AN UPDATE ON CANINE KENNEL COUGH

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Infectious respiratory disease continues to be one of the most troubling health related problems in performance dogs and also in all large populations of co-mingled dogs. Kennel cough (infectious tracheobronchitis) is characterized by the sudden onset of sneezing, spontaneous hacking, dry coughing that may induce gagging, and in many cases nasal-ocular discharge and mandibular lymphadenopathy.

Efforts to limit the occurrence of disease outbreaks in kennels and shelters through vaccination, ventilation, quarantine, and improved hygiene have been of limited success. Even with the many advances that have taken place in the practice of veterinary medicine and in the larger domain of biomedical research, the frequency of kennel cough outbreaks have not diminished and in certain situations have increased in both frequency and clinical severity.

Fortunately, the new insights that are now being provided by research into the molecular biology of infectious disease microorganisms and modern diagnostic methods which hold the promise of making progress which heretofore has not been possible or economically viable in veterinary medicine.

EPIDEMIOLOGY AND CLINICAL COURSE

A number of microbial etiologic causes have been hypothesized as being responsible or concurrently involved in causing connecting airway disease in dogs. These include such things as Bordetella bronchiseptica, Mycoplasma spp., canine-adenovirus type 1 or 2, canine-parainfluenzae virus, canine-herpesvirus, canine-distemper virus, canine-reoviruses, and most recently an equine-like influenza virus. Confusion concerning the cause of kennel cough relates to the limited response to the mucosa of the conducting airways, similarities in transmission and incubation periods, and a relatively poor response to treatment regardless of type. However, the only microorganism that can experimentally reproduce the clinical characteristics of kennel cough is B. bronchiseptica. Furthermore, whooping cough in humans caused by B. pertussis is characterized by clinical disease and treatment responses that are remarkably similar to those of kennel cough in dogs.

Both observational and experimentally derived data have provided valuable information concerning epidemiology and clinical course of a kennel cough outbreak. Transmission takes place by direct contact with contaminated materials (dishes, clothing, and handlers) as well as by aerosolized materials from sneezing and coughing dogs. Importantly, dogs who have recovered from clinical disease can harbor and potentially shed B. bronchiseptica for months. Epidemiologic, antibiotic resistance patterns, and genetic analysis suggest that the same strains of B. bronchiseptica can cause disease in both dogs and cats, and the transmission can take place between these animal species.

Signs of kennel cough typically develop between 5-10 days after the introduction into a disease free kennel. Depending on a number of poorly defined conditions the morbidity can be as high as 50% but fortunately the mortality is usually low except when complicated by secondary infection. Clinical signs can last for as little as a day or two, or take 1 to 2 weeks to fully resolve. The value of various treatment strategies in reducing the duration or severity of uncomplicated cases is not clear.

GENETIC ANALYSIS

While there are eight described Bordetella species only the three disease causing strains have been studied in detail. The recently completed genomic sequencing of B. bronchiseptica, B. pertussis, and B. parapertussis provides additional evidence that these bacteria are very closely related and at the same time important new insights into the biologic basis of host-specificity as well as a factual resource on which to develop more effective prophylaxes and therapeutics.

Evidence is now strong that B. bronchiseptica is closes to the evolutionary ancestor and that independent evolutionary events produced the other species. The genetic basis for the unique biological characteristics of B. bronchiseptica when compared B. pertussis and B. parapertussis is becoming apparent. For example, B. bronchiseptica is capable of persisting for long periods in the environment outside of an animal host and it also contains 1719 genes that are not present in at least one of the others. B. bronchiseptica is motile because it has a complete set of flagellar genes while the other two are non-motile because of genetic defects in these genes. Thus, motility is not necessary for B. pertussis to cause disease, at least in humans. In addition, things that were once thought to be important virulence determinates such as toxin production, type-III secretion systems, fimbria, and capsule are now being questioned because of their absence in strains that still retains the ability to cause clinically significant disease.

MODERN LABORATORY DIAGNOSIS

Confirming the etiologic diagnosis of kennel cough is challenging for a number of reasons. In the case of B. bronchiseptica (as is also the case for B. pertussis in humans) nasopharyngeal sampling is the more reliable means to get a positive results, whether in clinically ill or healthy carrier dogs. In either case the sensitivity is significantly reduced if sampling follows the use of antibiotics. In addition, successful isolation is greatly enhanced when submitted to a laboratory with considerable experience in the isolation and identification of B. bronchiseptica. The difference in successful isolation between laboratories receiving the duplicate samples can be greater that 100 percent.

Major advances have occurred in the use of genetic-base characterizations to identify individual strains of B. bronchi-septica. These have included ribotyping, PFGE, RAPD, and ribosomal gene sequencing. With these are added to such techniques as enzyme electrophoresis, plasmid analysis, antibiotic resistance, and more standard phenotypic characterizations it is now possible to precisely track the epidemiologic characteristics of a kennel cough outbreak. These techniques are of particular value in the investigation of outbreaks because of the widespread use of live intranasal vaccines.

B. bronchiseptica isolates obtained from historical collections and recent disease outbreaks were evaluated using RAPD genomic fingerprinting and ribotyping. The purpose was to determine whether the strains isolated from healthy dogs were genetically related to each other, to vaccine strains, or to isolates from clinically apparent cases of kennel cough. The fingerprint patterns of the vaccine
strains and clinical isolates were defined previously. The strains isolated in these studies produced some common bands in their fingerprint patterns, but were not identical to either vaccine strains or strains from clinically ill dogs. Previously we found genetic diversity among vaccine strains and isolates from clinically ill dogs. The results of a number of the clinical studies now confirm this conclusion and extend the results by finding additional genetic diversity. The geographical areas of collection and the state of health of the dog may also contribute to the diversity of these isolates and those examined previously.

