Aetiology of Canine Infectious Respiratory Disease Complex and Prevalence of its Pathogens in Europe

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Summary
The canine infectious respiratory disease complex (CIRDC) is an endemic worldwide syndrome involving multiple viral and bacterial pathogens. Traditionally, Bordetella bronchiseptica (Bb), canine adenovirus type 2 (CAV-2), canine distemper virus (CDV), canine herpesvirus (CHV) and canine parainfluenza virus (CPiV) were considered the major causative agents. Lately, new pathogens have been implicated in the development of CIRDC, namely canine influenza virus (CIV), canine respiratory coronavirus (CRCoV), canine pneumovirus (CnPnV), Mycoplasma cynos and Streptococcus equi subspecies zooepidemicus. To better understand the role of the different pathogens in the development of CIRDC and their epidemiological relevance in Europe, prevalence data were collected from peer-reviewed publications and summarized. Evidence of exposure to Bb is frequently found in healthy and diseased dogs and client-owned dogs are as likely to be infected as kennelled dogs. Co-infections with viral pathogens are common. The findings confirm that Bb is an important cause of CIRDC in Europe. CAV-2 and CDV recovery rates from healthy and diseased dogs are low and the most likely explanation for this is control through vaccination. Seroconversion to CHV can be demonstrated following CIRDC outbreaks and CHV has been detected in the lower respiratory tract of diseased dogs. There is some evidence that CHV is not a primary cause of CIRDC, but opportunistically re-activates at the time of infection and exacerbates the disease. The currently available data suggest that CIV is, at present, neither a prevalent nor a significant pathogen in Europe. CPiV remains an important pathogen in CIRDC and facilitates co-infection with other viral and bacterial pathogens. CnPnV and CRCoV are important new elements in the aetiology of CIRDC and spread particularly well in multi-dog establishments. M. cynos is common in Europe and is more likely to occur in younger and kennelled dogs. This organism is frequently found together with other CIRDC pathogens and is significantly associated with more severe respiratory signs. S. zooepidemicus infection is not common and appears to be a particular problem in kennels. Protective immunity against respiratory diseases is rarely complete, and generally only a reduction in clinical signs and excretion of pathogen can be achieved through vaccination. However, even vaccines that only reduce and do not prevent infection carry epidemiological advantages. They reduce spread, increase herd immunity and decrease usage of antimicrobials. Recommending vaccination of dogs against pathogens of CIRDC will directly provide epidemiological advantages to the population and the individual dog.

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Keywords: canine infectious respiratory disease complex; dog; pathogen; vaccination
Introduction

The canine infectious respiratory disease complex (CIRDC) is an endemic worldwide syndrome involving multiple viral and bacterial pathogens (LeRoith et al., 2012). Host and environmental factors play a role in the development of the disease and its severity. CIRDC has been referred to historically as ‘kennel cough’ or ‘canine infectious tracheobronchitis’ and is described as an acute, highly contagious respiratory infection of dogs. The disease is characterized by sudden onset, paroxysmal, dry, ‘honking’ cough with variable expectoration and naso-ocular discharge (Ford and Vaden, 1998). Signs last for days to weeks and are mild to moderate in most dogs. In puppies and dogs with immunosuppression or other concurrent diseases, CIRDC can be complicated by bronchopneumonia, resulting in more severe signs such as dyspnoea, weight loss, pyrexia and even death (Radhakrishnan et al., 2007).

CIRDC can affect dogs of all ages and causes sporadic illness as well as outbreaks (Dear, 2014). It commonly spreads where large numbers of dogs are housed in close confinement (e.g. in shelters or boarding kennels) or are gathered (e.g. at dog shows and training classes) (Erles et al., 2004). The disease is rapidly transmitted through droplets or asymptomatic carriers as most of its pathogens are ubiquitous. It is thought that, in most cases, viral infections initially damage the epithelium of the upper respiratory tract (Ford and Vaden, 1998), allowing secondary bacterial infections to add to the destruction and inflammation in the upper respiratory tract. As the host immune response is initiated, spread of the infection into the lower respiratory tract is normally prevented and the infection eventually cleared. Under adverse circumstances however, the infection may reach the lower airways and cause pneumonia or chronic respiratory disease.

Many bacteria and viruses can be involved in CIRDC. Traditionally, Bordetella bronchiseptica (Bb), canine adenovirus type 2 (CAV-2), canine distemper virus (CDV), canine herpesvirus (CHV) and canine parainfluenza virus (CPIV) were considered the major causative agents. Lately, new pathogens have been implicated in the development of CIRDC, namely canine influenza virus (CIV), canine respiratory coronavirus (CRCoV), canine pneumovirus (CnPnV), Mycoplasma cynos and Streptococcus equi sub-species zooepidemicus (S. zooepidemicus). Finally, canine bocavirus and canine hepacivirus have been isolated and/or loosely associated with respiratory disease in dogs (Priestnall et al., 2014), but are not considered further in this review.

Biology and Pathophysiology of Pathogens in CIRDC

Bb is a gram-negative, aerobic coccobacillus (reviewed by Goodnow, 1980). Many mammals are...
susceptible to infection with Bb including dogs. Research has concentrated on Bb infections in pigs (atrophic rhinitis), dogs (CIRDC), laboratory animals (bronchopneumonia) and increasingly man (Woolfrey and Moody, 1991; Ducours et al., 2017). The bacterium can act as a primary pathogen in dogs (Wright et al., 1973) or cause CIRDC concurrently with other bacteria and/or viruses.

Bb belongs to the genus *Bordetella* together with *Bordetella pertussis* and *Bordetella parapertussis*, which cause whooping cough in man (reviewed by Mattoo and Cherry, 2005). Of the three species, only Bb survives in the environment and appears to be able to spread through amoeba (Taylor-Mulneix et al., 2017). On entering a mammalian host, the bacterium expresses virulence factors including adhesins such as filamentous haemagglutinin (FHA), pertactin, tracheal colonization factor and fimbriae that facilitate adhesion to host cells (Edwards et al., 2005), and toxins, such as adenylate cyclase haemolysin, dermonecrotic toxin and tracheal cytotoxin that damage the ciliated epithelium (Bock and Gross, 2001). The signals for the in-vivo change from the ‘environmental’ to the ‘host’ phase are unknown. In *vitro*, however, growth at 37°C was determined to be a trigger.

Bb is a common pathogen and able to cause CIRDC without the help of respiratory viruses (Schulz et al., 2014b; Viitanen et al., 2015). The incubation time is between 2 and 10 days (Bemis et al., 1977). Affected animals develop a dry paroxysmal cough, nasal discharge and, only in severe cases, depression, pneumonia and death. The morbidity is high, but the disease is rarely fatal. Histopathology has shown that infection is limited to the ciliated mucosa where an inflammatory response with an influx of polymorphonuclear cells can be observed (Thompson et al., 1976; Bemis et al., 1977). Through adhesion to the cilia and the excetration of toxins the bacterium is thought to cause ciliostasis and failure of mucociliary clearance (Woolfrey and Moody, 1991). Disease duration varies from a few days to several weeks or longer in more severely affected dogs (Thompson et al., 1976; Canonne et al., 2016). Bb is shed from the respiratory tract of infected animals for a variable length of time, sometimes many months. Immunity to Bb involves the local mucosal production of specific immunoglobulin (Ig) A and IgG antibodies, although serum IgG responses are also made and these are more readily used to monitor the efficacy of vaccination in an experimental setting (Chalker et al., 2003b). Treatment includes antibiotics that reach therapeutic concentrations in the respiratory tract (Datz, 2003).

CAV-2 is an non-enveloped DNA virus belonging to the family Adenoviridae. The virus is highly related to canine hepatitis virus (CAV-1) and shares approximately 75% of the nucleotide sequence (reviewed by Buonavoglia and Martella, 2007). CAV-2 is limited to the respiratory tract and to a lesser degree the intestinal epithelium, while CAV-1 causes systemic infection. There is one report of the molecular detection of CAV-2 in the brain of a puppy with neurological signs (Benetka et al., 2006), but in this case no histopathological changes were detected in the brain suggesting viral pathology.

CAV-2 was first isolated from a dog with laryngotracheitis in Canada in 1961 (Ditchfield et al., 1962). It is endemic worldwide and its hosts include wild carnivores and marine mammals. On entry into the host via droplets, non-ciliated epithelial cells of the upper respiratory tract become infected. Viral replication peaks after 3–6 days and then declines as host immunity develops. Infection with CAV-2 alone is generally mild and self-limiting, but can be complicated by co-infections. Immunity to CAV-1 cross-protects against CAV-2 and vice versa. Neutralizing antibody levels correlate with protection and can be used to evaluate the need for vaccination.

CDV is an enveloped negative sense single-stranded RNA virus belonging to the family Paramyxoviridae in the genus *Morbillivirus*, together with measles virus (reviewed by Martella et al., 2008), and usually causes severe systemic disease in carnivores. CDV initially replicates in lymphoid cells of the respiratory tract before disseminating throughout the body 3–4 days after infection. This viraemic phase is characterized clinically by fever. The virus then infects and multiplies in epithelial cells of various organs and the central nervous system (CNS) (Elia et al., 2015). From 10 days after infection, respiratory, gastrointestinal and/or dermatological signs are observed such as ocular and nasal discharge, dyspnoea, diarrhoea, vomiting and hyperkeratosis of the foot pads.

