In: **Recent Advances in Canine Infectious Diseases**, L.E. Carmichael (Ed.)
Publisher: International Veterinary Information Service (www.ivis.org)

Canine Respiratory Bordetellosis: Keeping up with an Evolving Pathogen (13 Jan 2000)

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Introduction

In light of advances in molecular microbiology and vaccinology it may seem surprising that *Bordetella bronchiseptica* (Bb) continues to be a significant respiratory tract pathogen in dogs. Improvements in the prevention and control of canine respiratory bordetellosis are within our reach, however, the scarcity of resources committed to this goal continues to be a problem. In this report, we provide a review of the clinical aspects of respiratory bordetellosis in dogs, discuss recent progress in understanding canine Bb at the molecular level, and consider particularly promising new approaches for reliable disease prevention.

Canine respiratory bordetellosis was first recognized in association with epizootics of canine distemper that occurred in the early 1900's. Despite the availability of several types of vaccines, Bb continues to be a major cause of canine respiratory disease. This was particularly evident throughout 1999 when outbreaks of respiratory tract disease (Kennel Cough) plagued racing Greyhounds even though the racing Greyhound industry has rigorous vaccination policies to prevent infectious respiratory tract disease. Conservative estimates place 1999 Greyhound racing revenue losses associated with this disease complex in the millions of dollars. Less dramatic but nevertheless significant disease outbreaks are a relatively common occurrence in boarding kennels, animal shelters, research facilities, and veterinary clinics. Our studies, as well as the experience of veterinarians and dog owners, leave little doubt that current vaccines do not adequately protect dogs against bordetellosis.

Etiology and Pathogenesis

Bb remains the principal etiologic agent of infectious tracheobronchitis (ITB) in dogs. It is the only agent capable of inducing classic kennel cough disease under both natural and experimental conditions. However, it should be noted that canine parainfluenza virus (CPI) and canine adenovirus type-2 (CAV-2) sometimes play an initiating or complicating role in ITB. CPI and CAV-2 most often cause mild or subclinical infections. In the presence of Bb, the clinical disease caused by those agents becomes more severe. The potential role of canine herpesvirus, reovirus, mycoplasma, fungi, and parasites in ITB is poorly defined; however, *Mycoplasma* sp. have been shown to augment the severity of tracheobronchitis caused by Bb or viruses.

Bb is gram-negative bacterium exquisitely well adapted to colonizing the host's respiratory tract. In addition to dogs, the host range of Bb includes pigs, cats, laboratory animals, and human beings. Bb is closely related to B. pertussis (Bp), the etiologic agent of whooping cough (pertussis) in infants and children. Taxonomic studies indicate that Bp recently evolved from Bb, and, that Bp differs from Bb primarily in its ability to cause disease in humans only.

Bb is transmitted through aerosolization of the organism in respiratory secretions and by contaminated fomites. In most cases the infection remains localized in the respiratory tract and dissemination to other organs does not occur. Clinical evidence of systemic involvement beyond that attributable to the upper respiratory system indicates that more severe disease is present and necessitates a more detailed diagnostic evaluation.

Congregating dogs of different ages and various levels of susceptibility to Bb, CPI, and CAV-2 are the most

common pre-requisites for ITB. This makes the disease complex difficult to prevent in humane societies, boarding and training kennels, veterinary hospitals, research institutions, and facilities where performance or show dogs are housed. In addition, it is now clear that there is considerable genetic diversity as well as differences in disease causing potential among the various strains of Bb which have been isolated from healthy dogs and dogs with ITB.

