Canine Infectious Respiratory Disease Complex: Host, Pathogen, and Environmental Interactions

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OVERVIEW
An important step in the pathogenesis of Canine Infectious Respiratory Disease (CIRD) involves the colonization of the upper airway mucosa by primary respiratory pathogens. In the susceptible host and the proper environment, these primary respiratory pathogens are capable of bypassing the mechanical barriers, evading the innate immune response, and disrupting mucociliary clearance, thereby allowing both primary and secondary bacterial and viral pathogens to colonize and infect the upper and lower respiratory tract. An understanding of the complex relationship between these primary respiratory pathogens and the respiratory immune system is crucial to the development of strategies to effectively treat and prevent CIRD. The objective of this presentation is to provide an update on our current understanding of the interactions between the canine immune system and the classical and emerging respiratory pathogens underlying this disease complex. We will first review the components of the intact mechanical, innate, and adaptive canine respiratory immune system in health. We will discuss the mechanisms by which primary respiratory pathogens, like *Bordetella bronchiseptica*, and *Mycoplasma cynos*, can evade or bypass the immune system. We will discuss the potential role of emerging pathogens (e.g., Canine Influenza Virus, Canine Respiratory Coronavirus) in disease pathogenesis. Finally, we will discuss both immunologic (vaccination, natural immunity) and non-immunologic (premise control, environmental management) strategies to effectively prevent CIRD.

RESPIRATORY DEFENSE SYSTEM
The normal, intact respiratory defense system can be divided into three distinct levels. The first level consists of mechanical barriers, including the mucus and epithelial lining fluid that overlies the airway epithelium. These mechanical barriers serve to prevent inhaled pathogens from attaching to the epithelial surface, thereby inhibiting their ability to infect the host. If inhaled pathogens are able to bypass the mechanical barriers and engage the epithelial surface, the second level of the respiratory defense system, the innate immune system, can become activated. The innate immune response is triggered by binding of pathogen-associated-molecular-patterns (PAMPs) on the surface of the pathogen by receptors on the epithelial cell surface. Binding of these receptors stimulates the release of preformed mediators, including interferons, enzymes, and chemoattractant molecules, which function to inhibit infection and prime or amplify adaptive immunity. Thus, the innate immune response serves as an important bridge toward the development of the adaptive immune response. The adaptive immune system involves antigen presentation to t-helper lymphocytes in mucosal-associated lymphoid tissues (MALTs), which subsequently drive development of local IgA and systemic IgG-producing plasma cells and antigen-specific cytotoxic t-lymphocytes (CTL). It is the adaptive immune response that provides both immunologic specificity and long-term immunologic memory.

Mechanical barriers and the innate immune response are present in all patients, but may vary in their efficacy from patient to patient as a result of concurrent respiratory disease processes.
As an example, dogs with chronic bronchitis may have airway epithelial hyperplasia and squamous metaplasia, along with alterations of mucus production and mucus quality that together impair normal mucociliary clearance functions. While these functional aspects of the respiratory immune system do not confer immunologic specificity or memory, they both help to stimulate or amplify the adaptive immune system. The barrier lining the nasal airways, the trachea, and the first several generations of bronchi is a ciliated respiratory epithelium. This airway surface possesses motile cilia that effectively drag the overlying mucus blanket, along with any trapped particles or organisms from the inhaled air, directly in contact with mucosal-associated lymphoid tissues (MALTs) in the caudal aspect of the nasal cavity and in the upper portions of the tracheobronchial tree. Activation of the innate immune response recruits antigen presenting cells to the site of initial pathogen contact, which can mediate the early steps necessary for immunoglobulin production and CTL generation.