RATIONAL VACCINE DEVELOPMENT

Vaccination has been inappropriately viewed as the answer to all infectious diseases, regardless of the underlying cause. Assuming the vaccine has the ability to provide reliable protection against the disease agent, its effectiveness can be significantly altered if nutrition, environment, management, social, and genetic factors are not optimal. Vaccines are tested and their safety and efficiency determine under optimal conditions, which are rarely if ever present. In fact, under such extreme conditions avirulent or modified-live vaccines can actually cause disease.

Vaccination programs designed to prevent whooping cough have been successful. However, recent increases in the occurrence of disease in vaccinated people caused by strains that have a modified version of the critical protein "pertacin" have raised questions concerning the need to update the current whooping cough vaccine. Vaccination against B. bronchiseptica is an attractive approach because a respiratory tract pathogen in a variety of species including dogs, is the only microorganism that can be routinely isolated from dogs with kennel cough, and is also the only microorganism that can induce kennel cough in dogs following experimental exposure.

Outbreaks of kennel cough in well-vaccinated racing greyhounds (and other dogs) indicate that the disease continues to be a significant problem and that a better vaccine is needed. At their worst, outbreaks in well-vaccinated dogs at tracks and kennels throughout the country result in significant economic losses to the industry and are at the very least, a periodic nuisance to dog owners, kennel managers, and track managers. A recently published study involving 972 healthy dogs in a humane shelter examined the value of intranasal vaccination against B. bronchiseptica and canine-parainfluenzae, with or without canine-adenovirus type 2 found no statistically valid difference between vaccinated and non-vaccinated dogs in preventing the occurrence of spontaneous coughing. The study also found that the risk of KC increased by 3% for each additional day the dogs were in the shelter.

Current vaccines to prevent kennel cough include poorly characterized low-virulence live strains, whole-cell bacterins, and undefined antigenic extracts. Concerns about the efficacy and safety of current kennel cough vaccines have caused us to initially focus on the development of a multivalent, acellular vaccine to prevent canine bordetellosis. Acellular products have profoundly improved the safety and efficacy of vaccines to control B. pertussis infection (whooping cough) in humans. These vaccines have improved efficacy by as much as 48% and have significantly reduced adverse reactions. Eleven of thirteen acellular whooping cough vaccines evaluated in the Multicenter Acellular Pertussis Trial contained filamentous hemagglutinin as a component. The additional critical antigen contained in acellular whooping cough vaccines is pertactin.

Filamentous hemagglutinin (FHA) is a secreted (but membrane associated) protein conserved within the genus Bordetella. The structural gene for the FHA of B. pertussis (thaB) has been cloned and sequenced. FHA is essential for bacterial adherence to eukaryotic cells. Additionally, the immunologic response against FHA is protective in animal models of infection with B. pertussis. While the protective benefits of FHA have been recognized for some time, the immunodominant regions have only recently been identified.

By constructing a genomic DNA library of a canine B. bronchiseptica field isolate, and screening the library for FHA expression using polyclonal anti-FHA antiserum prepared it was possible to clone and sequenced the immunodominant region of FHA. The fragment has been subcloned, affinity tagged, and the fusion protein purified using a prokaryotic expression system. The 66 kDa recombinant fusion protein has the advantage of containing the type I domain peptide epitopes as well as conformational epitopes. The truncated fusion protein is recognized by convalescent serum of dogs following experimental infection with a heterologous strain of B. bronchiseptica. With this recombinant protein we now have an opportunity to capitalize on the wealth of information made available by the introduction of the new acellular whooping cough vaccines to produce more effective kennel cough vaccines because it has all the features necessary to be safe, antigenic, and offer a critical component of the protective immune response in dogs.

Pertactin is the other protein used by B. bronchiseptica to adhere to the respiratory tract. Pertactin gets its name from the fact that it is the only protein that is capable, by itself, of inducing protective immunity against disease. Variation in the nucleotide sequence, predicted amino-acid sequence, and size of the pertactin proteins expressed in canine B. bronchiseptica isolates is the most likely explanation for why current vaccines are not virulent and also explains why they do not provide reliable protection. These findings have recently been confirmed by other research groups working with swine strains of B. bronchiseptica as well as with strains of B. pertussis isolated from cases of whooping cough. It is now clear that currently, the canine vaccine strains of B. bronchiseptica and field isolates from vaccinated dogs with kennel cough do not express the same types of pertactin protein.

Pertactin variants have been cloned and expressed in a fashion similar what was used for FHA. While purified FHA and pertactin proteins have been produced for use in ELISA to follow the immune response following vaccination, the cost of using these in a vaccine was judged to be too great for a commercial vaccine. As a result, various bacterin formulations of recombinant E. coli expressing recombinant FHA and pertactin have been produced and evaluated for safety and immunogenicity.

Testing of various formulations using USDA protocols found whole-cell recombinant antigen vaccines to be safe (no systemic reactions at ten times the normal dose). The vaccine formulations induce significant but variable degrees of increased immunity to the protective antigens of B. bronchiseptica (FHA and Pertactin). Depending on the formulation, excellent immune responses to FHA and pertactin (measured by purified protein specific antigen ELISA) were induced by subcutaneous immunization of dogs.
When taken together, excellent progress is being made towards the development of a vaccine that is safe and induces optimal immunity against kennel cough. We have already reached several important milestones including determining that 1) the new vaccine is safe when tested according to USDA protocols and that 2) the vaccine formulations induce a significant immune response against the recombinant *B. bronchiseptica* antigens (pertactin and FHA). These are important findings because of the novel approach we are using to produce and deliver the recombinant protein antigens.

**Reference** available from the author upon request.