The ability of Bb to colonize ciliated respiratory epithelial cells is not surprising when the mechanisms used by Bb to accomplish this goal are reviewed. Both fimbrial and non-fimbrial adhesins, structures functioning in attaching the bacterium to the host, are involved in colonization of the host tissues. The fimbriae of Bb are hairlike appendages that extend from the cell membrane of the bacteria. By recognizing specific receptors within the respiratory tract, these protein structures allow Bb to attach to the host and they play a role in determining the specific tissues which are colonized (ciliated respiratory tract epithelium) as well as host species specificity. Two non-fimbrial adhesins essential for the attachment of Bb to epithelial cells include filamentous hemagglutinin (FHA) and pertactin (Prn). These proteins are found primarily in the outer-membrane of the bacteria and facilitate colonization by recognizing specific host cell receptors. Interestingly, a new generation of human vaccines containing these proteins has revolutionized the prevention of whooping cough in humans. The progress being made on these proteins from canine Bb isolates (see below: last section) may contribute to a new generation of canine vaccines.

Once colonization has been established, Bb utilizes several exotoxins (adenylate cyclase-hemolysin, dermonecrotic toxin, and tracheal cytotoxin) and endotoxin to damage the respiratory tract and impair the host's ability to eliminate infection. Together these factors disrupt ciliated cells, disable responding phagocytic cells, suppress both the humoral and cell mediated immune response and, for the most part, are believed to be responsible for the clinical signs displayed by dogs with ITB.

Recent studies on Bb suggest that revisions will occur in the way infection by this organism is now prevented. Bb has historically been considered an obligate extracellular pathogen that only colonizes the surface of the respiratory tract and is unable to survive outside the host. Dr. Jeff Miller and his colleagues (Department of Microbiology and Immunology, UCLA School of Medicine) have provided recent insight into systems in Bb that appear to orchestrate a complex interaction between bacterium, host, and the environment. A master control system (BvgAS) appears to act by sensing the environment of the bacteria, allowing the organism to successfully colonize the animal, invade cells, and survive under conditions of severe nutrient deprivation. While Bb has not been recovered from uncontaminated natural environments, the environmental persistence of Bb has been documented and environmental contamination should be considered a source of infection for susceptible animals. Additionally, the discovery of a secretion system (type III secretion system) in Bb capable of delivering bacterial proteins directly to the cytosol of host cells may offer unique targets for vaccine antigens and prevention of canine bordetellosis. From the study of other pathogenic bacteria that have type III secretion systems, it is increasingly clear that these systems are of fundamental importance in the disease process and are regulated by a host of complex environmental signals, including the number of bacteria present.

Clinical Signs and Diagnosis

The most characteristic clinical sign associated with ITB is a dry, hacking, paroxysmal cough. The cough is typically exacerbated by exercise and can be produced by palpation of the tracheal or laryngeal regions. A history of exposure to other dogs in a kennel, hospital, or group situation, in association with a hacking cough is usually sufficient to make a diagnosis. Nasal discharge may or may not be present.

Identification of the etiologic agent(s) involved in ITB is not essential in most situations. Individual animals will likely respond to appropriate therapy and an effective quarantine program will halt further spread of disease. Nevertheless, both individual cases and outbreaks involving groups of dogs can benefit from organism identification and evaluation.

Because the trachea can respond to infectious agents in only a limited number of ways, visual examination of the trachea is less likely to be useful in the diagnosis of bordetellosis than clinicopathologic evaluations of the same area. Several methods are available to obtain diagnostically significant samples from the trachea. They include endoscopy, bronchoalveolar lavage, transtracheal wash, and guarded swabs introduced directly through the mouth or through an endotracheal tube.

In general, tracheal specimens yield more reliable results by eliminating problems associated with identifying Bb among the bacterial flora normally present in the oropharynx. However, this problem can be controlled and the potential of isolating Bb enhanced significantly by use of Schaedler's enrichment broth, charcoal blood

agar with cephalexin, and extending incubation times. Regrettably, most veterinary diagnostic laboratories do not apply these improved isolation techniques routinely; they, therefore, should be requested when samples are submitted. If tracheal culture is not possible, deep nasal swabs (using a mini-tip culturette) are preferred over pharyngeal swabs. Swabs should be collected for cytology as well as bacterial culture and antimicrobial sensitivity testing. Additionally, viral culturettes can be collected for CPI and CAV-2 isolation; however, serologic identification may be preferred for those agents. Veterinarians should contact their diagnostic laboratory prior to obtaining samples for virology.