The nasal cavity, trachea, and bronchi are the principal sites for pathogen colonization and infection in dogs with CIRD. Activation of MALTs in these same regions is an important first step in the generation of an adaptive immune response. These MALTs possess follicles of B-cells surrounded by parafollicular T-cell zones, and are populated by antigen presenting cells (APCs). The APCs on the airway present CIRD antigens to T-cells, which then direct the B-cells to produce immunoglobulin A (IgA) and immunoglobulin G (IgG). IgA and IgG confer mucosal immunity through the processes of immune exclusion and immune elimination. IgA produced locally by MALTs is translocated through epithelial cells and resides on the airway surface in the epithelial lining fluid. IgA is very effective in binding airborne pathogens and inhibiting pathogen attachment to the airway surface (immune exclusion), but is not an effective opsonin, does not activate complement, and is relatively short-lived. IgG is principally a circulating (or humoral) immunoglobulin, but can be recruited to the airway mucosal surfaces during following pathogen colonization. IgG is a potent opsonin, is a potent activator of complement (immune elimination), and is produced by plasma cells with longer half-lives. Both IgA and IgG are important in mediating immune responses against extracellular pathogens. CTLs, which recognize processed intracellular antigens in the context of MHC-I, are important in mediating immunity against intracellular pathogens. All three work together in order to provide variable degrees of protection against CIRD infection in either naturally infected or effectively vaccinated patients.

**PATHOGENESIS OF CIRD**

CIRD, or “kennel cough,” or “canine shipping fever,” is a complex, highly contagious respiratory infection that is spread primarily through aerosolized respiratory secretions. Aerosolized viral and bacterial pathogens in the complex initially colonize the respiratory epithelium lining the nasal cavity, trachea, and bronchi. Because most dogs are infected through exposure to aerosolized secretions, there is often a predictable temporal relationship between exposure to other dogs and the onset of clinical symptoms (anywhere from 3-10 days in most cases). The communicable nature of CIRD makes it a frequent cause of morbidity in shelters, kennels, boarding facilities, “doggie” day care centers, and veterinary clinics. While most dogs are infected through direct exposure to aerosolized respiratory secretions, dogs can also be exposed to CIRD pathogens indirectly through fomites. Potential fomites in CIRD transmission include improperly sanitized hospital surfaces (exam tables, cages, scales, waiting areas), toys, endotracheal tubes, medical equipment, and hospital personnel or personal protective equipment (e.g., contaminated scrubs).

The primary pathogens in the CIRD complex colonize the ciliated respiratory epithelium in the upper airway. Once they gain access to the tissues, many of these pathogens possess virulence
factors that allow them to disrupt mucociliary clearance, often by altering ciliary function or by causing injury to ciliated cells. Mucociliary clearance dysfunction subsequently allows other pathogenic or opportunistic bacteria and viruses to colonize the airway surface, maintain longer residence time, and complicate the infection. Many viral and bacterial principal pathogens are known to disrupt ciliary function or morphology (e.g., Canine parainfluenza virus), and many others are suspected to do so on the basis of their possession of known virulence factors (e.g., *Mycoplasma cynos*).

**“Old Friends”–Established Pathogens in CIRD**

**Canine parainfluenza virus and canine adenovirus.**

Canine parainfluenza virus is the most commonly isolated pathogen in the CIRD complex. Several vaccines are available for prevention of clinical infection. Appropriately vaccinated dogs will develop high titers against canine parainfluenza virus that may persist for at least three years, and vaccination is effective in minimizing or preventing the clinical signs associated with infection. However, vaccinated dogs subsequently exposed to canine parainfluenza virus may transiently shed virus, possibly leading to false positive results in PCR panels. Another viral pathogen, canine adenovirus-2, is occasionally isolated as a co-factor in multiple pathogen infections, but rarely causes overt respiratory symptoms as a single agent infection. Vaccination for canine adenovirus-2 is included as a core vaccine for prevention of canine infectious hepatitis (CAV-1). Both canine adenovirus and canine parainfluenza virus typically cause mild, self-limiting respiratory infections exhibiting minimal systemic symptoms[1].