If the virus cannot be contained by the immune response, neurological signs due to demyelination may also be observed from 20 days after infection (Gillespie and Rickard, 1956). Neurological signs generally become progressively worse, as the virus is able to persist in the CNS. A rare neurological manifestation of CDV infection is ‘old dog encephalitis’, which is observed in vaccinated adult dogs. It is thought that the virus is able to persist in the CNS following infection despite the development of immunity and does not start to trigger neurological signs until adulthood (Axthelm and Krakowka, 1998).

Distemper can be challenging to diagnose if clinical signs are not multisystemic (Chvala et al., 2007).
Lymphopenia is a characteristic aspect of CDV infection that is normally not shared by the other respiratory pathogens. Immunity against CDV is fully protective and long lasting (Schultz et al., 2010). Neutralizing antibody levels correlate with protection and can be used to evaluate the need for vaccination.

CHV is an enveloped DNA virus belonging to the family Herpesviridae and was first identified as a canine pathogen in 1965 (Carmichael et al., 1965). Its host range is restricted to canids. The virus causes fetal or perinatal death if infection of immunologically naive animals occurs during pregnancy or shortly after birth (reviewed by Buonavoglia and Martella, 2007; Evermann et al., 2011). In pups over 2 weeks of age and adult dogs, clinical signs are rarely observed following infection. The sites of entry are the respiratory and genital tracts. From there the virus moves to the sensory ganglia and becomes latent. If immunity wanes due to stress, disease, immunosuppressive therapy, pregnancy or old age, the virus is reactivated and starts shedding from many mucosal surfaces. Reactivation normally does not cause clinical signs, but in some individuals it has also been implicated in CIRDC, ocular disease, reproductive disorders, genital lesions and even systemic disease (Kumar et al., 2015).

Influenza A viruses are negative sense segmented single-stranded RNA viruses belonging to the family Orthomyxoviridae (reviewed by Buonavoglia and Martella, 2007). There are different viruses for each species and these usually only spread within the species; however, some subtypes are able to cross between species. The virus is enveloped so is not stable for long in the environment and contains eight separate gene segments, which facilitate the exchange of segments between different strains in a process called reassortment. Strains are identified and classified by their surface protein subtypes, haemagglutinin (H1 to H16) and neuraminidase (N1 to N9). Historically, dogs were considered resistant to influenza infection. In 2004, however, CIV was detected in greyhounds in Florida following repeated outbreaks of respiratory disease over a number of years (reviewed by Harder and Vahlenkamp, 2010). The virus is transmitted from dog to dog, and seroprevalence can be as high as 49% in the USA. The original Florida isolate was a H3N8 subtype and stemmed from an equine influenza strain that circulated in the USA in the early 1990s. Another CIV, subtype H3N2, has been circulating among dogs in southeastern Asia since 2007, with a seroprevalence of up to 33%. H3N2 CIV has also been associated with outbreaks of respiratory disease in dogs in the USA since 2015 (Voorhees et al., 2017). These two influenza A subtypes are unique in that they are both efficiently transmitted horizontally among co-mingled dogs and both cause respiratory disease in susceptible dogs, with morbidity rates as high as 80%. Canine infections with other subtypes (e.g. H5N1, H1N1 and H3N1) have been reported sporadically, but only rarely associated with respiratory disease.

Following experimental infection, CIV causes inflammation and necrosis of the ciliated epithelium of the respiratory tract as well as in the major organs (Castleman et al., 2010). Morbidity can reach 100% and is usually characterized by upper respiratory disease with fever for 10–14 days. In a minority of dogs, peracute death due to haemorrhagic bronchopneumonia has been described following CIV infections that were complicated by bacterial co-infections (Yoon et al., 2005). The severity of the infection depends on the challenge CIV strain. Inactivated virus vaccines (monovalent or bivalent) have been produced to protect dogs against H3N8 and H3N2 influenza A virus infection (Parrish and Huber Voorhees, 2019).

CPiV belongs to the family Paramyxoviridae, subfamily Rubulavirinae, comprising also distantly related human parainfluenza viruses 2 and 4, and mumps virus (reviewed by Ellis and Krakowa, 2012; ICTV, 2018). Although the virus is generally still referred to as CPiV in veterinary medicine, the correct nomenclature evolved from ‘parainfluenza virus 5’ (Rima et al., 2014) to ‘mammalian orthorubulavirus 5’, and this name is consistent with the isolation of the virus from various mammals, including man and dogs (ICTV, 2018). The virus is highly contagious and therefore endemic worldwide. It was first isolated together with other pathogens in 1967 from laboratory dogs with respiratory disease. Transmission occurs via droplets. Morbidity depends on the density of the dog population as the virus does not survive for long in the environment. The site of entry is the respiratory tract. CPiV is known to mainly affect the surface epithelium of the respiratory tract and to rarely cause systemic infection (Appel and Binn, 1987). Clinical signs involve mild respiratory signs 2–8 days after infection that last less than a week unless the disease is complicated by other pathogens (Viitanen et al., 2015). Very young, geriatric or immunocompromised dogs may also show systemic signs. There are occasional reports of CPiV being isolated from tissues outside the respiratory tract, but these are exceptions (Binn et al., 1979; Buonavoglia and Martella, 2007). It is assumed that immunity to CPiV involves local mucosal production of specific antibody, but most published studies measure systemic antibody responses (Ellis and Krakowa, 2012). Mucosal and parenteral CPiV vaccines are available.
CnPnV belongs to the family Pneumoviridae, a new virus family related to the family Paramyxoviridae, which includes CDV and CPIV. In the genus Orthopneumovirus, CnPnV is closely related to human respiratory syncytial virus and bovine respiratory syncytial virus. CnPnV was first isolated in the USA from kennelled dogs with respiratory disease in 2010 and has since been detected in Europe (Renshaw et al., 2010; Mitchell et al., 2013; Decaro et al., 2016). Little is known about the pathogenesis of CnPnV infection in dogs. Experimental infection of mice showed that CnPnV replicated in lung tissue and caused lethal disease at higher doses; infection induced antibody responses and protective immunity (Percopo et al., 2011).

CRCoV was first isolated from the respiratory tract of dogs with CIRDC from a UK shelter (Erles et al., 2003) and is a relatively new addition to the possible causes of CIRDC. It is an enveloped single-stranded positive RNA virus and belongs to the family Coronaviridae, genus Betacoronavirus, which also includes bovine coronavirus and human coronavirus, implicated in shipping fever and severe acute respiratory syndrome (SARS), respectively. CRCoV is genetically distinct from canine enteric and pantropic alpha coronavirus. CRCoV is probably transmitted via droplets and initially infects ciliated epithelial cells in the trachea and lymphoid cells of the tonsils (reviewed by Priestnall et al., 2014). This leads to reduction in mucociliary clearance and facilitates secondary infections. Infected dogs show the typical signs of CIRDC and shed infectious virus for up to 6 days.

Mycoplasma spp. lack a bacterial cell wall and are thus distinct from other bacteria (reviewed by Chalker et al., 2004). They are difficult to grow in culture and consequently are often underdiagnosed. To date, there are 15 species of Mycoplasma found in dogs and many more have been described in other animals and man. Mycoplasma spp. are normal commensals of the upper respiratory tract, which has complicated investigations into their virulence. M. cynos was first described in 1972 following isolation from the lungs of a dog with pneumonia. Since then M. cynos has been detected in many other cases of CIRDC, often concurrently with viral infections, confounding the interpretation of its role in the pathogenesis of CIRDC (reviewed by Priestnall et al., 2014; Maboni et al., 2018). However, Zeugswetter et al. (2007) reported an outbreak of M. cynos mono-infection in a litter of 3-week-old golden retriever pups and a recent study supports the role of M. cynos as a primary pathogen in the lower respiratory tract (Jambhekar et al., 2019).

S. zooepidemicus is a β-haemolytic Lancefield group C streptococcus. It is part of the normal bacte-

Prevalence of CIRDC

The most recent prevalence figures on respiratory disease in dogs are provided by the Small Animal Veterinary Surveillance Network (SAVSNET), which held approximately 1.7 million electronic health records from 227 veterinary practices in the UK (Singleton et al., 2019). Between January 2018 and February 2019, 0.9% of dogs were presented for respiratory signs. More detailed information on the consultations was obtained from a randomly selected subgroup of 2,404 canine patients. This showed that the most common presenting sign was coughing (68%) and that the majority of patients were presented for the first time (52.2%) and after a period of illness of up to 1 week (47.2%). In 71.2% of cases, the observed clinical signs were considered to be respiratory in origin by the attending veterinarian. This equates to approximately 0.64% of dogs presented annually to veterinary practices. The proportion of dogs with CIRDC is likely to be lower, as respiratory signs can
also be due to non-infectious causes such as neoplastic disease, allergic reactions or brachycephaly (O’Neill et al., 2015).