Bacterial culture results can be difficult to interpret, especially if the samples were from the nose or oropharynx. The bacteriology laboratory should be asked to report the results in a quantitative, or semiquantitative manner. Bb obtained form the trachea should be considered significant. On the other hand, Bb isolated from nasal or pharyngeal swabs requires more careful evaluation. Bb isolated in pure culture, or in high numbers (moderate to heavy growth on direct plating) from nasal swabs is usually significant. Insignificant or low numbers of Bb from nasal cultures should be considered in light of history and clinical signs and the cultures repeated if necessary. Cytologic evaluation of bordetellosis is typified by neutrophilic inflammation. A Gram stain and examination of the slide for organisms can offer important preliminary information.

Therapy and Prevention

The decision whether or not to initiate antibiotic therapy for ITB must be made on an individual basis. In general, ITB is self-limiting and does not require antibiotic therapy. However, the prophylactic use of antibiotics may be recommended following exposure to dogs with ITB. Several factors, including penetration of the drug into bronchial secretions and perhaps penetration of the antibiotic into host cells, need to be considered for successful treatment of airway infection with Bb.

Antibiotic selection should be based on the results of microbiologic culture and antimicrobial sensitivity testing. Pending (or in the absence of) culture results antibiotics should be selected based on a presumptive Bb infection. Antibiotics considered most effective against Bb include tetracyclines, chloramphenicol and related compounds, and macrolides (erythromycin, clarithromycin, and azithromycin). Those thought to be less effective would include fluoroquinolones, cephalosporins (especially third generation), and trimethoprim-sulfamethoxazole.

Strict rest, avoidance of excitement, and nominal exercise are indicated to minimize cough-precipitating situations and avoid perpetuating airway irritation. Antitussives (narcotic and non-narcotic) and bronchodilators are also recommended when pharmacological intervention is required to control coughing. The use of anti-inflammatory doses (0.25 to 0.5 mg / kg, q 12 h) of corticosteroids is controversial. However, because many of the antibiotics effective against Bb are bacteriostatic, the concurrent use of corticosteroids and these antibiotics should be avoided.

Prevention of ITB should be attempted using a strategy that combines minimizing exposure to infectious agents and optimizing immunity through vaccination. Exposure can be minimized by isolating dogs that have a recently been exposed to a kennel or group boarding situation. Because Bb may survive severe nutrient depravation, attention should be given to hygiene and sanitation, especially in group housing environments. Animal caretakers and owners should be educated on the survival of pathogens in the environment and reminded that most chemical disinfectants are inactivated in the presence of organic material (feces, urine, pus, exudates, discharges, and blood). Kennel management should review the properties of the disinfectant being used in their facility. Ideally, a disinfectant should be selected that is rapidly bactericidal (within seconds to minutes of contact time) and active against enveloped (CPI) and nonenveloped (CAV-2) viruses. Managing animal housing at a racetrack, or any large kennel situation, can be difficult. Large boarding facilities and performance animal kennel directors may benefit from consulting an environmental engineer. Environmental professionals can be helpful by minimizing animal crowding, optimizing hourly air exchanges and humidity levels, and ensuring proper flow of air with respect to the population of animals being housed. The Compendium of Veterinary Products lists nine vaccines for the prevention of ITB and bordetellosis. Presently, Shering-Plough markets two products that differ only in the delivery technology employed. Pfizer Animal Health also markets two products (one a Bb bacterin alone, the other a Bb bacterin combined with canine distemper virus, CAV-2, and CPI). Additional manufactures and distributors of bordetellosis vaccines include Performer, Bio-Ceutic, Intervet, and Fort Dodge.

