

## Co-infections with Respiratory Viruses in Dogs with Bacterial Pneumonia

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**Background:** Bacterial pneumonia (BP) is an inflammation of the lower airways and lung parenchyma secondary to bacterial infection. The pathogenesis of BP in dogs is complex and the role of canine respiratory viruses has not been fully evaluated.

**Objectives:** The aim of this study was to investigate the occurrence of viral co-infections in dogs with BP and to assess demographic or clinical variables as well as disease severity associated with viral co-infections.

**Animals:** Twenty household dogs with BP caused by opportunistic bacteria and 13 dogs with chronic (>30 days) tracheobronchitis caused by *Bordetella bronchiseptica* (BBTB).

**Methods:** Prospective cross-sectional observational study. Diagnosis was confirmed by clinical and laboratory findings, diagnostic imaging, and cytologic and microbiologic analysis of bronchoalveolar lavage or transtracheal wash fluid. Canine parainfluenza virus (CPIV), canine adenovirus, canine herpes virus, canine influenza virus, canine distemper virus, canine respiratory coronavirus (CRCoV) and canine pneumovirus, as well as *B. bronchiseptica* and *Mycoplasma* spp. were analyzed in respiratory samples using PCR assays.

**Results:** CPIV was detected in 7/20 and CRCoV in 1/20 dogs with BP. Respiratory viruses were not detected in dogs with BBTB. There were no significant differences in clinical variables between BP dogs with and without a viral co-infection.

**Conclusion and Clinical Importance:** Respiratory viruses were found frequently in dogs with BP and may therefore play an important role in the etiology and pathogenesis of BP. Clinical variables and disease severity did not differ between BP dogs with and without viral co-infection.

**Key words:** Canine; Canine infectious respiratory disease; CIRDC.

**B**acterial pneumonia (BP) is an acquired inflammation of the lower airways and lung parenchyma secondary to bacterial infection.<sup>1</sup> Because BP is difficult to induce experimentally in healthy animals, the pathogenesis is considered complex and involves several underlying mechanisms.<sup>2</sup> Possible predisposing factors for the development of BP, such as diseases leading to aspiration, decreased ciliary function, or immunodeficiency, have been described in Dogs.<sup>3</sup> However, the role of canine respiratory viruses in the development of BP and the possible connection between canine infectious respiratory disease (CIRD) and BP have not yet been fully evaluated.

Canine infectious respiratory disease, also known as infectious tracheobronchitis or kennel cough, is considered 1 of the most common infectious diseases in dogs worldwide.<sup>4</sup> It is highly contagious and affects the larynx, trachea, bronchi, and nasal mucosa, causing acute respiratory signs, mainly cough, which usually are self-limiting.<sup>4</sup> Because of the contagious nature of CIRD, it is most prevalent in dense dog populations such as in

### Abbreviations:

BAL	bronchoalveolar lavage
BALF	bronchoalveolar lavage fluid
BBTB	tracheobronchitis caused by <i>Bordetella bronchiseptica</i>
BP	bacterial pneumonia
CAP	community-acquired pneumonia
CAV-2	canine adenovirus type 2
CDV	canine distemper virus
CIRD	canine infectious respiratory disease
CIV	canine influenza virus
CHV	canine herpes virus
CnPnV	canine Pneumovirus
CPIV	canine parainfluenza virus
CRCoV	canine respiratory coronavirus
CRP	C-reactive protein
TTW	transtracheal wash

kennels and rehoming centers.<sup>4</sup> Canine infectious respiratory disease has a multifactorial etiology, and several respiratory viruses as well as bacterial pathogens have been shown to contribute to the disease complex.<sup>4–6</sup> Canine parainfluenza virus (CPIV) and canine adenovirus type 2 (CAV-2) were first detected in dogs with contagious tracheobronchitis in the 1960s, and since then these viruses have been considered as the principal etiologic agents in CIRD.<sup>7–10</sup> Canine herpes virus (CHV) also was detected in dogs with respiratory signs decades ago, but its role in CIRD remains controversial.<sup>11–14</sup> In addition to these pathogens, novel respiratory viruses recently have been reported. Canine respiratory coronavirus (CRCoV) was first identified in 2003 in dogs at a rehoming center in the United Kingdom.<sup>15</sup> Subsequently, evidence of contagious tracheobronchitis caused by CRCoV has been reported worldwide.<sup>16–20</sup> Canine influenza virus (CIV) was first described in a racing Greyhound population in