Similar SAVSNET surveys were performed in 2014–2015 and 2017 (Sanchez-Vizcaino et al., 2016; Arsevska et al., 2018). The proportion of canine patients presented for respiratory signs was 1.7% in 2014–2015 and 1.3% in 2017, compared with 0.9% in 2018. As before, coughing was the main clinical sign and the majority of dogs had shown the signs for <1 week.

O’Neill et al. (2014) investigated the prevalence of disorders in a representative subset of electronic health records from UK primary-care veterinary practices (Vet Compass) between 2009 and 2013 with the aim of detecting differences between purebred and crossbred dogs. Records of 3,884 dogs, mostly purebred (79.4%), were included in the survey. The prevalence of upper respiratory tract disease was 5.7% without a significant difference between purebred (5.6%) and crossbred (6.4%) dogs.

A pet owner survey was performed in 2014 on 43,005 purebred dogs from the UK (Wiles et al., 2017). The advantage of such studies is that disease episodes for which no veterinary advice was sought are included in the dataset. On the other hand, misinterpretation is possible as disorders were not always clinically confirmed. Furthermore, owners may forget disease episodes as all data are based on memory recall. Respiratory disease was reported in 2.8% of dogs, with kennel cough (0.26%), regular reverse sneezing (0.15%), unspecified respiratory signs (0.14%) and brachycephalic airway obstruction syndrome (0.12%) as the most commonly reported conditions. Boxers were significantly more likely to be affected by kennel cough than any of the other surveyed breeds.

Adams et al. (2010) reported a mortality rate of 1.2% due to respiratory disease in 15,881 pedigree dogs between 1994 and 2014. The reported causes were ‘unspecified disease or failure’ (0.4%), ‘pneumonia’ (0.3%), ‘laryngeal paralysis’ (0.2%), ‘choking’ (0.1%), ‘bronchitis’ (0.1%), ‘tracheal collapse’ (0.1%) and ‘other’ (0.1%). Another client-based study covering 5,663 dogs between 2005 and 2014 found no mortality due to respiratory disease (Lewis et al., 2018). These findings indicate that respiratory disease is rarely lethal in the pet dog population.

CIRDC prevalence data from other European countries are sparse. Balboni et al. (2014) selected health data on 51 client-owned dogs at a teaching hospital in Italy in 2012. Cases were included in the study if owners agreed to have their pets sampled non-invasively (i.e. rectal swabs and spontaneous urinary samples). The majority of dogs were adults (76%) and purebred (71%). Just over half of the sampled dogs showed no clinical signs (55%). Respiratory signs were observed in 7.8% of dogs. A Danish study from 1997 collected, among other information, health data on 4,295 purebred dogs registered with the Danish Kennel Club (Proschowsky et al., 2003). Nearly 11% of dogs had shown a respiratory disease event during their lifetime, but the responses were not validated by a veterinarian. Male dogs were significantly more likely to be affected by respiratory disease (12.2%) than female dogs (9.6%), as were some breeds, including Schnauzer (17.9%), scent hounds (17.5%) and sighthounds (16.4%).

The prevalence of CIRDC is expected to be higher in kennels where the risk of infection is increased due to turnover of animals, intensive housing and stress (Pesavento and Murphy, 2014). Chalker et al. (2003a) investigated CIRDC in a rehoming kennel and found respiratory signs in 66% of dogs with 12% of dogs showing severe signs. The proportion of dogs with CIRDC increased after arrival at the kennel from 21.1% in week 1 to >70% in weeks 2–4. After the fourth week, the proportion of dogs with CIRDC decreased again.

**Prevalence of Bordetella bronchiseptica**

European publications reporting the prevalence of Bb in healthy and diseased dogs were reviewed (Table 1). The seroprevalence of Bb was 22% in healthy, unvaccinated adult dogs from Sweden between 2000 and 2001 (Eglund et al., 2003). Exposure rates may even be higher as exposure does not always result in seroconversion and antibody titres decline over time if not boosted through repeated infections (Jacobs et al., 2005).

Bacterial isolation from healthy dogs ranged from 0.0% to 45.6% in Austria, Germany and Belgium between 2009 and 2016 (Schulz et al., 2014b; Stejskal et al., 2017; Canonne et al., 2018). The variability may be explained by the different samples collected in the different studies. Bb is known to reside in the upper respiratory tract of healthy animals (Gueirard et al., 1998) so the lack of detection by Stejskal et al. (2017), in which nasal and pharyngeal swabs were analysed by both culture and PCR, is surprising. Isolation from the lower respiratory tract is usually associated with disease (Thompson et al., 1976) so isolation would be less likely from bronchoalveolar lavage fluid (BALF) samples from healthy dogs used by Canonne et al. (2018).
The detection rates of Bb in dogs with respiratory disease were also variable and ranged from 3.3% to 78.7%. An unusually low detection rate was reported by Stejskal et al. (2017), suggesting perhaps a high threshold of detection in their assays. Rheinwald et al. (2015) detected Bb in 5.2% of BALF samples from dogs with CIRDC in Germany, the second lowest detection rate of all listed studies. In this retrospective study, microbiology results of 493 dogs with signs of respiratory disease were reviewed for isolation of Bb over 22 years (1989–2011). In contrast, there was a detection rate of 78.7% reported for Bb in a subsequent study at the same institution by Schulz et al. (2014b). Samples for this study were collected over a 12-month period from 2011 to 2012, which may have been a time of unusually high Bb prevalence in the South of Germany. Another reason may have been the lower age of the sampling population (median 3.5 years, range 3 months–16 years) compared with a median of 6 years in the study by Rheinwald et al. (2015) and the more sensitive detection method.

Against expectations, dogs from private households were significantly more likely to be infected with Bb than dogs from shelters in the study by Schulz et al. (2014b).

The second highest detection rate was reported in an Italian study of 50 diseased dogs (Corona et al., 2013). A total of 52% of dogs with CIRDC tested positive for Bb. Of these, 76% (13/17) of the samples collected from dogs <1 year of age were positive for Bb, 44% (8/18) of the samples collected from dogs between 1 and 7 years of age were positive for Bb and 33% (5/15) of the samples collected from dogs >7 years of age were positive for Bb.

Bb was detected in dogs with pneumonia (14.7%, Woehrer et al., 2016), eosinophilic bronchopneumonia (25%) and chronic bronchitis (10%) (Canonne et al., 2018). Woehrer et al. (2016) found Bb together with Pasteurella multocida in five puppies; in one of these puppies concurrently with CRCoV and in another puppy concurrently with CRCoV and M. canis. Bb was also detected in five adult

| Country  | Year          | Population                                      | Method                                      | Detection rate | Reference       |
|----------|---------------|-------------------------------------------------|---------------------------------------------|----------------|-----------------|
| Sweden   | 2000–2001     | 302 healthy, non-vaccinated dogs ≥2 years old    | Bb specific IgG in serum by ELISA           | 22.0%          | Eglund et al. (2003) |
| Germany  | 1989–2011     | 493 dogs with CIRDC                             | Bb in BALF by culture                       | 5.2%           | Rheinwald et al. (2015) |
| Austria  | 1997–2007     | 68 dogs with pneumonia                          | Bb in lung samples by IHC                   | 14.7%          | Woehrer et al. (2016) |
| Germany  | 2004–2009     | 84 dogs with CIRDC                             | Bb in BALF by culture                       | 20.2%          | Steinfeld et al. (2012) |
| EU       | 2008–2010     | 215 dogs with CIRDC                             | Bb by culture                               | 22.8%          | Morrissey et al. (2016) |
| Italy    | Not recorded  | 50 dogs with CIRDC                             | Bb in BALF by culture                       | 52.9%          | Corona et al. (2013) |
| Germany  | 2011–2012     | 61 dogs with CIRDC                              | Bb in nasal and pharyngeal swabs by RT-PCR  | 78.7%          | Schulz et al. (2014b) |
| Italy    | 2011–2013     | 78 dogs with CIRDC                             | Bb in nasal and pharyngeal swabs by RT-PCR  | 10.3%          | Decaro et al. (2016) |
| Austria  | 2013–2015     | 214 dogs with CIRDC                            | Bb in nasal and throat swabs by PCR and culture | 3.3%          | Stejskal et al. (2017) |
| Poland   | 2014–2015     | 40 dogs with CIRDC                             | Bb in URT swabs and tracheal fluid by PCR   | 30.0%          | Kaczorek et al. (2016) |
| Belgium  | 2009–2016     | 24 dogs with eosinophilic bronchopneumonia     | Bb in BALF by qPCR                          | 25.0%          | Canonne et al. (2018) |
|          |               | 21 dogs with chronic bronchitis                 |                                             | 10.0%          |                 |
|          |               | 15 healthy dogs                                 |                                             | 13.0%          |                 |
| UK       | 2016–2019     | 1,602 canine respiratory samples               | Bb in samples by qPCR                       | 12.9–17.3%     | Singleton et al. (2019) |

BALF, bronchoalveolar lavage fluid; Bb, Bordetella bronchiseptica; CIRDC, canine infectious respiratory disease complex; ELISA, enzyme linked immunosorbent assay; EU, European Union; IHC, immunohistochemistry; PCR, polymerase chain reaction; qPCR, quantitative PCR; RT-PCR, reverse transcriptase PCR; UK, United Kingdom; URT, upper respiratory tract.
dogs, two of which showed concurrent infection with *P. multocida*. The findings confirm that Bb participates in lung infections, but can also be the sole pathogen, even in adult dogs.

