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INFECTIOUS DISEASE: REVIEW ARTICLE

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.

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

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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* subspecies *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 excretion 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 in mild cases to 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 naïve 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* monoinfection 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 bacterial flora of the upper respiratory tract and lower genital tract of horses (reviewed by Priestnall and Erles, 2011). The bacterium also causes opportunistic infections in horses (e.g. abscesses, endometritis) and dogs. Sporadic illness resembling CIRDC as well as outbreaks of often lethal haemorrhagic pneumonia have been observed. Kennelled dogs are at particular risk of peracute outbreaks (Priestnall *et al.*, 2014).

The pathogenesis of *S. zooepidemicus* infection is thought to involve exotoxins, which act as superantigens and significantly augment the host immune response, comparable with human streptococcal toxic shock syndrome. Although *S. zooepidemicus* has zoonotic potential, transmission from horses or dogs to man is rarely reported. Experimental challenges with *S. zooepidemicus* only caused clinical disease if dogs were challenged concomitantly with CIV, underlining the complexity of the interactions between the different pathogens of CIRDC (Priestnall *et al.*, 2014).

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. These data included serological findings and detection or isolation results from healthy dogs and dogs with respiratory disease published since the year 2000. The literature was searched using Google and PubMed databases from 2000 to 2019 with search terms including: 'dog', 'canine', 'respiratory disease', 'respiratory pathogen' and the names of the specific organisms described in this review.

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.*,

Table 1
Prevalence of *Bordetella bronchiseptica* in European studies 2000–2019

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 throat swabs by RT-PCR	52.0%	Corona *et al.* (2013)
Germany	2011–2012	61 dogs with CIRDC 90 healthy dogs	Bb in nasal and pharyngeal swabs by RT-PCR	78.7% 45.6%	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 50 healthy dogs	Bb in nasal and throat swabs by PCR and culture	3.3% 0.0%	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 21 dogs with chronic bronchitis 15 healthy dogs	Bb in BALF by qPCR	25.0% 10.0% 13.0%	Canonne *et al.* (2018)
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.

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 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.*, 1985; Macartney *et al.*, 1988).

Table 2
Prevalence of canine adenovirus type 2 in European studies 2000–2019

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%	Woehrer *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	100% 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 13 dogs with chronic Bb infection	CAV-2 in BALF/TTW by RT-PCR	0.0% 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.

Table 3
Prevalence of canine distemper virus in European studies 2000–2019

Country	*Year*	*Population*	*Method*	*Detection rate*	*Reference*
Austria	1997–2007	68 dogs with pneumonia	CDV in lung samples by IHC	16.2%	Woehrer *et al.* (2016)
Austria	2013–2015	214 dogs with CIRDC 50 healthy dogs	CDV in nasal and tonsil swabs by PCR	0.5%	Hiebl *et al.* (2019)
Germany	2011–2012	61 dogs with CIRDC 90 healthy dogs	CDV in nasal and pharyngeal swabs by RT-PCR	0.0% 0.0%	Schulz *et al.* (2014b)
Italy	2011–2013	78 dogs with CIRDC	CDV in nasal and pharyngeal swabs by RT-PCR	0.0%	Decaro *et al.* (2016)
Finland	2011–2013	20 dogs with bacterial pneumonia 13 dogs with chronic Bb infection	CDV in BALF/TTW by RT-PCR	0.0% 0.0%	Viitanen *et al.* (2015)

BALF, bronchoalveolar lavage fluid; Bb, *Bordetella bronchiseptica*; CDV, canine distemper virus; CIRDC, canine infectious respiratory disease complex; IHC, immunohistochemistry; RT-PCR, reverse transcriptase polymerase chain reaction; TTW, transtracheal wash.

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. 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

Table 4
Prevalence of canine herpesvirus in European studies 2000–2019

Country	*Year*	*Population*	*Method*	*Detection rate*	*Reference*
UK	Not recorded	211 humanely destroyed shelter dogs	CHV in tracheal samples by RT-PCR CHV in lung samples by RT-PCR	12.8% 9.6%	Erles *et al.* (2004)
UK	2001–2002	54 dogs in kennel (A) with CIRDC 26 dogs in kennel (B) with CIRDC	CHV in tonsillar swabs by RT-PCR	0.0% 0.0%	Erles and Brownlie (2005)
Slovakia	Not recorded	20 dogs with CIRDC 10 healthy dogs	CHV-specific antibodies in sera by ELISA	60.0% 0.0%	Vojtek *et al.* (2010)
Germany	2011–2012	90 healthy dogs 61 dogs with CIRDC	CHV in nasal and pharyngeal swabs by RT-PCR	0.0% 0.0%	Schulz *et al.* (2014b)
Italy	2011–2013	78 dogs with CIRDC	CHV in nasal and pharyngeal swabs by RT-PCR	0.0%	Decaro *et al.* (2016)
Finland	2011–2013	20 dogs with bacterial pneumonia 13 dogs with chronic Bb infection	CHV in BALF/TTW by RT-PCR	0.0% 0.0%	Viitanen *et al.* (2015)
Poland	2014–2015	40 dogs with CIRDC	CHV in URT swabs and tracheal lavage fluid by PCR	80.0%	Kaczorek *et al.* (2016)

