. A study of rat pneumonia. J. Bacteriol. **37:**351-352.
72. **Beer, J.** 1960. Uber das vorkommen und uber die rolle von *Bordetella (B.) bronchiseptica* bei lungenerkrankungen einiger haus-und laboratoriumstiere. Zentralbl. Bakteriol. Parasitenk. **177:**208-214.
73. **Rosen, S., H. S. Hunt, and M. A. Bernarde.** 1954. Oxytetracycline (Terramycin) as a therapeutic agent for pneumonia caused by *Brucella bronchiseptica* in rats. J. Am. Vet. Med. Assoc. **124:**300.
74. **Wickert, W. A., S. Rosen, H. A. Dawson, and H. R. Hunt.** 1958. *Brucella bronchiseptica* vaccine for rats. J. Am. Vet. Med. Assoc. **133:**363-364.
75. **Switzer, W. P., C. J. Mare, and E. O. Hubbard.** 1966. Incidence of *Bordetella bronchiseptica* in wildlife and man in Iowa. Am. J. Vet. Res. **27:**1134-1136.
76. **Yoda, H., K. Nakayama, T. Yusa, S. Sato, S. Fukuda, K. Matsumoto, and M. Nakagawa.** 1976. Bacteriological survey on *Bordetella bronchiseptica* in various animal species. Exp. Anim. **25:**7-11.
77. **Boot, R., R. H. G. Bakker, H. Thuis, and J. L. Veenema.** 1993. An enzyme-linked immunosorbent assay (ELISA) for monitoring guinea pigs and rabbits for *Bordetella bronchiseptica* antibodies. Lab. Animals **27:**342-349.
78. **Wullenweber, M. and R. Boot.** 1994. Interlaboratory comparison of enzyme-linked immunosorbent assay (ELISA) and indirect immunofluorescence (IIF) for detection of *Bordetella bronchiseptica* antibodies in guinea pigs. Lab Anim. **28:**335-339.
79. **Fujiwara, K., Y. Tanishima, and M. Tanaka.** 1979. Seromonitoring of laboratory mouse and rat colonies for common murine pathogens. Exp. Anim. **28:**297-306.
80. **Boot, R., H. van-Herck, and J. van-der-Logt.** 1996. Mutual viral and bacterial infections after housing rats of various breeders within an experimental unit. Lab. Anim. **30:**42-45.
81. **McGowan, J. P.** 1911. Some observations on a laboratory epidemic, principally among dogs and cats, in which the animals affected present the symptoms of the disease called "distemper". J. Pathol. **15:**372-426.
82. **Burek, J. D., G. C. Jersey, C. K. Whitehair, and G. R. Carter.** 1972. The pathology and pathogenesis of *Bordetella bronchiseptica* and *Pasteurella pneumotropica* infection in conventional and germfree rats. Lab. Anim. Sci. **22:**844-849.
83. **Kimman, T. G. and M. S. Kamp.** 1986. Induced atrophic rhinitis in rats. Am. J. Vet. Res. **47:**2426-2430.
84. **Mattoo, S., J. F. Miller, and P. A. Cotter.** 2000. Role of *Bordetella bronchiseptica* fimbriae in tracheal colonization and development of a humoral immune response. Infect. Immun. **68:**2024-2033.
85. **Brockmeier, S. L.** 1999. Early colonization of the rat upper respiratory tract by temperature modulated *Bordetella bronchiseptica*. FEMS Microbiol. Lett. **174:**225-229.
86. **Stockbauer, K. E., B. Fuchslocher, J. F. Miller, and P. A. Cotter.** 2001. Identification and characterization of BipA, a *Bordetella* Bvg-intermediate phase protein. Mole. Microbiol. **39:**65-78.
87. **Martinez de Tejada, G., J. F. Miller, and P. A. Cotter.** 1996. Comparative analysis of the virulence control systems of *Bordetella pertussis* and *Bordetella bronchiseptica*. Mol. Microbiol. **22:**895-908.
88. **Yuk, M. H., E. T. Harvill, and J. F. Miller.** 1998. The BvgAS virulence control system regulates type III secretion in *Bordetella bronchiseptica*. Mol. Microbiol. **28:**945-959.
89. **Keegan, J. J.** 1920. The pathology of epidemic pneumonia in mice and guinea pigs. Arch. Intern. Med. **26:**570-593.