The latest UK survey on the prevalence of Bb reported a detection rate of 14.5% in respiratory samples (Singleton et al., 2019). The survey included 1,602 canine samples that had been analysed by four laboratories in the UK over a 3-year period from January 2016 to February 2019. The greatest proportion of positive samples was found in winter (17.3%), followed by summer (14.1%), autumn (13.6%) and spring (12.9%). Spatial trends could not be detected as areas with high and low proportions of positive samples were observed all over the UK.

In summary, evidence of exposure to Bb is frequently found in healthy and diseased dogs and client-owned dogs are as likely to be infected as kennelled dogs. Co-infections with viral pathogens are common. Bb was also found in respiratory diseases that are not typically part of the CIRDC spectrum. The findings confirm that the pathogen is still an important cause of CIRDC in Europe.

### Prevalence of Canine Adenovirus Type 2

European publications reporting the prevalence of CAV-2 in healthy and diseased dogs were reviewed (Table 2). Two investigations assessed the presence of CAV-2 in the respiratory tract of healthy dogs or dogs with clinical signs other than respiratory signs in Germany (Schulz et al., 2014a,b) and Italy (Balboni et al., 2014). Schulz et al. (2014b) found CAV-2 in only one healthy dog (1.1%), while Balboni et al. (2014) detected CAV-2 in half of the healthy dogs and 63.2% of the dogs with clinical signs. Vaccination status was unlikely to explain the high prevalence in healthy Italian dogs since >90% of the study population was vaccinated.

The same group reported a 100% prevalence of CAV-2 in dogs with respiratory signs, but the number of diseased dogs in the study was small (n = 4) and samples had been collected within a 5-week period during early summer when CAV-2 was perhaps circulating in the local dog population (Balboni et al., 2014). Moreover, the samples analysed in this study were faecal swabs and urine samples, which are not the traditional sources for CAV-2 detection. The virus is mainly found in the respiratory tract and associated lymphoid tissues, although detection in faeces is reported (Hamelin et al., 1983; Macartney et al., 1988).

In general, CAV-2 recovery rates from healthy and diseased dogs were low and the most likely explanation for this is control through vaccination. Erles et al. (2004) did not detect CAV-2 in a shelter dog population where all dogs were vaccinated on arrival.

| Country | Year   | Population                                      | Method                                      | Detection rate | Reference          |
|---------|--------|------------------------------------------------|---------------------------------------------|----------------|--------------------|
| UK      | Not recorded | 95 vaccinated shelter dogs               | CAV-2 in tracheal and lung samples by RT-PCR | 0.0%           | Erles et al. (2004) |
| Austria | 1997–2007 | 68 dogs with pneumonia                     | CAV-2 in lung samples by ISH                | 0.0%           | Wöhrer et al. (2016) |
| Austria | 2013–2015 | 214 dogs with CIRDC; 50 healthy dogs       | CAV-2 in nasal and tonsil swabs by RT-PCR   | 0.5%           | Hiebl et al. (2019) |
| Germany | 2011–2012 | 61 dogs with CIRDC; 90 healthy dogs        | CAV-2 in nasal and pharyngeal swabs by RT-PCR  | 0.0% 1.1%     | Schulz et al. (2014b) |
| Italy   | 2012    | Four dogs with respiratory signs; 28 healthy dogs; 19 dogs with signs other than respiratory signs | CAV-2 in rectal swabs and urine samples by PCR | 50.0% 63.2% | Balboni et al. (2014) |
| Italy   | 2011–2013 | 78 dogs with CIRDC                       | CAV-2 in nasal and pharyngeal swabs by RT-PCR | 0.0%           | Decaro et al. (2016) |
| Finland | 2011–2013 | 20 dogs with bacterial pneumonia; 15 dogs with chronic Bb infection | CAV-2 in BALF/TTW by RT-PCR | 0.0%           | Viitanen et al. (2015) |
| Poland  | 2014–2015 | 40 dogs with CIRDC                        | CAV-2 in URT swabs and tracheal lavage fluid by PCR | 0.0%           | Kaczorek et al. (2016) |

BALF, bronchoalveolar lavage fluid; Bb, *Bordetella bronchiseptica*; CAV-2, canine adenovirus type 2; CIRDC, canine infectious respiratory disease complex; ISH, in-situ hybridization; PCR, polymerase chain reaction; RT-PCR, reverse transcriptase PCR; TTW, transtracheal wash; URT, upper respiratory tract.
Another explanation could be the short period of viral shedding following infection (Buonavoglia and Martella, 2007), which may hamper detection unless samples are collected at onset of clinical signs.

**Prevalence of Canine Distemper Virus**

European publications reporting the prevalence of CDV in healthy and diseased dogs were reviewed (Table 3). Reports into the prevalence of CDV in healthy dogs or dogs with CIRDC were sparse. In Germany (Schulz et al., 2014b) and Finland (Viitanen et al., 2015) neither healthy dogs nor dogs with CIRDC were infected with CDV. Woehrer et al. (2016) found CDV in 16.2% of Austrian dogs with pneumonia when investigating historical samples by immunohistochemistry. All CDV-positive dogs were ≤12 months of age and many had concurrent neurological and/or gastrointestinal signs. Di Francesco et al. (2012) reported a CDV prevalence of 56.6% in Italian dogs that suffered from respiratory signs coupled with neurological and/or gastrointestinal signs. An investigation into a CDV outbreak amongst dogs imported from Hungary in 2015 showed that seven of 11 dogs with distemper had clinical signs involving at least two organ systems, mainly the gastrointestinal and respiratory tracts (Willi et al., 2015). Even if CDV is rarely diagnosed in dogs in Europe, the infection remains in the wild population, as for example in foxes (Garigliany et al., 2018), with a constant risk of infection of the canine population.

These findings suggest that CDV usually causes systemic disease, but distemper presenting mainly as respiratory disease has been described (Chvala et al., 2007). CDV involvement in CIRDC appears to be rare due to vaccination. Mitchell et al. (2017) showed that vaccination against CDV was associated with a significantly lower risk of CIRDC and severe respiratory signs.

**Prevalence of Canine Herpesvirus**

European publications reporting the prevalence of CHV in healthy dogs and dogs with respiratory signs were reviewed (Table 4). CHV has been implicated as a causative agent of CIRDC because experimental infections have resulted in respiratory signs (Karpas et al., 1968; Appel et al., 1969). In Belgium, approximately half of investigated dogs, including healthy dogs and dogs with various ailments, were found to be seropositive (Ronsse et al., 2002). In Germany, Italy, Lithuania and the UK, seropositivity or PCR positivity ranged from 0.0% to 27.7% in healthy adult dogs (Erles et al., 2004; Erles and Brownlie, 2005; Manteufel et al., 2008; Musayeva et al., 2013; Pratelli et al., 2014; Schulz et al., 2014b; Bottinelli et al., 2016). In some instances, seropositivity was higher in kennelled dogs or increased following a stay at a kennel (Erles et al., 2004; Erles and Brownlie, 2005; Musayeva et al., 2013). In Norway, 80% of healthy adult dogs were seropositive for CHV (Krogenæsa et al., 2012). Seropositivity is higher in dogs with reproductive problems than in healthy dogs (Van Gucht et al., 2001; Dahlbom et al., 2009; Cobzariu et al., 2018).

Erles et al. (2004) found CHV in 12.8% of tracheal samples and 9.6% of lung samples of shelter dogs that were humanely destroyed because of behavioural...
problems or severe (including respiratory) diseases. The presence of CHV was more likely if dogs had moderate to severe signs of CIRDC, but this was not significant. In another study (Erles and Brownlie, 2005), CHV was not isolated from tonsillar swabs of dogs from two training kennels, which occasionally had outbreaks of CIRDC; however, there was seroconversion against CHV following these outbreaks. Vojtek et al. (2010) investigated the presence of antibodies against CHV in 20 dogs with CIRDC and found that 60% were positive, while none of the healthy control dogs tested seropositive. Kaczorek et al. (2016) isolated CHV from 80% of dogs with CIRDC and found that 60% were positive, while none of the healthy control dogs tested seropositive.

Kaczorek et al. (2016) isolated CHV from 80% of dogs with CIRDC and found that detection was more successful with tracheal lavage samples than swabs from the upper respiratory tract. Unfortunately this study did not contain a control population. Schulz et al. (2014b) failed to find CHV in healthy dogs and dogs with CIRDC when testing nasal and pharyngeal swabs. A Finnish study of dogs with bacterial pneumonia and chronic bronchitis did not yield any CHV when BALF and transtracheal washes were tested (Viitanen et al., 2015).

In summary, seroconversion to CHV can be demonstrated following CIRDC outbreaks and CHV has been detected in the lower respiratory tract of diseased dogs. Whether CHV is the primary cause of CIRDC or, as a latent herpesvirus, opportunistically reactivates at the time of infection with other pathogens of the complex is unclear. Kaczorek et al. (2016) found CHV in combination with other pathogens in 71.9% of dogs with CIRDC compared with 28.1% of dogs with only CHV. Samples were not tested for all possible pathogens, leaving doubt as to whether CHV infection alone induced the clinical signs. That CHV predominantly plays an exacerbating rather than initiating role in CIRDC is corroborated by the findings of Erles et al. (2004) who detected CHV later than other viral infections in a shelter population of dogs and in more moderate and severe cases of CIRDC.