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

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 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)

Table 5
Prevalence of canine influenza virus in European studies 2000–2019

Country	*Year*	*Population*	*Method*	*Detection rate*	*Reference*
Italy	1997–2011	562 sera from adult dogs	CIV-specific antibodies by cELISA, 124 positive and negative samples tested by H3N8/H3N2-specific HI	3.56% 0.36% (H3N8) 37.9% (H3N2)	Pratelli and Colao (2014)
Italy	2004–2008	224 farm and rescue dogs	CIV-specific antibodies by cELISA	0.0%	Piccirillo *et al.* (2010)
Italy	2009	964 sera from healthy dogs	CIV-specific antibodies by cELISA, positive samples re-tested by H1N1-specific HI	3.0% 0.7%	Dundon *et al.* (2010)
Germany	2010–2011	736 dog sera	CIV-specific antibodies by cELISA, positive samples re-tested by N1-specific cELISA and SN	0.95% 0.14%	Damiani *et al.* (2012)
Germany	2010–2011	272 healthy dogs 35 dogs with acute CIRDC	CIV H3N8-specific antibodies in sera by ELISA Positive samples re-tested by IFA and subtype-specific HI CIV in nasal and pharyngeal swabs by RT-PCR	0.37% 2.86% 0.0% 0.0%	Schulz *et al.* (2014a)
Germany	2011–2012	90 healthy dogs 61 dogs with CIRDC	CIV in nasal and pharyngeal swabs by RT-PCR	0.0% 0.0%	Schulz *et al.* (2014b)
Finland	2011–2013	20 dogs with bacterial pneumonia 13 dogs with chronic Bb infection	CIV in BALF/TTW by RT-PCR	0.0% 0.0%	Viitanen *et al.* (2015)
Italy	2011–2013	78 dogs with CIRDC	CIV in nasal and pharyngeal swabs by RT-PCR	0.0%	Decaro *et al.* (2016)
Austria	2013–2015	214 dogs with CIRDC 50 healthy dogs	CIV in nasal and tonsil swabs by PCR	0.0%	Hiebl *et al.* (2019)
Europe	2011–2013	525 dogs exposed to CIRDC	CIV in URT swabs by RT-PCR ($n = 511$) CIV antibodies in sera by cELISA ($n = 220$)	0.0% 2.7%	Mitchell *et al.* (2017)

BALF, bronchoalveolar lavage fluid; Bb, *Bordetella bronchiseptica*; CIRDC, canine infectious respiratory disease complex; CIV, canine influenza virus; cELISA, competitive enzyme-linked immunosorbent assay; HI, haemagglutination inhibition test; IFA, immunofluorescence antibody test; RT-PCR, reverse transcriptase polymerase chain reaction; SN, serum neutralization test; TTW, transtracheal wash; URT, upper respiratory tract.

(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

Table 6
Prevalence of canine parainfluenza virus in European studies 2000–2019

Country	*Year*	*Population*	*Method*	*Detection rate*	*Reference*
UK	Not recorded	211 humanely destroyed shelter dogs	CPiV in tracheal samples by RT-PCR	19.4%	Erles *et al.* (2004)
			CPiV in lung samples by RT-PCR	10.4%	
		150 shelter dogs on arrival	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%	Schulz *et al.* (2014b)
		90 healthy dogs		7.8%	
Italy	2011–2013	78 dogs with CIRDC	CPiV in nasal and oropharyngeal swabs by RT-PCR	20.5%	Decaro *et al.* (2016)
Finland	2011–2013	20 dogs with bacterial pneumonia	CPiV in BALF/TTW by RT-PCR	35.0%	Viitanen *et al.* (2015)
		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%	Kaczorek *et al.* (2016)
Austria	2013–2015	214 dogs with CIRDC	CPiV in nasal and tonsil swabs by RT-PCR	6.5%	Hiebl *et al.* (2019)
		50 healthy dogs			

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.

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 65% 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%–67.5% 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

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		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.

appears to facilitate co-infection with other viral and bacterial pathogens.