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the United States in 2004, and it was later shown that CIV is widespread in the USA.<sup>21–23</sup> Canine pneumovirus (CnPnV) recently was discovered in dogs with respiratory disease in the United States, but the role of CnPnV as a causative agent in CIRDC still is not fully known.<sup>24–26</sup> In addition to viral agents, some bacterial pathogens such as *Bordetella bronchiseptica*, *Mycoplasma* spp., and more recently *Streptococcus equi* sp. *zooepidemicus*, have been shown to contribute to the etiology of CIRDC.<sup>27–32</sup> Although not considered part of the CIRDC complex, canine distemper virus (CDV) is an important respiratory pathogen causing severe systemic disease characterized by a variety of clinical signs, including respiratory signs.<sup>33</sup>

Viral-bacterial co-infections in the respiratory tract are well documented in humans with community-acquired pneumonia (CAP), and epidemiologic data as well as laboratory studies support the conclusion that respiratory viruses predispose to the development of secondary bacterial infections.<sup>34,35</sup> Several mechanisms have been shown to contribute to this situation: Viruses destroy the respiratory epithelium and facilitate bacterial adhesion, viral infection up-regulates expression of molecules that bacteria utilize as receptors, and virus-induced immunosuppression promotes secondary bacterial infection.<sup>35,36</sup> Recent advances in molecular diagnostic techniques have greatly increased understanding of the etiology of CAP in humans. Viruses affecting the lower respiratory tract currently are recognized as causative and complicating factors in up to 40% of humans with CAP.<sup>37–39</sup>

Preceding or concurrent infections with CIRDC viruses also have been considered as a possible etiological factor in dogs with BP, although there currently are only isolated reports suggesting this connection.<sup>1,40,41</sup> In dogs, BP most often is caused by opportunistic bacteria that belong to the normal oral flora.<sup>42,43</sup> However, published studies on viral-bacterial co-infections in dogs almost exclusively describe dogs housed in dense populations, such as kennels or rehoming centers, that are infected with respiratory viruses and bacteria belonging to the CIRDC complex.<sup>6,7,44</sup> Nevertheless, it is likely that respiratory viruses also are an important etiologic factor in dogs with BP caused by opportunistic bacteria in a similar manner as has been reported in humans.

The purpose of this study was to investigate the occurrence of viral respiratory infections in household dogs diagnosed with BP and to assess the possible demographic or clinical variables associated with viral co-infections as well as to assess the impact of viral co-infections in the severity of the disease.

## Materials and Methods

### Study Design

The study was conducted as a prospective cross-sectional observational study. A prior sample size calculation was not performed.

### Study Population

Privately owned household dogs diagnosed with BP at the Veterinary Teaching Hospital of the University of Helsinki between

March 2011 and October 2013 were eligible for inclusion in the study. Dogs with prolonged (duration of clinical signs >30 days) tracheobronchitis caused by *Bordetella bronchiseptica* (BBTB) were included as controls for the virus analysis.

All dogs living in or being recently (<4 weeks ago) exposed to environments with high infection pressure (eg, boarding kennels, rescue shelters) were excluded. Dogs that had been vaccinated against CDV, CAV-2, CPIV, or *B. bronchiseptica* <4 weeks before inclusion in the study were excluded.