### Prevalence of Canine Influenza Virus

European publications reporting the seroprevalence of influenza A viruses in healthy and diseased dogs were reviewed (Table 5). Apart from an historical outbreak in the UK (Daly et al., 2008) the reported seroprevalence against CIV was low in Europe before 2013. In Italy, up to 3.56% of dogs had antibodies against CIV’ (Dundon et al., 2010; Piccirillo et al., 2010; Pratelli and Colao, 2014). In Germany the seroprevalence was up to 2.86% (Damiani et al., 2012; Schulz et al., 2014a). A survey of dogs from France, Hungary, Italy, Greece, The Netherlands and Spain showed a seroprevalence of 2.7% by enzyme-linked immunosorbent assay (ELISA) (Mitchell et al., 2017), but re-testing of positive samples by subtype-specific haemagglutination inhibition test did not always confirm positivity (Dundon et al., 2010; Damiani et al., 2012; Pratelli and Colao, 2014; Schulz et al., 2014b). This could be due to false-
positive results in the ELISA or the presence of antibodies to subtypes that were not tested for.

In accordance with the low seroprevalence of CIV in Europe, CIV was not detected in respiratory tract samples from any tested healthy dog or dog with respiratory disease in Europe between 2010 and 2019 (Schulz et al., 2014a, b; Viitanen et al., 2015; Decaro et al., 2016; Mitchell et al., 2017; Hiebl et al., 2019). Reasons for this could be the sampling time points and/or the studied populations. Viral shedding occurs early after infection for 1–6 days and is missed if swabs are collected too late (Castleman et al., 2010). CIV is more likely to circulate in animals from multi-dog households and kennels (Buonavoglia and Martella, 2007), which may not have been included in sufficient numbers in the above studies. Currently available data suggest that CIV is, at present, neither a prevalent nor a significant pathogen of CIRDC in Europe.

**Prevalence of Canine Parainfluenza Virus**

European publications reporting the prevalence of CPIV in healthy and diseased dogs were reviewed (Table 6). CPIV is found commonly in samples of dogs with and without respiratory signs. Schulz et al. (2014b) detected CPIV in 7.8% of healthy German dogs. Erles et al. (2004) detected CPIV in 19.4% of tracheal samples and 10.4% of lung samples of dogs humanely destroyed at a UK shelter. Since 63% of these dogs showed respiratory signs before humane destruction, the results cannot be compared directly with...
those of a healthy population. The higher detection rate in tracheal samples (19.4%) compared with lung samples (10.4%) is characteristic for the virus as its target tissue is mainly ciliated epithelium of the upper respiratory tract (Appel and Binn, 1987).

In dogs with CIRDC or bronchial pneumonia, CPiV was detected in 6.5% of animals (Schulz et al., 2014b; Viitanen et al., 2015; Decaro et al., 2016; Kaczorek et al., 2016; Hiebl et al., 2019).

CPiV prevalence in healthy and diseased dogs was compared in one study and shown to be significantly higher if dogs had CIRDC (Schulz et al., 2014b). In contrast, Erles et al. (2004) reported tracheal CPiV in 20% of dogs without respiratory signs and 19% of dogs with respiratory signs. The lack of an association between clinical signs and CPiV detection in this study can have many explanations. Dogs with pre-existing immunity to CPiV through vaccination and past exposure may still become infected with CPiV field strains, but are less likely to show clinical signs (Emery et al., 1976). Since 44% of the dogs in the study by Erles et al. (2004) had antibodies against CPiV on arrival at the shelter, they would have been protected from clinical signs, although virus shedding may have still occurred. Another explanation is that dogs with CPiV-positive samples and without clinical signs were humanely destroyed during an early stage of infection when clinical signs were not yet detectable. Furthermore, close-contact housing in the shelter and circulation of a field CPiV strain amongst the shelter dogs may have distorted detection rates in this study.

Decaro et al. (2016) investigated the presence of CPiV in dogs with acute CIRDC, dogs exposed to CIRDC and CIRDC convalescent dogs and reported detection rates of 20.5%, 4.5% and 2.6%, respectively, suggesting that CPiV is more commonly found in dogs with signs of CIRDC than in dogs without such signs. Statistical analysis of the association between a respiratory score and the presence of CPiV approached significance in this study ($P = 0.063$). In another study, approximately one third of dogs with bacterial pneumonia carried CPiV (Viitanen et al., 2015). The authors speculated that initial infection with CPiV may predispose dogs to lung infections with opportunistic bacteria. Kaczorek et al. (2016) reported the highest prevalence of CPiV in dogs with CIRDC of 67.5%, but no healthy control group was included in this study.

CPiV infection was often accompanied by the presence of other pathogens. Common combinations were: (1) CPiV with Bb, which occurred more often in dogs with CIRDC than in healthy dogs ($P < 0.001$, Schulz et al., 2014b), (2) CPiV with CRCoV (Erles et al., 2004; Schulz et al., 2014b; Decaro et al., 2016), and (3) co-infection with CAV-2 and/or CHV (Erles et al., 2004; Kaczorek et al., 2016).

Therefore, there is evidence to support CPiV as still being an important pathogen in CIRDC in Europe, despite widespread vaccination. Furthermore, CPiV appears to facilitate co-infection with other viral and bacterial pathogens.

### Table 6

| Country | Year | Population | Method | Detection rate |
|---------|------|------------|--------|---------------|
| UK      | Not recorded | 211 humanely destroyed shelter dogs | CPiV in tracheal samples by RT-PCR | 19.4% |
|         |      | 150 shelter dogs on arrival | CPiV in lung samples by RT-PCR | 10.4% |
|         |      | 90 healthy dogs | CPiV-specific antibodies by ELISA | 44.0% |
| Germany | 2011–2012 | 61 dogs with CIRDC | CPiV in nasal and pharyngeal swabs by RT-PCR | 37.7% |
|         |      | 90 healthy dogs | RT-PCR | 7.8% |
| Italy   | 2011–2013 | 78 dogs with CIRDC | CPiV in nasal and oropharyngeal swabs by RT-PCR | 20.5% |
| Finland | 2011–2013 | 20 dogs with bacterial pneumonia | CPiV in BALF/TTW by RT-PCR | 35.0% |
|         |      | 13 dogs with chronic Bb infection | | 0.0% |
| Poland  | 2014–2015 | 40 dogs with CIRDC | CPiV in URT swabs and tracheal lavage fluid by PCR | 67.5% |
| Austria | 2013–2015 | 214 dogs with CIRDC | CPiV in nasal and tonsil swabs by RT-PCR | 6.5% |

BALF, bronchoalveolar lavage fluid; Bb, *Bordetella bronchiseptica*; CIRDC, canine infectious respiratory disease complex; CPiV, canine parainfluenza virus; ELISA, enzyme-linked immunosorbent assay; PCR, polymerase chain reaction; RT-PCR, reverse transcriptase PCR; TTW, transtracheal wash; URT, upper respiratory tract.
Prevalence of Canine Pneumovirus

European publications reporting the prevalence of CnPnV in healthy and diseased dogs were reviewed (Table 7). CnPnV was discovered as a causative agent of CIRDC in the USA in 2010. Since then, its prevalence in Europe has also been confirmed (Mitchell et al., 2013, 2017; Decaro et al., 2016). Mitchell et al. (2013) analysed 625 canine serum samples from the UK and Ireland for antibodies against CnPnV and found that 50.2% were seropositive. A Europe-wide study including France, Greece, Hungary, Italy, The Netherlands and Spain confirmed that CnPnV was also circulating in these countries (Mitchell et al., 2017). Seroprevalences varied considerably between countries with France showing the highest prevalence of 70.1% followed by The Netherlands (60.3%), Hungary (43.3%), Spain (37.7%), Greece (27.1%) and Italy (21.5%).

Infection with CnPnV appears to occur from 6 months of age onwards as dogs <6 months were seronegative (Mitchell et al., 2013). Seronegative dogs convert within 3 weeks of arrival at a kennel, indicating that housing dogs in close proximity facilitates the spread of CnPnV (Mitchell et al., 2013). Mitchell et al. (2017) showed that the seroprevalence of CnPnV was significantly higher in shelter dogs (54.8%) than client-owned dogs (21.8%).

Dogs that seroconverted against CnPnV following entry into a kennel were significantly more at risk of developing CIRDC and significantly more likely to show severe disease than dogs that had pre-existing antibodies (Mitchell et al., 2013, 2017). Decaro et al. (2016) found a lower prevalence of CnPnV in dogs with CIRDC (6.41%) than Mitchell et al. (2017; 22.2%) and no association with severe clinical signs. The differences may be explained by the higher proportion of client-owned dogs in the former study. CnPnV was not detected in dogs with bacterial pneumonia or chronic Bb infections (Viitanen et al., 2015), suggesting that CnPnV does not play a significant role in these more chronic respiratory diseases.