90. **Bemis, D. A.** Unpublished data.
91. **Heilmann, P., M. Kruger, and F. Horsch.** 1985. Pathohistological research on the white mouse after infection with swine strains of *Bordetella bronchiseptica*. 1. Pathohistological research results using intravenously infected mice. Arch. Exp. Veterinarmed. **39:**85-100.
92. **Magyar, T.** 1990. Virulence and leinotoxicity of *Bordetella bronchiseptica* in mice. Vet. Microbiol. **25:**199-207.
93. **Akerley, B. J., P. A. Cotter, and J. F. Miller.** 1995. Ectopic expression of the flagellar regulon alters development of the *Bordetella*-host interaction. Cell **80:**611-620.
94. **Lendvai, N., T. Magyar, and G. Semjen.** 1992. Cross protection studies on *Bordetella bronchiseptica* in mice using an intracerebral challenge model. Vet. Microbiol. **31:**191-196.
95. **Mendoza, S., A. Ciprian, J. Camacho, and J. A. Montaraz.** 1993. Comparison of three biological assays for the detection of toxigenic properties of *Bordetella bronchiseptica* associated with atrophic rhinitis. Vet. Microbiol. **36:**215-219.
96. **Sekiya, K., M. Kawahira, and Y. Nakase.** 1983. Protection against experimental *Bordetella bronchiseptica* infection in mice by active immunization with killed vaccine. Infect. Immun. **41:**598-603.
97. **Hoffman**, **A., M. Kruger, and F. Horsch.** 1984. Differentiation of *Bordetella bronchiseptica* strains. 3. Brief study of different reaction of various mouse lines to *Bordetella bronchiseptica* strains. Arch. Exp. Veterinarmed. **38:**101-104.
98. **Goodnow, R. A., S. C. Causey, S. J. Geary, and W. S. Wren.** 1983. Comparison of an infective avirulent and canine virulent *Bordetella bronchiseptica*. Am. J. Vet. Res. **44:**207-211.
99. **Goodnow, R. A., C. D. Lehr, F. J. Shade, and J. L. Wisecarver.** 1977. Mouse potency assay for *Bordetella bronchiseptica* bacterins. J. Clin. Microbiol. **6:**337-339.
100. **Rutter, J. M., L. M. Francis, and B. F. Sansom.** 1982. Virulence of *Bordetella bronchiseptica* from pigs with or without atrophic rhinitis. J. Med. Microbiol. **15:**105-116.
101. **Bacon, M., R. F. Bacon, and C. H. Drake.** 1958. Bacterial infections in wild rodents of eastern and central Washington. J. Infect. Dis. **102:**5-13.
102. **Smith, I. M., A. J. Baskerville, E. Brothwell, and J. Oliphant.** 1982. Immunogenicity of killed *Bordetella bronchiseptica* vaccines in the mouse. Res. Vet. Sci. **32:**248-252.
103. **Magyar, T. and R. Glavits.** 1990. Immunopathological changes in mice caused by *Bordetella bronchiseptica* and *Pasteurella multocida*. Acta. Vet. Hung. **38:**203-210.
104. **Karasek, E. and M. Kruger.** 1987. Suitability of laboratory mouse strains for virulence testing of *Bordetella bronchiseptica*. Arch. Exp. Veterinarmed. **41:**389-397.
105. **Kendrick, P. L., E. B. Nadolski, G. Eldering, and J. Baker.** 1953. Antigenic relationships of *Hemophilus pertussis*, the Parapertussis bacillus and *Brucella bronchiseptica* as shown by cross-protection tests in mice. J. Bacteriol. **66:**166-169.
106. **Ross, R., J. Munoz, and C. Cameron.** 1969. Histamine-sensitizing factor, mouse-protective antigens and other antigens of some members of the *Genus Bordetella*. J. Bacteriol. **99:**57-64.
107. **Graeff-Wohlleben, H., S. Killat, A. Banemann, N. Guiso, and R. Gross.** 1997. Cloning and characterization of an Mn-containing superoxide dismutase (sod A) of *Bordetella pertussis*. J. Bacteriol. **179:**2194-2201.