### Diagnostic Testing and Sample Collection

A full clinical examination was performed, with special emphasis on the respiratory tract. Thoracic radiographs (laterolateral and ventrodorsal or dorsoventral views) were obtained at presentation and assessed by the same radiologist (AL), who was blinded to the patient data. Hematology, serum biochemistry, serum C-reactive protein (CRP) and fecal analysis were performed. Arterial blood gas analysis for partial pressures of oxygen and carbon dioxide were measured and the alveolar-arterial gradient was calculated. Bronchoscopy was performed with a 4.9-mm flexible endoscope,<sup>4</sup> and airway samples for cytology, semiquantitative aerobic and anaerobic bacterial culture, qualitative *Mycoplasma* spp. culture and PCR analysis were obtained by weight-adjusted (1 ml/kg 0.9% NaCl) bronchoalveolar lavage (BAL).<sup>45</sup> Transtracheal wash (TTW) sampling was used in dogs with BP in which anesthesia was considered unsafe because of the severity of the disease.<sup>46</sup>

Bacterial pneumonia was diagnosed when there were typical acute signs (at least 3 of the following: fever, lethargy, dyspnea, tachypnea, cough) and findings compatible with BP on thoracic radiographs.<sup>1,42,43</sup> A bacterial origin was proved by cytological confirmation of bacterial infection in respiratory samples (>2 intracellular bacteria/oil immersion field), positive bacterial culture ( $\geq 10^3$  colony-forming units/mL in airway samples),<sup>47</sup> or rapid response to antibiotics and full clinical and radiographic normalization with antibiotic treatment. All dogs with BP were followed until full cure. Prolonged BBTB was diagnosed when there was a cough lasting for >30 days and a positive bacterial culture of *B. bronchiseptica* (>10<sup>3</sup> colony-forming units/mL) in bronchoalveolar lavage fluid (BALF) and an absence of other diagnosed airway pathology.

### Sample Handling and Analysis

Hematology, serum biochemistry, arterial blood gas analysis, serum CRP, and fecal analysis as well as cytologic and microbiologic analysis of respiratory samples were performed as previously described.<sup>48</sup> Semiquantitative bacterial culture of BALF and TTW fluid was performed as follows: A 10- $\mu$ L volume of the specimen was streaked onto agars. Bacterial counts were quantified by calculating the colonies on a plate and multiplying the number of colonies by the dilution factor. The limit of detection was 100 colony-forming units/mL. The remaining respiratory samples (BALF, TTW) and serum were immediately frozen and stored at  $-80^{\circ}\text{C}$ . CPIV, CAV-2, CHV, CIV, CDV, and CRCoV, as well as *Mycoplasma* spp. and *B. bronchiseptica*, were analyzed by real-time PCR analysis at a commercial reference laboratory.<sup>b</sup> CnPnV was analyzed by RT-PCR at the laboratory<sup>c</sup> responsible for the discovery of this pathogen.<sup>25</sup>

### Statistical Analysis

Normality testing was based on Shapiro-Wilk's test of normality and normal  $Q-Q$  plots. The differences between groups with negative and positive virus PCR were evaluated using the independent samples Student's  $t$ -test (normally distributed variables) and

Mann–Whitney *U*-test (non-normally distributed variables). The Mann–Whitney *U*-test also was used to evaluate whether there were differences in BALF and TTW differential cell counts in all dogs with BP. *P*-values < .05 were considered statistically significant. All statistical analyses were performed using commercial statistical software.<sup>d</sup>

### Ethical Approval and Owner Consent

This study was approved by the Ethics Committee of the University of Helsinki. Owner consent was obtained from the owners of the dogs before participation.

## Results

### Dogs

Altogether, 33 dogs were included in the study, consisting of 20 dogs diagnosed with BP (10/20 male and 10/20 female) and 13 with BBTB (8/13 male and 5/13 female). The age distribution and weights of dogs with BP are presented in Table 1. Dogs in both groups represented various breeds without over-representation of any single breed.

Other simultaneous respiratory diseases or bacterial infections were identified in 4/12 dogs (chronic bronchitis 2/4, tracheoesophageal fistula 1/4, oral abscess 1/4). Laryngeal examination was not performed in dogs with BP and therefore aspiration etiology could not be fully excluded. All dogs with BP presented as acute (median duration of clinical signs, 1 day; range, 0.5–7 days; interquartile range, 1–3.8 days) emergency cases of varying severity (14/20 dogs required hospitalization for a median duration of 1.8 days; interquartile range, 1–4.6 days), and none of the dogs died or was euthanized. All dogs with BBTB were clinically stable and were

presented with chronic cough (median duration of clinical signs, 120 days; interquartile range, 60–186 days).