Decaro et al. (2016) found co-infections of CnPnV with CRCoV or Bb and M. canis in two of five dogs (40%) in Italy. Kennelled dogs in the UK showed co-infection of CnPnV with CRCoV and/or CPiV in 69.5% of cases (Mitchell et al., 2013). Mitchell et al. (2017) were able to demonstrate that the presence of CRCoV doubled the likelihood of a positive result for CnPnV. Therefore, CnPnV can be considered as an important new pathogen in CIRDC, often found in co-existence with CRCoV. CnPnV spreads particularly well in multi-dog establishments. Exposure appears to result in protective immunity against clinical signs suggesting that vaccination may be effective against this pathogen.

### Table 7
Prevalence of canine pneumovirus in European studies 2000–2019

| Country       | Year          | Population                          | Method                                      | Detection rate | Reference       |
|---------------|---------------|-------------------------------------|---------------------------------------------|----------------|-----------------|
| UK and Ireland | 1999–2001     | 215 kennelled dogs                  | CnPnV-specific antibodies by ELISA          | 26.0–93.5%     | Mitchell et al. (2013) |
|               | 1999–2001     | 205 humanely destroyed kennelled dogs | CnPnV in tracheal samples by RT-PCR         | 14.2%          |                 |
|               | 2005          | 625 serum samples from clinical patients | CnPnV-specific antibodies by ELISA        | 50.2%          |                 |
| Italy         | 2011–2013     | 78 dogs with CIRDC                  | CnPnV in nasal and oropharyngeal swabs by RT-PCR | 6.41%          | Decaro et al. (2016) |
| Finland       | 2011–2013     | 20 dogs with bacterial pneumonia    | CnPnV in BALF/TTW by RT-PCR                | 0.0%           | Viitanen et al. (2015) |
|               |               | 13 dogs with chronic Bb infection   | CnPnV in BALF/TTW by RT-PCR                | 0.0%           |                 |
| EU            | 2011–2013     | 525 dogs exposed to CIRDC           | CnPnV in URT swabs by RT-PCR (n = 511)     | 23.4%          | Mitchell et al. (2017) |
|               |               |                                    | CnPnV antibodies in sera by ELISA (n = 220) | 41.7%          |                 |

BALF, bronchoalveolar lavage fluid; Bb, Bordetella bronchiseptica; CIRDC, canine infectious respiratory disease complex; CnPnV, canine pneumovirus; ELISA, enzyme-linked immunosorbent assay; RT-PCR, reverse transcriptase polymerase chain reaction; TTW, transtracheal wash; URT, upper respiratory tract.

Prevalence of Canine Pneumovirus

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Decaro et al. (2016) found co-infections of CnPnV with CRCoV or Bb and M. canis in two of five dogs (40%) in Italy. Kennelled dogs in the UK showed co-infection of CnPnV with CRCoV and/or CPiV in 69.5% of cases (Mitchell et al., 2013). Mitchell et al. (2017) were able to demonstrate that the presence of CRCoV doubled the likelihood of a positive result for CnPnV. Therefore, CnPnV can be considered as an important new pathogen in CIRDC, often found in co-existence with CRCoV. CnPnV spreads particularly well in multi-dog establishments. Exposure appears to result in protective immunity against clinical signs suggesting that vaccination may be effective against this pathogen.
Prevalence of Canine Respiratory Coronavirus

European publications reporting the prevalence of CRCoV in healthy and diseased dogs were reviewed (Table 8). Antibodies to CRCoV were found in Italian dog samples from 1999 onwards (Priestnall et al., 2007). The seroprevalence ranged from 8% to 30% depending on the year. Another Italian study, which investigated retrospectively the presence of CRCoV antibodies in canine sera collected between 1994 and 2006, reported positive samples from 2005 onwards at a seroprevalence of 32.1% in 2005 and 26.8% in 2006. In the UK, adult dogs are significantly more likely to be seropositive for CRCoV than dogs <1 year of age (Priestnall et al., 2006, 2007). Presumably this can be explained by increasing contact with other dogs and therefore a rising risk of exposure to CRCoV with advancing age. Mitchell et al. (2017) reported that the seroprevalence in shelter dogs was higher (55.6%) than in client-owned dogs. A German study also found a higher seroprevalence in shelter dogs (55.6%) compared to client-owned dogs (30.4%).

**Table 8**

| Country   | Year     | Population                      | Method                             | Detection rate | Reference               |
|-----------|----------|---------------------------------|------------------------------------|----------------|-------------------------|
| Austria   | 1997−2007| 68 dogs with pneumonia           | CRCoV in lung samples by RT-PCR    | 15.6%          | Woehrer et al. (2016)   |
| Austria   | 2013−2015| 214 dogs with CIRDC, 50 healthy dogs | CRCoV in nasal and tonsil swabs by RT-PCR | 7.3%          | Hiebl et al. (2019)     |
| Italy     | 1999−2006| 590 dog sera                     | CRCoV-specific antibodies in serum by ELISA | 20.0%         | Priestnall et al. (2007) |
| Italy     | 2005−2006| 216 dog sera                     | CRCoV-specific antibodies by ELISA  | 32.1%          | Decaro et al. (2007)    |
| Italy     | 2004−2006| 109 canine lung samples          | CRCoV in lung samples by RT-PCR    | 0.92%          | Decaro et al. (2016)    |
| Italy     | 2011−2013| 78 dogs with CIRDC               | CRCoV in nasal and oropharyngeal swabs by RT-PCR | 8.97%         |                        |
| UK        | Not recorded | 111 shelter dogs                  | CRCoV-specific antibodies in sera by ELISA | 30.1−99.0%    | Erles (2003)            |
| UK        | 2001−2002 | 90 kennelled dogs (A), 62 kennelled dogs (B), 64 kennelled dogs (C) | CRCoV-specific antibodies in blood by ELISA, CRCoV in swabs by RT-PCR | 22.2−83.0%, 54.2−90.0% | Erles and Brownlie (2005) |
| UK and Ireland | Not recorded | 896 dog sera                      | CRCoV-specific antibodies in serum by ELISA | 35.6%          | Priestnall et al. (2006) |
| Austria   | Not recorded | 129 client-owned dogs with CIRDC | CRCoV-specific antibodies in serum by IFA, CRCoV in oropharyngeal swabs by RT-PCR (n = 34) | 61.2%, 8.8%    | Spiss (2012), Schulz et al. (2014b) |
| Germany   | 2011−2012| 61 dogs with CIRDC, 90 healthy dogs | CRCoV in nasal and oropharyngeal swabs by RT-PCR | 9.8%, 0.0%     | Viitanen et al. (2015)  |
| Finland   | 2011−2013| 20 dogs with bacterial pneumonia, 15 dogs with chronic Bb infection | CRCoV in BALF/TTW by RT-PCR | 5.0%          |                        |
| Europe    | 2011−2013| 525 dogs exposed to CIRDC        | CRCoV in URT swabs by RT-PCR       | 7.7%           | Mitchell et al. (2017)  |

BALF, bronchoalveolar lavage fluid; Bb, Bordetella bronchiseptica; CIRDC, canine infectious respiratory disease complex; CRCoV, canine respiratory coronavirus; ELISA, enzyme-linked immunosorbent assay; IFA, immunofluorescence antibody test; PCR, polymerase chain reaction; RT-PCR, reverse transcriptase PCR; TTW, transtracheal wash; URT, upper respiratory tract.
dogs (36.7%). Following arrival at a kennel, the majority of seronegative dogs seroconverted within 3 weeks, so that the seroprevalence in some kennels reached nearly 100% (Erles et al., 2003; Erles and Brownlie, 2005). When the seroprevalence for CRCoV was monitored in two kennels for 2 years, it was found that seroconversion was often preceded by an outbreak of CIRDC (Erles and Brownlie, 2005). Seronegative dogs had a significantly higher risk of developing CIRDC following arrival than seropositive dogs (Erles et al., 2003). Furthermore, CRCoV is frequently detected in the respiratory tract of dogs with respiratory disease. Woehrler et al. (2016) found 15.5% of pathological samples from dogs with pneumonia contained CRCoV. Some of the positive samples were co-infected with Bb and/or bacterial pathogens. Decaro et al. (2007) detected CRCoV in one dog (0.92%) in Italy that had died of a canine parvovirus infection. Higher detection rates were observed by Decaro et al. (2016) when investigating dogs that were acutely affected by CIRDC (8.97%), had been exposed to dogs with CIRDC (1.28%) or were CIRDC convalescent (5.26%). Just under half