**Canine distemper virus.**

Canine distemper virus is another important cause of CIRD. Initial symptoms in infected dogs are frequently localized to the upper and lower respiratory tract, and in many cases, symptoms are limited to the respiratory tract (cough, nasal discharge, fever). In systemically infected dogs, multiple organs may become infected. Characteristic lesions in systemically infected dogs include ocular (periocular dermatitis, conjunctivitis, keratitis), dermal (footpad and nasal hyperkeratosis), and neurologic (acute and/or progressive myeloencephalitis) manifestations. Canine distemper virus is unique among viral causes of CIRD due to its longer incubation period. Dogs infected with canine distemper may exhibit symptoms several weeks after exposure, while dogs infected with canine parainfluenza virus of canine adenovirus may exhibit symptoms within a few days after exposure[2].

**Bordetella bronchiseptica.**

*Bordetella bronchiseptica*, an aerobic, gram negative bacterium, is the most commonly isolated bacterial cause of CIRD. There are hundreds of isolates of *Bordetella bronchiseptica* in the environment with variable virulence, pathogenicity, and host distribution. Unlike many of the other causes of CIRD, which tend to be relatively host-adapted, *Bordetella bronchiseptica* is uniquely able to infect dogs, people, as well as other mammals. Because of this, dogs naturally infected with *Bordetella bronchiseptica* or recently vaccinated with a modified live Bordetella bronchiseptica vaccine may pose a zoonotic risk to immunocompromised people, although the risk is likely very low, and evidence supporting dog-to-human transmission is very weak and largely circumstantial.

Among the virulence factors possessed by *Bordetella bronchiseptica* are filamentous hemagglutinin and fimbriae, which facilitate bacterial attachment to cilia on respiratory epithelial cells, and a type-3 secretion mechanism that allows for translocation of cytotoxins that mediate ciliary stasis into the cytosol of airway epithelial cells[3, 4].
Emerging Pathogens in CIRD

Canine respiratory coronavirus.
Canine respiratory coronavirus is an enveloped RNA virus that was first associated with acute respiratory infections in shelter dogs in England over ten years ago. Canine respiratory coronavirus is distinct from the canine enteric coronavirus, and immunity to the enteric form does not provide cross-protection against the respiratory virus. Canine respiratory coronavirus infection is usually associated with mild, self-limiting respiratory disease, but has been associated with outbreaks of severe respiratory disease in shelters and boarding facilities. Exposure of dogs to canine respiratory coronavirus is widespread in North America, with >50% of dogs demonstrating serum antibodies reactive against the virus. Canine respiratory coronavirus may facilitate infection with other CIRD pathogen, as up to 12% of dogs infected with this virus are co-infected with canine parainfluenza virus, canine influenza virus, Mycoplasma spp., and Bordetella bronchiseptica[5, 6].

Canine influenza virus.
Up until December 2014, Canine Influenza Virus infection was due to a H3N8 Canine Influenza A virus that emerged in the canine population following a mutation of the equine influenza virus. This mutation allowed horse-to-dog transmission of the virus. Infections in dogs have now been documented in over 40 states, as well as in Canada, Australia and England. In 2015, the first cases of infection by a novel H3N2 Canine Influenza Virus were reported in Chicago. Since those initial reports, cases of H3N2 Canine Influenza have now been reported in over 30 states and Canada, with “hotspots” of ongoing infection occurring in Chicago, Atlanta, and the northeastern United States. To date, the H3N8 and H3N2 Canine Influenza Viruses are the only two known to be capable of infecting and causing disease in dogs and capable of being transmitted from an infected dog to a vulnerable dog. The H3N2 virus has also been reported to be transmitted from dogs to cats, and may cause clinical disease in vulnerable cats.