108. **Gueirard, P., P. Minoprio, and N. Guiso.** 1996. Intranasal inoculation of *Bordetella bronchiseptica* in mice induces long-lasting antibody and T-cell mediated immune responses. Scand. J. Immunol. **43:**181-192.
109. **Harvill, E. T., P. A. Cotter, and J. F. Miller.** 1999. Pregenomic comparative analysis between *Bordetella bronchiseptica* RB50 and *Bordetella pertussis* Tohama I in murine models of respiratory tract infection. Infect. Immun. **67:**6109-6118.
110. **Harvill, E. T., P. A. Cotter, M. H. Yuk, and J. F. Miller.** 1999. Probing the function of *Bordetella bronchiseptica* adenylate cyclase toxin by manipulating host immunity. Infect. Immun. **67:**1493-1500.

111. **Harvill, E. T., A. Preston, P. A. Cotter, A. G. Allen, D. J. Maskell, and J. F. Miller.** 2000. Multiple roles for *Bordetella* lipopolysaccharide molecules during respiratory tract infection. Infect. Immun. **68:**6720-6728.
112. **Magyar, T., G. Semjen, and Z. Osvath.** 1985. Investigation of adhesive and nonadhesive *Bordetella bronchiseptica* strains in a suckling-mouse model. Acta. Vet. Hung. **33:**137-141.
113. **McMillan, D. J., E. Medina, C. A. Guzman, and M. J. Walker.** 1999. Expression of urease does not affect the ability of *Bordetella bronchiseptica* to colonize and persist in murine respiratory tract. F.E.M.S. Microbiol. Lett. **178:**7-11.
114. **Yuk, M. H., E. T. Harvill, P. A. Cotter, and J. F. Miller.** 2000. Modulation of host immune responses, induction of apotosis and inhibition of NF-kB activation by the *Bordetella* type III secretion system. Mol. Microbiol. **35:**991-1004.
115. **Nagano, H., T. Nakai, Y. Horiguchi, and K. Kume.** 1988. Isolation and characterization of mutant strains of *Bordetella bronchiseptica* lacking dermonecrotic toxin-producing ability. J. Clin. Microbiol. **26:**1983-1987.
116. **Sawata, A. and K. Kume.** 1982. Nasal turbinate atrophy in young mice inoculated with *Bordetella bronchiseptica* of pig origin. Am. J. Vet. Res. **43:**1845-1847.
117. **Sekiya, K., Y. Futaesaku, and Y. Nakase.** 1988. Electron microscopic observations on tracheal epithelia of mice infected with *Bordetella bronchiseptica*. Microbiol. Immunol. **32:**461-472.
118. **Heilmann, P., M. Kruger, and F. Horsch.** 1985. Pathohistological research on the white mouse after infection with swine strains of *Bordetella bronchiseptica*. 2. Pathohistological research results using intratracheally infected mice. Arch. Exp. Veterinarmed. **39:**101-120.
119. **Ohgitani, T., T. Okabe, and N. Sasaki.** 1991. Characterization of hemagglutination from *Bordetella bronchiseptica*. Vaccine **9:**653-658.
120. **Muller, G., and P. Heilmann.** 1987. Comparative local and systemic immunization against experimentally-induced *Bordetella bronchiseptica* pneumonia in ICR mice. Z-Erkr-Atmungsorgane. **169:**136-146.
121. **Mendoza-Elvira, S., A. Ciprian-Carrasco, F. Sotres-Carreras, V. Lara-Saghon, J. Camacho-Machin, J. Tortora-Perez, and J. A. Montaraz-Crespo.** 1991. Pulmonary clearance of *Bordetella bronchiseptica* in mice infected by aerosol. Veterinaria-Mexico. **22:**57-61.
122. **Novotny, P., A. P. Chubb, K. Cownley, J. A. Montaraz, and J. E. Beesley.** 1985. *Bordetella* adenylate cyclase: a genus specific protective antigen and virulence factor. Dev. Biol. Stand. **61:**27-41.
123. **Kruger, M., O. Hecht, and F. Horsch.** 1986. Differentiation of *Bordetella bronchiseptica* strains. 4. Pathogenic significance of heat-labile dermonecrotic toxins of *Bordetella bronchiseptica* for the development of atrophic rhinitis in baby mice. Arch. Exp. Veterinarmed. **40:**157-163.