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 inclient-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 8
Prevalence of canine respiratory coronavirus in European studies 2000–2019

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	7.5%	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)
	2004–2006	109 canine lung samples	CRCoV in lung samples by RT-PCR	0.92%	
Italy	2011–2013	78 dogs with CIRDC	CRCoV in nasal and oropharyngeal swabs by RT-PCR	8.97%	Decaro *et al.* (2016)
UK	Not recorded	111 shelter dogs	CRCoV-specific antibodies in sera by ELISA	30.1–99.0%	Erles *et al.* (2003)
		119 humanely destroyed shelter dogs	CRCoV in tracheal samples by RT-PCR	26.9%	
UK	2001–2002	90 kennelled dogs (A) 62 kennelled dogs (B) 64 kennelled dogs (A)	CRCoV-specific antibodies in blood by ELISA CRCoV in swabs by RT-PCR	22.2–83.0% 54.2–90%% 4.7%	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	61.2%	Spiss (2012)
			CRCoV in oropharyngeal swabs by RT-PCR ($n = 34$)	8.8%	
Germany	2011–2012	61 dogs with CIRDC 90 healthy dogs	CRCoV in nasal and pharyngeal swabs by RT-PCR	9.8% 0.0%	Schulz *et al.* (2014b)
Finland	2011–2013	20 dogs with bacterial pneumonia	CRCoV in BALF/TTW by RT-PCR	5.0%	Viitanen *et al.* (2015)
		13 dogs with chronic Bb infection		0.0%	
Europe	2011–2013	525 dogs exposed to CIRDC	CRCoV in URT swabs by PCR	7.7%	Mitchell *et al.* (2017)
			CRCoV antibodies in sera by ELISA	47.0%	

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.

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 38.3% in 2005 and 26.8% in 2006. In the UK, 22.2%–54.2% of dogs showed antibodies against CRCoV, with considerable regional variability (Erles *et al.*, 2003; Erles and Brownlie, 2005; Priestnall *et al.*, 2006), which appeared to be correlated to the population density.

Seroprevalence was found to increase with age and exposure to other dogs. 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 (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. Woehrer *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*) 11.8% (*M. canis*)	Scholten *et al.* (2017)
			M spp. specific antibodies by DIBA	73.5% (*M. cynos*) 70.6% (*M. canis*)	
UK	Not recorded	42 dogs from a shelter	*M. cynos*-specific seroconversion by western blotting	29.0% (CIRDC) 7.0% (healthy)	Rycroft *et al.* (2007)
Austria	1997–2007	68 dogs with pneumonia	M spp. in lung samples by RT-PCR	2.9%	Woehrer *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) 9.7% (healthy)	Chalker *et al.* (2004)
	2001–2002	153 dogs from training kennel (B)		0.0% (CIRDC) 0.9% (healthy)	
Belgium	2006–2014	17 dogs with Bb infection 10 healthy dogs	*M. cynos* in BALF by culture and qPCR	53.0% 20.0%	Canonne *et al.* (2016)
Germany	2010–2012	29 dogs with respiratory disease	M spp. in BALF and pharyngeal swabs by culture and PCR	91.7% (pharyngeal) 37.9% (BALF)	Schulz *et al.* (2015)
		16 dogs without respiratory disease		86.7% (pharyngeal) 18.8% (BALF)	
Italy	2011–2013	78 dogs with CIRDC	*M. cynos* in nasal and oropharyngeal swabs by RT-PCR	7.69%	Decaro *et al.* (2016)
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 50 healthy dogs	*M. cynos* in nasal and throat swabs by PCR and culture	2.3% 0.0%	Stejskal *et al.* (2017)
Belgium	2009–2016	24 dogs with eosinophilic bronchopneumonia	*M. canis* and *M. cynos* in BALF by qPCR	25.0% (*M. canis*) 8.3% (*M. cynos*)	Canonne *et al.* (2018)
		21 dogs with chronic bronchitis		9.5% (each M spp.)	
		15 healthy dogs		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
Prevalence of streptococci in European studies 2000–2019

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	209 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	2011–2013	78 dogs with CIRDC	*S. equi* subsp. *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.

2015), eosinophilic bronchopneumonia (25% *M. canis*, 8.3% *M. cynos*) and chronic bronchitis (8.3% each *M. canis* and *M. cynos*; Canonne *et al.*, 2018). Stejskal *et al.* (2017) showed that respiratory signs were significantly more likely if *M. canis* or *Mycoplasma spumans* were isolated from nasal swabs.

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 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 (Ruch-Gullie *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.

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Conflict of Interest Statement

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