Like canine respiratory coronavirus, canine influenza viruses exhibit a short latency period of ~24 hours. Viral replication and shedding occurs within one day of exposure in infected dogs. The incubation period of canine influenza virus is longer (2-5 days), resulting in 1-4 day period during which dogs shedding virus may be asymptomatic. For this reason, the viruses are very effectively transmitted between infected and vulnerable dogs in overcrowded environments. The period of peak viral shedding of H3N8 canine influenza virus is short for clinically affected dogs (<7 days). For that reason, PCR testing may yield false negative results for H3N8 in dogs exhibiting symptoms for several weeks. In these patients, paired serologic antibody titers against canine influenza are a more appropriate diagnostic method [7, 8]. Conversely, the period of viral shedding for dogs infected with the H3N2 Canine Influenza virus can be as long as 4 weeks.

Mycoplasma cynos
Mycoplasmas are among the primary pathogens implicated in Atypical Respiratory Infections in people. These refer to bacterial respiratory infections associated with milder, chronic respiratory disease and prolonged infections. Mycoplasma canis and M. felis are common inhabitants of the laryngeal mucosa and nasopharynx of healthy dogs and cats. Their role as pathogens in CIRD is unclear. However, Mycoplasma cynos has been associated with pneumonia and lower respiratory infections in puppies, and has been documented to experimentally induce respiratory disease in dogs. Mycoplasmas are fastidious organisms and
are very difficult to culture, but PCR techniques can be very useful to document *Mycoplasma cynos* infections if appropriate samples are collected[9].

*Streptococcus equi subspecies zooepidemicus.*  
*Streptococcus equi* subsp. *zooepidemicus*, or “strep zoo”, is a β-hemolytic Streptococcal species that has been recently associated with widespread outbreaks of severe, often fatal, respiratory disease in shelter dogs. Dogs infected with Streptococcus equi initially resemble dogs infected with other components of the CIRD complex. However, many infected dogs will progress to develop hemorrhagic pneumonia. The later manifestations of disease are similar to those exhibited by people suffering from toxic shock syndrome, suggesting that the clinical aspects of disease may be associated with Streptococcal toxins. Reported mortality rates in infected dogs during outbreaks are as high as 50%. The risk of infection in dogs in communal settings is considerable, while reports of *Streptococcus equi* infections in individual pet dogs are rare. *Streptococcus equi* can be isolated from infected, affected dogs as well as from healthy horses, and both species pose a potential zoonotic risk to people. Cases of both dog-to-human and horse-to-human transmission of *Streptococcus equi* have been reported[10-12].

**Canine herpesvirus**  
Canine herpesvirus is one causative agent of a rapidly progressive, often fatal condition in neonatal dogs known as “fading puppy syndrome.” In young puppies, canine herpesviral infections can cause ocular, dermal, and genital lesions along with a severe interstitial pneumonia. Affected puppies may die within 24 hours of the onset of symptoms, and some dogs may die acutely with no overt symptoms. The mortality rate in puppies 1-3 months old has been reported as high as 100%, while deaths are rare in puppies older than six months old. Latently infected adult female dogs are believed to be the primary source of infection in young puppies.

Canine herpesviral infections in adult dogs may manifest as symptoms consistent with CIRD, including nasal discharge and a non-productive cough, along with ocular and genital lesions similar to those exhibited in infected puppies. Symptoms in adult dogs are usually self-limiting, requiring no intervention or general supportive care. Canine herpesvirus can enter a latency period in infected adult dogs, and may exhibit recrudescence later in life, although the these events are typically less severe[13].

**Other emerging pathogens potentially associated with CIRD**  
Mammalian reoviruses, canine minute viruses, and canine pneumoviruses have all been isolated from dogs exhibiting upper and lower respiratory symptoms. Mammalian reoviruses are capable of infecting all mammals, and have been isolated from the respiratory and gastrointestinal tracts of dogs exhibiting co-morbid respiratory and gastrointestinal symptoms. Canine minute viruses are another etiologic agent in “fading puppy syndrome,” and can cause manifestations in puppies similar to those caused by canine herpesvirus. Surviving littermates may exhibit upper respiratory localizing symptoms. Canine pneumovirus has been isolated from pharyngeal and nasal specimens of CIRD-affected dogs, predominantly as co-infections with canine parainfluenza virus, canine influenza virus, and canine respiratory coronavirus. While each of these agents has at least been temporally associated with respiratory disease in dogs, their roles as primary pathogens in the CIRD complex remains uncertain[14, 15].