124. **Magyar, T., R. Glavits, G. D. Pullinger, and A. J. Lax.** 2000. The pathological effect of the *Bordetella* dermonecrotic toxin in mice. Acta. Veterinaria Hungarica **48:**397-406.
125. **Ishikawa, H. and W. Sato.** 1997. Role of *Bordetella bronchiseptica* sialic acid-binding hemagglutinin as a putative colonization factor. J. Vet. Med. Sci. **59:**43-44.
126. **West, N. P., H. Jungnitz, J. T. Fitter, J. D. McArthur, C. A. Guzman, and M. J. Walker.** 2000. Role of phosphoglucomutase of *Bordetella bronchiseptica* in lipopolysaccharide biosynthesis and virulence. Infect. Immun. **68:**4673-4680.
127. **Tuomanen, E. I., J. Nedelman, J. O. Hendley, and E. L. Hewlett.** 1983. Species specificity of *Bordetella* adherence to human and animal ciliated respiratory epithelial cells. Infect. Immun. **42:**692-695.
128. **Smith, I. M. and A. J. Baskerville.** 1979. A selective medium facilitating the isolation and recognition of *Bordetella bronchiseptica* in pigs. Res. Vet. Sci. **27:**187-192.
129. **Register, K. B., M. R. Ackermann, and D. W. Dyer.** 1995. Non-radioactive colony lift-hybridization assay for detection of *Bordetella bronchiseptica* infection in swine. J. Clin. Microbiol. **33:**2675-2678.
130. **Taneda, A., S. Futo, S. Mitsuse, Y. Seto, M. Okada, and T. Sakano.** 1994. Oligonucleotide probes for *Bordetella bronchiseptica* based on 16S ribosomal RNA sequences. Vet. Microbiol. **42:**297-305.
131. **Nygren, M., E. Reizenstein, M. Ronaghi, and J. Lundeberg.** 2000. Polymorphism in the pertussis toxin promoter region affecting the DNA-based diagnosis of *Bordetella* infection. J. Clin. Microbiol. **38:**55-60.
132. **Hozbor, D., F. Fouque, and N. Guiso.** 1999. Detection of *Bordetella bronchiseptica* by the polymerase chain reaction. Res. Microbiol. **150:**333-341.
133. **Farrell, D. J., M. McKeon, G. Daggart, M. J. Loeffelholz, C. J. Thompson and T. K. S. Mukkur.** 2000. Rapid-cycle PCR method to detect *Bordetella pertussis* that fulfills all concensus recommendations for use of PCR in diagnosis of pertussis. J. Clin. Microbiol. **38:**4499-4502.
134. **Goodnow, R. A.** 1980. Biology of *Bordetella bronchiseptica*. Microbiol. Rev. **44:**722-738.
135. **Woolfrey, B. F. and J. A. Moody.** 1991. Human infections associated with *Bordetella bronchiseptica*. Clin. Microbiol. Rev. **4:**243-255.
136. **Shek, W. R.** Unpublished data.
137. **Schneider, B., D. Stubs, and R. Gross.** 2002. Identification and genomic organization of gene loci negatively controlled by the virulence regulatory BvgAS two-component system in *Bordetella bronchiseptica*. Mol. Genet. Genomics. **267:**526-535.
138. **Porter, J. F., R. Parton, and A. C. Wardlaw.** 1991. Growth and survival of *Bordetella bronchiseptica* in natural waters and in buffered saline without added nutrients. Appl. Environ. Microbiol. **57:**1202-1206.
139. **Porter, J. F. and A. C. Wardlaw.** 1993. Long-term survival of *Bordetella bronchiseptica* in lakewater and in buffered saline without added nutrients. FEMS Microbiol. Lett. **110:**33-36.
140. **Hoidal, J. R., G. D. Beall, F. L. Rasp, Jr., B. Holmes, J. G. White, and J. E. Repine.** 1978. Comparison of the metabolism of alveolar macrophages from humans, rats, and rabbits: response to heat-killed bacteria or phorbol myristate acetate. J. Lab. Clin. Med. **92:**787-794.
141. **Confer, D. L. and J. W. Eaton.** 1982. Phagocyte impotence caused by an invasive bacterial adenylate cyclase. Science **217:**948-950.
142. **Clifford, C. B.** Unpublished data.