The advantages and disadvantages of Bb vaccines have recently been reviewed. In general, the products consist of either a whole cell bacterin (Pfizer Animal Health), extracted cellular antigens (Performer), or

avirulent live cultures (Schering-Plough, Pfizer, Bio-Ceutic, Intervet, and Fort Dodge). Several products also include a modified live CPI (Bio-Ceutic, Schering-Plough, Intervet, and Fort Dodge); one product includes a modified live CAV-2 (Fort Dodge).

Parenteral products have the advantage of being easy to administer. Unfortunately, current parenteral Bb vaccines can cause local and systemic reactions and are inhibited by colostral immunity. Intranasal vaccines are less convenient to administer, however, their efficacy in the face of colostral immunity may be an advantage. Avirulent live Bb cultures are thought to stimulate local secretory antibodies; however, there is little information on the specificity of the immune response stimulated by those products. Recent studies have revealed that avirulent live Bb vaccine strains are genetically distinct from field isolates and may express altered levels, or immunologically distinct variants, of critical antigens. While the duration of immunity, and effectiveness against different Bb field strains in different populations of dogs are not known, experience suggests that the protection is strain and population specific, and that the duration of immunity can be as short as 3 months.

Product(s) selected to prevent ITB should be used according to the instructions of the manufacturer. While most pet dogs are adequately protected using parenteral modified live CPI and CAV-2 vaccines (conveniently combined with most distemper vaccines), a more aggressive approach is required for performance animals and other high risk dogs. The idea of simultaneously maximizing systemic and secretory immunity seems a reasonable approach for high exposure situations, but additional research is needed. The pressure on Bb to survive (evolve) may necessitate a new generation of vaccines. New vaccines should containing current field isolates, or purified antigens derived from them, in order to optimize protection against natural challenge with Bb in the 21st century.

Recent Advances in Understanding and Preventing Canine Bordetellosis

Vaccine reactions and outbreaks of pertussis (Bp) among highly vaccinated groups of infants led researchers to question the safety and efficacy of standard whole cell whooping cough vaccines. The result of this work has been the development of a new group of acellular whooping cough vaccines based on purified antigens like FHA and Prn. Studies of the new vaccines have shown that they are considerably more effective and significantly safer than standard whole cell pertussis vaccines. The close relationship between Bp and Bb would suggest that similar improvements can be made in vaccines to prevent canine bordetellosis. However, it should be emphasized that Bp and Bb are immunologically distinct species and care should be taken to produce and purify vaccine antigens from canine Bb isolates.

The canine vaccines discussed above have been on the market for nearly 20 years. With respect to a bacterial species, this is an enormous amount of time to evolve and adapt to a changing environment. Bb has long been thought of as a clonal pathogen with only limited genetic and antigenic diversity. If true, this would imply that vaccines based on a single isolate should offer protection against a majority of field strains. Recent evaluation of canine Bb isolates suggests that there is a great deal of genetic and antigenic diversity. DNA fingerprinting indicates that most the current avirulent live vaccine strains of Bb are closely related (or identical); however, considerable genetic diversity has been found among field strains. Furthermore, antigenic variability between vaccine strains and field isolates has been confirmed for a number of critical antigens including FHA and Prn. Recently, a purified protein from a canine Bb isolate has been used as a vaccine antigen in rabbits. The protein was shown to be safe and antigenic and induced antibodies that inhibit the attachment of Bb to canine cells by as much as 65%. While evaluation of the safety and efficacy of this antigen in dogs is required (studies in progress) these preliminary studies suggest that a safe and effective acellular vaccine for the prevention of canine bordetellosis is possible. Without continued funding for companion animal infectious disease research, and a commitment by industry to improve existing products, the promise of providing a vaccine against canine ITB that is as reliable and safe as the new generation whooping cough vaccines will not be fully appreciated.

Acknowledgements: - The authors thank the Kansas Racing and Gaming Commission, Intervet Inc., Shering-Plough Animal Health Corp., and Pfizer Animal Health Inc. for financial support and for their commitment to improving ITB vaccines.

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