**CLINICAL PRESENTATION OF CIRD**  
Most dogs infected with CIRD pathogens exhibit “uncomplicated” infections, characterized by mild to moderate, upper respiratory localizing symptoms. Many will experience sneezing and
nasal discharge as an early symptom, followed often by an acute onset of a non-productive, “honking” cough. Coughing is usually paroxysmal, with episodes lasting for minutes in severe cases. There is often a known or suspected exposure event associated with direct contact with other dogs or indirect contact with fomites within 3-10 days of the onset of symptoms. Despite what can often be severe upper airway localizing signs, these dogs are typically otherwise healthy, and infections are usually self-limiting (with the notable exceptions of canine distemper virus and canine influenza virus). In a subset of these dogs, an initially “uncomplicated” infection can become “complicated”, and associated with more mucoid or purulent nasal and pulmonary secretions, fever, anorexia, productive coughing, and occasionally respiratory distress. “Complicated” infections are often seen in puppies, immunocompromised patients (either through concurrent disease processes or as a result of drug therapy), and dogs with concurrent respiratory disease (e.g., chronic bronchitis with bronchiecctasis). Severely affected patients can die as a result of bronchopneumonia secondary to CIRD infections, demonstrating the importance of airway imaging early in the course of complicated infections.

**DIAGNOSIS**

Dogs with uncomplicated cases of CIRD can often be managed without extensive diagnostic testing, on the basis of their signalment, history, and physical examination findings. Documentation of the presence of the pathogen along with an immune response to the pathogen are necessary to confirm diagnosis, however, this is rarely performed in uncomplicated cases. Diagnostic testing may be indicated in uncomplicated cases which fail to respond to appropriate therapy, or in patients in whom the risk of progression to a complicated case is high (e.g., dogs with chronic lower airway disease).

Bacterial components can be documented via airway cytology and culture of respiratory secretions obtained via trans-tracheal or endotracheal lavage. Both bacterial and viral components of the complex can be documented through the use of respiratory polymerase chain reaction (PCR) panels. Pathogens in these panels include *Bordetella bronchiseptica*, *Mycoplasma cynos*, *Streptococcus equi*, canine adenovirus, canine distemper virus, canine herpes virus, canine parainfluenza virus, canine pneumovirus, canine respiratory coronavirus, and H1N1 influenza virus. It is important to note that not all pathogens are offered by all vendors. Collection of samples for PCR should include DEEP nasal, pharyngeal, and tonsillar swabs, as well as fluid or tissue samples from respiratory tract sampling, and serum samples. Interpretation of PCR results requires some degree of scrutiny, and should be considered in light of the patient.

Dogs with complicated cases should have respiratory sampling done when possible, as these dogs may be infected with CIRD pathogens or opportunistic pathogens. Systemic assessments should include complete blood counts and thoracic imaging.

**TREATMENT AND PREVENTION OF CIRD**

Dogs with uncomplicated cases of CIRD may require no therapy at all, as many cases are self-limiting within a few days to a couple of weeks. Dogs presenting with non-productive coughing may respond favorably to cough suppression both for patient comfort and to prevent cough-induced airway damage. Antibiotic therapy can be useful in cases caused or complicated by bacterial CIRD pathogens, both by shortening the duration of illness and decreasing the shedding of bacteria in the environment. In these cases, antibiotic selection should include drugs with an antimicrobial spectrum including gram negative bacteria (*Bordetella bronchiseptica*) and mollicutes (*Mycoplasma cynos*), and should be maintained for
two weeks. Examples of these include tetracyclines, fluoroquinolones, azalides, and macrolides.