### Clinical Findings

Body temperature, respiratory rate, serum CRP, results from arterial blood gas analysis and hematology, as well as information on the duration of clinical signs and duration of hospitalization in dogs with BP are presented in Table 1. Fecal analysis results were available for 15/20 dogs with BP, and they were all negative for lung worms and intestinal parasites. Radiographic findings in dogs with BP are presented in Table 2. Demographic and clinical data in dogs with and without viral co-infection were compared and results are presented in Tables 1 and 3. Dogs with viral co-infections were significantly heavier ( $P = .037$ ) than dogs with negative virus PCR results. Duration of clinical signs or hospitalization, age of the dogs, body temperature, respiratory rate, serum CRP, hematology, arterial blood gas analysis, and BALF and TTW cytology results did not differ significantly between dogs with and without viral co-infection.

### Respiratory Samples

In the PCR analysis of BALF or TTW fluid, CPIV was detected in 7/20 (35%; 95% confidence interval, 14–56%) and CRCoV in 1/20 (5%; 95% confidence interval, 0–15%) dogs with BP. CAV-2, CHV, CIV, CDV, or CnPnV were not detected in any of the samples. Dogs with positive virus PCR did not have other diseases predisposing to BP. Respiratory viruses were not detected in dogs with BBTB.

**Table 1.** Comparison of demographic and clinical findings in dogs with bacterial pneumonia with positive and negative respiratory virus PCR results.

	Negative Respiratory Virus PCR (n = 12)	Positive Respiratory Virus PCR (n = 8)	<i>P</i> -Value
Sex	Male 5/12 Female 7/12	Male 5/8 Female 3/8	
	Mean ± SD	Mean ± SD	
Weight (kg)	25.0 ± 16.4	41.7 ± 15.7	.037
Body temperature (°C)	39.6 ± 0.9	39.2 ± 0.8	.31
Respiratory rate (breaths/min)	59 ± 21	45 ± 20	.15
Arterial paO <sub>2</sub> (mmHg)	74.9 ± 11.1	79.1 ± 14.1	.49
Arterial paCO <sub>2</sub> (mmHg)	29.3 ± 3.9	30.9 ± 1.1	.19
Alveolar-arterial O <sub>2</sub> gradient	41.3 ± 11.8	33.5 ± 14.4	.23
	Median (IQR)	Median (IQR)	
Age, years	5.8 (1.3–8.4)	0.9 (0.7–1.4)	.057
Duration of clinical signs (days)	1.0 (1–3.5)	3.0 (1.0–5.3)	.48
Duration of hospitalization (days)	1.8 (0.0–4.8)	1.0 (0.3–1.5)	.43
Serum C-reactive protein (mg/L)	140 (84–192)	63 (52–178)	.98
Blood leukocyte count (10 <sup>9</sup> /L)	14.6 (12.5–22.0)	10.6 (7.4–22.9)	.34
Segmented neutrophil count (10 <sup>9</sup> /L)	11.7 (9.1–15.6)	7.8 (5.2–17.5)	.27
Band neutrophil count (10 <sup>9</sup> /L)	0.5 (0.08–2.0)	0.3 (0.0–0.9)	.48
Lymphocyte count (10 <sup>9</sup> /L)	0.7 (0.4–1.7)	1.5 (0.4–3.6)	.52
Eosinophil count (10 <sup>9</sup> /L)	0.3 (0.0–0.6)	0.2 (0.0–0.6)	.91
Monocyte count (10 <sup>9</sup> /L)	1.0 (0.3–1.7)	0.7 (0.5–1.5)	.97
Basophil count (10 <sup>9</sup> /L)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	.68

**Table 2.** Radiographic findings in dogs with bacterial pneumonia with positive and negative respiratory virus PCR results.