Table 9
Prevalence of Mycoplasma species in European studies 2000–2019

| Country | Year       | Population                                  | Method                                                | Detection rate          | Reference                |
|---------|------------|---------------------------------------------|-------------------------------------------------------|-------------------------|--------------------------|
| Slovenia | 2008–2013  | 34 healthy dogs                             | M spp. in oral swabs by PCR                           | 2.9% (M. cynos)         | Scholten et al. (2017)   |
|         |            |                                             | M spp. specific antibodies by DIBA                    | 11.8% (M. canis)        |                          |
|         |            |                                             | M. cynos-specific seroconversion by western blotting  | 73.5% (M. cynos)        |                          |
|         |            |                                             |                                                       | 70.6% (M. canis)        |                          |
|         |            |                                             |                                                       | 29.0% (CIRDC)           |                          |
|         |            |                                             |                                                       | 7.0% (healthy)          |                          |
| UK      | Not recorded| 42 dogs from a shelter                       |                                                       |                         |                          |
|         |            |                                             |                                                       |                         |                          |
|         |            |                                             |                                                       |                         |                          |
| Austria | 1997–2007  | 68 dogs with pneumonia                      | M spp. in lung samples by RT-PCR                      | 2.9%                    | Woehrler et al. (2016)   |
| UK      | 1999–2002  | 210 humanely destroyed dogs from shelter (A)| M spp. in BALF and tracheal samples by culture and PCR| 23.9% (CIRDC)           | Chalker et al. (2004)    |
|         | 2001–2002  | 153 dogs from training kennel (B)           |                                                       | 9.7% (healthy)          |                          |
|         |            |                                             |                                                       | 0.0% (CIRDC)            |                          |
|         |            |                                             |                                                       | 0.9% (healthy)          |                          |
| Belgium | 2006–2014  | 17 dogs with Bb infection                   | M. cynos in BALF by culture and qPCR                  | 53.0%                   | Canonne et al. (2016)    |
|         |            | 10 healthy dogs                             |                                                       | 20.0%                   |                          |
| Germany | 2010–2012  | 29 dogs with respiratory disease           | M spp. in BALF and pharyngeal swabs by culture and PCR| 91.7% (pharyngeal)      | Schulz et al. (2015)     |
|         |            |                                             |                                                       | 37.9% (BALF)            |                          |
|         |            | 16 dogs without respiratory disease         |                                                       | 86.7% (pharyngeal)      |                          |
|         |            |                                             |                                                       | 18.8% (BALF)            |                          |
|         |            |                                             |                                                       | 7.69%                   |                          |
| Italy   | 2011–2013  | 78 dogs with CIRDC                         | M. cynos in nasal and oropharyngeal swabs by RT-PCR   | 40.0%                   | Decaro et al. (2016)     |
|         |            |                                             |                                                       | 23.1%                   |                          |
| Finland | 2011–2013  | 20 dogs with bacterial pneumonia           | M spp. in BALF/TTW by RT-PCR                          | 40.0%                   | Viitanen et al. (2015)   |
|         |            | 13 dogs with chronic Bb infection          |                                                       | 23.1%                   |                          |
| EU      | 2011–2013  | 525 dogs exposed to CIRDC                  | M spp. in URT swabs by PCR                            | 0.9%                    | Mitchell et al. (2017)   |
|         |            |                                             | M. cynos antibodies in sera by ELISA                  | 45.0%                   |                          |
| Austria | 2013–2015  | 214 dogs with CIRDC                        | M. cynos in nasal and throat swabs by PCR and culture | 2.3%                    | Stejskal et al. (2017)   |
|         |            | 50 healthy dogs                            |                                                       | 0.0%                    |                          |
| Belgium | 2009–2016  | 24 dogs with eosinophilic bronchopneumonia  | M. canis and M. cynos in BALF by qPCR                 | 25.0% (M. canis)        | Canonne et al. (2018)    |
|         |            | 21 dogs with chronic bronchitis            |                                                       | 8.3% (M. cynos)         |                          |
|         |            | 15 healthy dogs                            |                                                       | 9.5% (each M spp.)      |                          |
|         |            |                                             |                                                       | 13.0% (each M spp.)     |                          |
|         |            |                                             |                                                       |                         |                          |

BALF, bronchoalveolar lavage fluid; Bb, Bordetella bronchiseptica; CIRDC, canine infectious respiratory disease complex; DIBA, dot-immunobinding assay; ELISA, enzyme-linked immunosorbent assay; M, Mycoplasma; PCR, polymerase chain reaction; RT-PCR, reverse transcriptase PCR; qPCR, quantitative PCR; TTW, transtracheal wash; URT, upper respiratory tract.
of the CRCoV-positive dogs in this study showed co-infections with CPiV, CnPnV, Bb and/or Mycoplasma species.

Schulz et al. (2014b) found CRCoV in 9.8% of dogs with CIRDC and in no healthy control dogs. The difference in detection rates was significant. Furthermore, all CRCoV-positive dogs in that study had co-infections with Bb. Similar detection rates were obtained for dogs with CIRDC in Austria (8.8%, Spiss, 2012; 7.5%, Hiebl et al., 2019), Greece (8.6%), Hungary (7.4%), France (11.5%) and The Netherlands (13.6%) (Mitchell et al., 2017). The presence of CRCoV was associated with a significantly higher risk of developing severe signs of CIRDC in a European study (Mitchell et al., 2017).

CRCoV is widespread in Europe and more common where dogs have increased contact with other dogs. The virus is detected consistently in approximately 10% of cases with CIRDC and has been linked to an increase in severity of clinical signs, suggesting that it plays a role in the pathogenesis of CIRDC.

Prevalence of Mycoplasma species

European publications reporting the prevalence of mycoplasma species in healthy and diseased dogs were reviewed (Table 9). Data on the seroprevalence of Mycoplasma spp. in dogs are sparse. Over two thirds of healthy working dogs in Slovenia were found to have antibodies against M. cynos and M. canis by dot-immunobinding assay (Suhadolc Scolten et al., 2017). In a European study of dogs with CIRDC, seroprevalence levels ranging from 20.7% to 61.9% were noted in different countries (Mitchell et al., 2017). Western blotting of paired canine serum samples from a shelter in the UK showed that dogs that developed respiratory signs were significantly more likely to seroconvert to M. cynos than dogs that remained healthy (Rycroft et al., 2007), supporting the view that M. cynos is associated with CIRDC.

Mycoplasma spp. are frequently detected in respiratory samples of healthy dogs. Detection rates in healthy dogs ranged from 0.9% to 91.7%. Stejskal et al. (2017) isolated Mycoplasma species from 78% to 93% of throat swabs from healthy dogs, with M. canis as the predominant species. Schulz et al. (2015) showed that rates were higher in pharyngeal swabs than in BALF samples. The findings indicate that Mycoplasma spp. are normal commensals of the upper respiratory tract. M. cynos was, however, only detected in dogs with respiratory disease in both studies.

Others were able to confirm that the presence of M. cynos is significantly associated with more severe respiratory signs (Chalker et al., 2004; Decaro et al., 2016) and that only dogs with high loads of M. cynos were diseased (Canonne et al., 2018). Chalker et al. (2004) demonstrated that there is a significant association between M. cynos infection and young age (<1 year), time spent at a shelter (>1 week) and CIRDC. Schulz et al. (2015) also showed a notable difference in the proportion of dogs with Mycoplasma spp. in BALF samples between healthy dogs (18.8%) and dogs with respiratory signs (37.9%), but the difference was not significant. Not all dogs with respiratory signs had infectious diseases, as dogs with airway collapse and reverse sneezing were also included, which may have reduced the statistical power of the study. Similarly, Canonne et al. (2016) could not demonstrate a significant difference in the presence of M. cynos between healthy and diseased dogs. Reasons for this may have been the small animal number in this study and antibiotic pre-treatment of diseased dogs, which may have reduced the number of positive dogs.

Woehrer et al. (2016) found mycoplasma in 2.9% of dogs with pneumonia in a retrospective study of histopathological samples. Considerably higher detection rates were achieved using BALF samples of dogs with bacterial pneumonia (40%; Viitanen et al.,

Table 10

| Country     | Year   | Population                  | Method                        | Detection rate | Reference              |
|-------------|--------|-----------------------------|-------------------------------|----------------|------------------------|
| UK and Ireland | 1998–2000 | 71 client-owned dogs with respiratory signs | Streptococcus spp. in BALF by culture | 1.4%           | Chalker et al., (2003a) |
|             | 1999–2001 | 269 humanely destroyed kennelled dogs |                             | 23.9%          |                        |
| Germany     | 1989–2011 | 493 client-owned dogs with respiratory signs | Streptococcus spp. in BALF by culture | 30.7%          | Rheinwald et al. (2015) |
| Italy       | 2011–2015 | 78 dogs with CIRDC | S. equi subspp. zooepidemicus in nasal and oropharyngeal swabs by RT-PCR | 0.0%           | Decaro et al. (2016)  |

BALF, bronchoalveolar lavage fluid; CIRDC, canine infectious respiratory disease; RT-PCR, reverse transcriptase polymerase chain reaction.
In two studies investigating dogs with Bb infections, Mycoplasma spp. were also found in 23.1% (Viitanen et al., 2015) and 53% (M. cynos only, Canonne et al., 2016) of animals. Schulz et al. (2015) and Decaro et al. (2016) also found co-infections of M. cynos with Bb. In the latter study co-infections of M. cynos with respiratory viruses (CPiV, CRCoV) were also reported.

Therefore, M. cynos is widespread in Europe and more common in younger dogs and kennelled dogs. The organism is frequently found in dogs with respiratory disease, often together with other CIRDC pathogens. M. cynos is significantly associated with more severe respiratory signs, suggesting that it exacerbates respiratory infections.