Dogs with CIRD cases complicated with bronchopneumonia should be treated with broad spectrum, lipid soluble antibiotics to cover both for CIRD pathogens as well as opportunistic bacteria. The typical duration of therapy for dogs with complicated infections is 6-8 weeks, or 2 weeks beyond radiographic resolution of alveolar infiltrates. Intravenous fluids, saline nebulization, coupage, and low-intensity exercise should all be employed where possible in order to generate and maintain a productive cough. Cough suppressants should not be employed in dogs with bronchopneumonia, as coughing is an important clearance mechanism for the lower airways.

Maternal antibodies provide an important source of protection for young puppies against CIRD pathogens during the first 6-8 weeks of life, after which point, antibody titers begin to wane gradually. Circulating maternal antibodies directed against canine parainfluenza and canine adenovirus may interfere with the efficacy of parenterally-administered vaccines in young puppies. For this reason, core vaccination for canine parainfluenza virus and canine adenovirus should begin near the expected time of maternal antibody waning (6-8 weeks), and be boosted until ~12 weeks of age or beyond in order to ensure vaccine efficacy.

Vaccines are currently available for only six of the potential pathogens in the CIRD complex (canine parainfluenza virus, canine adenovirus, canine distemper virus, canine influenza viruses H3N8 and H3N2, and *Bordetella bronchiseptica*). Vaccination against canine parainfluenza virus, canine distemper virus, and canine adenovirus are considered part of the core canine vaccine protocols. While it is not technically considered a “core” vaccine, this author routinely recommends vaccination of puppies and adults to provide protection against *Bordetella bronchiseptica*. Vaccination against canine influenza virus is frequently recommend for “high risk” dogs (e.g., frequent boarders, dogs participating in day care, participants in dog shows, travelers), but as the reports of canine influenza become more widespread, recommendations for vaccination against canine influenza virus may be considered for any dog spending time commingled with other dogs.

Premise control and environmental management are also important aspects of CIRD prevention. Facilities should aim to minimize both direct (e.g., commingling in waiting areas) and indirect (e.g., community water bowls) contact between known or suspected infected dogs and vulnerable dogs. Altering traffic patterns between waiting areas and isolation areas, reducing personnel traffic between hospitals and boarding facilities or day care facilities, and triaging coughing dogs outside or in separate entrances can minimize pathogen spread. Since personnel are a very important potential source of fomite transmission, use of personal protective equipment (PPE) when handling known or suspected infectious patients should be a core component of practices or boarding facilities.

When known infectious cases are managed, routine disinfection of re-usable equipment (cages, bowls, medical equipment, muzzles, mouth gags, etc) should be performed after use. Devices that come in contact with mucosal surfaces (e.g., endotracheal tubes) should be disinfected with chlorhexidine, glutaraldehyde, quaternary ammonium compound, or other effective disinfectants that are safe for mucosal contact. Heavily contaminated runs, cages, or rooms should be disinfected with freshly prepared dilute bleach (~10%) or accelerated peroxide products. All items or surfaces to be disinfected should be first cleaned of organic debris, as this may reduce the effectiveness of disinfectants. Surfaces should be coated with disinfectant
for a minimum of 10 minutes of contact time to ensure a 99% knockdown of surface contaminants.

Environmental management should include considerations for both the ambient environment and the patient environment. Goals of ambient environmental management should include provision of adequate ventilation (10-20 room air changes per hour; outside ventilation when possible), avoiding extremes of humidity and temperature, and in-line filtration systems for recirculated air. Patient environment should be adapted to minimize stress in commingled dogs, as prolonged stress may inhibit immune function. Early weaning (in breeding environments), isolation, and prolonged food and water restriction are potential stressors that should be avoided when possible for pet dogs.