	Radiographic Pattern	Number of Dogs	Location Number of Dogs
Negative virus PCR (n = 12)	Alveolar, single lobe	4/12	Right cranial lobe 1/4 Right caudal lobe 1/4 Left cranial cranial lobe 1/4 Left caudal cranial lobe 1/4
	Alveolar, multiple lobes	5/12	Right cranial lobe 3/5 Right middle lobe 1/5 Right caudal lobe 1/5 Left cranial cranial lobe 3/5 Left caudal cranial lobe 1/5 Left caudal lobe 1/5 Patchy alveolar 1/5
	Bronchointerstitial (graded moderate-severe)	3/12	
Positive virus PCR (n = 8)	Alveolar, single lobe	2/8	Right middle lobe 2/2
	Alveolar, multiple lobes	4/8	Right cranial lobe 2/4 Right middle lobe 3/4 Left cranial cranial lobe 2/4 Left caudal cranial lobe 2/4 Patchy alveolar 1/4
	Bronchointerstitial (graded moderate-severe)	2/8	

**Table 3.** Comparison of cytological analysis of bronchoalveolar lavage and transtracheal wash fluid in dogs with bacterial pneumonia with positive and negative respiratory virus PCR results.

	Negative Respiratory Virus PCR (n = 12)	Positive Respiratory Virus PCR (n = 8)	P-Value
	Median (IQR)	Median (IQR)	
Total cell count (10 <sup>9</sup> /L)	1.4 (0.1–10.6)	4.7 (0.2–31.7)	.57
Neutrophils (%)	66.1 (7.7–95.9)	65.2 (12.0–90.2)	.91
Eosinophils (%)	0.4 (0.0–9.0)	1.0 (0.1–3.5)	.62
Mast cells (%)	0.0 (0.0–1.8)	0.0 (0.0–0.0)	.57
Lymphocytes (%)	5.0 (0.7–16.6)	4.7 (0.4–20.4)	1.00
Macrophages (%)	23.7 (3.7–57.9)	22.1 (8.2–57.6)	1.00
Epithelial cells (%)	0.0 (0.0–0.0)	0.0 (0.0–0.7)	.57
Basophils (%)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	1.00
Plasma cells (%)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	1.00

Respiratory samples were retrieved using BAL in 13/20 and TTW in 7/20 dogs diagnosed with BP. BAL was used as a sampling method in all dogs with BBTB. Results from cytology analysis of BALF and TTW fluid in BP dogs are presented in Table 3. Because BALF and TTW fluid cytology did not differ significantly in other variables aside from the percentage of epithelial cells (median in BALF, 0.0; interquartile range, 0.0–0.0 versus median in TTW fluid, 0.7; interquartile range, 0.0–14.7;  $P = .008$ ), combined results are represented for the 2 sampling techniques.

Altogether, 11/20 dogs with BP had received antimicrobials before sampling. Significant bacterial growth ( $\geq 10^3$  colony-forming units/mL in BALF or TTW fluid) was identified in the primary culture in 11/20 samples (5/11 dogs with prior antimicrobial treatment). A single species was isolated in 8/11 samples, including *Pasteurella* sp. (1/10), *Escherichia coli* (2/10), *Streptococcus* sp. (2/10), *Haemophilus* sp. (1/10), *Mycoplasma* sp. (1/10),

and *Nocardiopsis* sp. (1/10), and 2 species in 3 samples, *Pasteurella* sp. and *Mycoplasma* sp.<sup>47</sup> Intracellular bacteria were seen in 6/11 dogs with significant bacterial growth in primary culture. In 1 dog with a negative primary culture and prior antimicrobial treatment, >2 intracellular bacteria/oil immersion field were demonstrated, and *Actinomyces* sp. was cultured after enrichment.<sup>47</sup> Positive bacterial growth was detected only after enrichment in 4/20 dogs (single species of bacteria, including *Streptococcus* sp. [1/4], *Pasteurella* sp. [1/4], *Haemophilus* sp. [1/4], and *Actinomyces* sp. [1/4]), 2 of these dogs had received prior antimicrobial treatment. A negative bacterial culture in airway samples was recorded in 4/20 dogs (2 had received prior antimicrobial treatment), but they showed an acute onset of respiratory signs and had new alveolar densities in thoracic radiographs as well as neutrophilia in BALF cytology. All of these dogs showed a rapid response to antibiotics, and a full clinical and radiographic cure was

achieved with antimicrobial treatment. CPIV was detected by PCR in 2/4 and *Mycoplasma* spp. in 1/4 dogs with negative culture results.