**Prevalence of Streptococcus species**

European publications reporting the prevalence of streptococcal species in healthy and diseased dogs were reviewed (Table 10). Rheinwald et al. (2015) showed that streptococci were the most frequently isolated bacterial species from BALF samples of dogs with respiratory disease (30.7%). In another study, S. zooepidemicus was found in 1.4% of BALF samples from pet dogs with respiratory signs (Chalker et al., 2003a). Streptococcus canis was not detected in those dogs, but was occasionally isolated from shelter dogs that were humanely destroyed (8.0%). Instead, shelter dogs mainly harboured S. zooepidemicus, particularly if they had severe respiratory signs.

Decaro et al. (2016) did not detect S. zooepidemicus in 78 Italian dogs with CIRDC. This is in accordance with the results of Chalker et al. (2003a) where only 1 of 71 pet dogs tested positive for S. zooepidemicus, supporting the notion that it is not a common pathogen and may be a particular problem in kennels (Chalker et al., 2003a).

**Diagnosis and Control of CIRDC**

The diagnosis of CIRDC is usually based on clinical signs such as described above (Singleton et al., 2019). History of being kennelled or exposure to other dogs with CIRDC can also point towards CIRDC. Testing of nasal, oropharyngeal or conjunctival swabs by PCR for viral and bacterial pathogens and by submission of samples for bacterial culture may be performed, but results can be difficult to interpret as many pathogens may be isolated from healthy and diseased dogs (Lappin et al., 2017).

Control of CIRDC involves vaccination and improvement of kennelling conditions, considering factors such as sanitation, population density, ventilation and quarantine procedures (LeRoith et al., 2012). Vaccines are available against Bb, CAV-2, CDV and CPiV. A vaccine is also available against CHV for use in bitches to prevent mortality in neonatal puppies. In the case of respiratory vaccines, vaccination does not always prevent infection and shedding from the respiratory tract. Immunity against CDV prevents infection and is therefore 100% protective against CDV involvement in CIRDC (Wilson et al., 2014). In contrast, immunity through vaccination against CAV-2 and CPiV reduces morbidity and shedding, but does not prevent field infections (Emery et al., 1976; Kontor et al., 1981; Wilson et al., 2014).

Vaccination against Bb has been available for many years. Initially, inactivated vaccines were administered parenterally with good results (reviewed by Ellis, 2015). To improve local immunity, modified-live intranasal vaccines were introduced, which showed comparable or improved efficacy to injectable formulations and were slightly safer (Ellis, 2015). Larson et al. (2013) found that intranasal vaccination reduced clinical signs, lung pathology and bacterial shedding following an experimental challenge, but did not analyse this finding statistically. Ellis et al. (2016) confirmed that intranasal vaccination significantly reduced clinical signs and bacterial shedding compared with non-vaccinated control animals.

Due to the difficulties associated with intranasal administration in some dogs, oral vaccination against Bb was introduced in 2011. Comparative studies between intranasal and oral formulations have shown variable results. Larson et al. (2013) found that the oral route induced higher levels of nasal IgA after vaccination, and that both vaccination routes equally reduced clinical signs, lung pathology and bacterial shedding following an experimental challenge. However, these findings were not verified statistically. Ellis et al. (2016) also compared intranasal and oral vaccines against Bb. They found that vaccination by both routes significantly reduced clinical signs, but that administration by the intranasal route was more efficacious at reducing bacterial shedding. The authors concluded that the intranasal formulation provided better efficacy than the oral formulation and attributed this to a greater antigen exposure of local lymphoid tissue following intranasal administration.
In dogs, there are three lymphoid tissues associated with the upper respiratory and digestive tracts: the pharyngeal tonsil in the nasopharynx dorsal to the auditory tubes, a small lingual tonsil at the base of the tongue, and the palatine tonsil in the lateral side of the oropharynx (Casteleyn et al., 2011). The lingual and palatine tonsils are likely to be exposed to oral vaccines and the palatine and pharyngeal tonsils are likely to be exposed to intranasal vaccines. Although lymphoid tissues are anatomically separated, all sites of the immune system are functionally connected through circulating immune cells. This is supported by the protection provided against respiratory disease through parentally administered vaccines.

Although vaccines against CAV-2, CPiV and Bb do not prevent clinical signs and shedding (Emery et al., 1976; Kontor et al., 1981; Hess et al., 2011; Wilson et al., 2014; Scott-Garrard et al., 2018), they still provide epidemiological advantages, reduce suffering and decrease the need for antibiotic treatment. The greater and longer the exposure to respiratory pathogens in the field, the more likely are infection and clinical disease (Foley et al., 2002; Mitchell et al., 2013). A reduction in shedding is therefore likely to result in less environmental contamination and slow the spread to other dogs. Field studies utilising intranasal vaccines against canine respiratory pathogens have demonstrated that their use leads to a decreased incidence of CIRDC. Glickman and Appel (1981) found that a trivalent vaccine containing Bb, CPiV and CAV-2 was 71.2% and 81.8% effective at reducing the incidence of coughing in a Beagle breeding facility during summer and winter, respectively. Another study demonstrated that intranasal vaccination in a shelter setting helped to reduce coughing by 24.4% for a trivalent vaccine and 20.7% for a bivalent vaccine (Edinboro et al., 2004). Finally, Mitchell et al. (2017) were able to demonstrate a significant level of protection from CIRDC and severe respiratory signs in dogs vaccinated against CDV, CAV-2 and CPiV. Others could only demonstrate a non-significant protective effect following vaccination against CPiV (Erles et al., 2004; Schulz et al., 2014b), perhaps because the study populations were too small to show significant differences.

Examples of vaccines with more significant effects at the population level than in an individual animal can be found in management systems where large numbers of animals are kept in close proximity (e.g. poultry farms, dairy herds, studs and feedlots). Theurer et al. (2015) demonstrated through a systematic review and meta-analysis that the risk of developing bovine respiratory disease in the field was significantly lowered through vaccination against causative viruses. Vaccinating horses regularly against equine herpesvirus (EHV) 1 and EHV 4, and equine influenza virus, is recommended as vaccination is currently considered the most effective control method in the field (Reed and Toribio, 2004; Lunn et al., 2009), even though vaccines against EHV1 have been found to only reduce clinical signs and shedding (Burrows et al., 1984).

Another epidemiological benefit of vaccination against CAV-2, CPiV and Bb involves spread of attenuated vaccine strains to non-vaccinated in-contact dogs. Vaccinated dogs shed vaccine strains for a variable duration after vaccination (Rugh-Ullie et al., 2016). Shedding can interfere with the diagnosis of field infection as the strains cannot usually be distinguished. An obvious advantage of vaccine strain shedding, however, is the spread to other in-contact dogs and stimulation of their immune systems to develop immunity or augment existing protection. Since the vaccine strains are attenuated, they do not usually cause disease in naïve in-contact dogs (Zoetis, data on file). Therefore, immunity in the canine population can be increased directly through vaccination and indirectly through contact of non-vaccinated dogs with vaccinated dogs.

Apart from epidemiological advantages, vaccination against CIRDC pathogens is directly beneficial for the dog as it reduces the occurrence of CIRDC and the severity of clinical signs (Mitchell et al., 2017). A reduction in clinical signs positively affects the welfare of the pet and its owner and will influence the length of antibiotic treatment prescribed. Antimicrobial use in animals can contribute to the emergence of resistant bacteria that can be transferred to people through direct contact. This may reduce the effectiveness of antimicrobials for treating human and animal diseases (Edo et al., 2017). Prudent use of antibiotics is therefore indicated and if patients only show mild, transient clinical signs of CIRDC, antimicrobial therapy will not generally be required.

In summary, 100% protective immunity against respiratory diseases is rare, and generally only a reduction in clinical signs and excretion of pathogen can be achieved through vaccination. Nevertheless, vaccination against respiratory diseases is recommended where large numbers of animals come into close proximity. Pets only exceptionally congregate in large groups (e.g. in kennels, at dog shows, at puppy classes or breeding facilities). Recommending vaccination against pathogens of CIRDC in these cases, according to the World Small Animal Veterinary Association guidelines (Day et al., 2016), will directly provide epidemiological advantages to the group and any involved dog.
Conclusions

CIRDC is an endemic worldwide syndrome involving multiple viral as well as bacterial pathogens. It is observed in up to approximately 1% of individual dogs yearly, but can have a morbidity of nearly 100% during outbreaks following stays at places were many dogs congregate such as kennels, dog shows and training classes. There are multiple viral and bacterial causes of CIRDC, which can all be primary pathogens, but often co-infect to complicate the disease. Vaccines against four of the causative pathogens are available that confer epidemiological advantages by reducing the spread of the pathogens, increasing herd immunity through shedding of the vaccine and decreasing the need for antibiotic treatment. The prevalence of the agents involved in CIRDC is changing as new pathogens emerge and the importance of traditional pathogens is shifting and continued surveillance is required throughout Europe to track the evolution of the syndrome.

Acknowledgments

The authors are members of the European Canine Infectious Respiratory Diseases Advisory Board. Primary research for this manuscript was conducted by L. Siedek and the authors undertook revision and editing of the initial draft of the content.

Conflict of Interest Statement

The European Canine Infectious Respiratory Diseases Advisory Board is sponsored by Zoetis. Members receive travel expenses and an honorarium for attendance at meetings.

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