Information on previous CDV, CAV-2, and CPIV vaccinations was available for all dogs with BP. All dogs with positive CPIV PCR in respiratory samples ( $n = 7$ ) had been vaccinated against CPIV <12 months previously (median, 6.5 months; interquartile range, 4.5–9.0 months). In total, 12/13 dogs with negative CPIV PCR in respiratory samples were vaccinated against CPIV (median, 13.0 months; interquartile range 2.9–24 months). There was no significant difference in the timing of CPIV vaccination between dogs with positive and negative respiratory virus PCR ( $P = .152$ ). All dogs participating in this study had previously been vaccinated against CAV and CDV.

*Bordetella bronchiseptica* PCR analysis was positive in 12/13 dogs with BBTB and 1/20 dogs with BP. All dogs with BBTB and none of the dogs with BP had positive growth of *B. bronchiseptica* in BALF or TTW fluid.

In addition to *Mycoplasma* spp. PCR analysis, a culture for *Mycoplasma* spp. was performed in 15/20 dogs with BP and 4/13 dogs with BBTB. *Mycoplasma* PCR was positive in 8/20 dogs with BP and 3/13 dogs with BBTB. *Mycoplasma* culture was positive in 4/15 dogs with BP and 2/4 dogs with BBTB. *Mycoplasma* PCR was positive in all dogs with BP and BBTB with positive culture results. Additionally, PCR was positive in 3 dogs with negative *Mycoplasma* culture results (2 dogs with BP and 1 with BBTB) and in 2 dogs with BP lacking culture results.

## Discussion

Bacterial pneumonia is a serious lower respiratory tract infection in dogs with substantial morbidity and risk of mortality. Although BP was described in Dogs decades ago, information on the mechanisms leading to the development of the disease still is limited. Factors such as diseases predisposing to aspiration, immunodeficiency, or ciliary dysfunction that lead to impairment of pulmonary defense mechanisms and thereby predispose to the development of BP have been described.<sup>3</sup> However, the role of preceding or concurrent infections with CIRD viruses has not been fully evaluated in dogs with BP, although it has been suspected to play a role in the etiology, as reported in humans with CAP.<sup>34–39</sup> Previously, respiratory viral-bacterial co-infections mostly have been reported in dogs housed in dense populations, such as kennels and rescue shelters, and bacteria accompanying viruses have been primary CIRD bacteria (*B. bronchiseptica*, *S. equi* sp. *zooepidemicus*, and *Mycoplasma* spp.).<sup>6,7,19,44</sup>

Our study indicates that respiratory viruses, primarily CPIV, frequently are also found in dogs with BP, which is caused by opportunistic bacteria. Therefore, it is likely that CIRD viruses can predispose dogs to opportunistic bacterial lung infections by increasing bacterial adhesion, as has been reported in humans.<sup>35</sup>

In this study, CPIV was the most common viral pathogen detected, which is in accordance with previous

reports describing viruses responsible for CIRD in different countries.<sup>6,13,19</sup> Novel CRCoV was detected in 1 dog with BP, further demonstrating that CRCoV has a worldwide distribution and also may be detected in Northern Europe.

Canine parainfluenza virus was prevalent despite recent vaccination, which can be considered indicative of poor vaccine-induced antibody coverage against CPIV. In contrast, CAV and CDV were not encountered in dogs vaccinated against these viruses. This finding is in accordance with previous reports. In a longitudinal study on respiratory viruses in a rehoming center in England, CPIV was commonly detected despite regular vaccinations, but CDV and CAV-2 were not encountered, most likely because of adequate vaccination coverage.<sup>13</sup> It remains unknown whether more efficient CPIV vaccines and possible CRCoV vaccinations could decrease the incidence of BP, as has been shown in humans, in whom protection against influenza and respiratory syncytial virus decreased the incidence of secondary bacterial infections.<sup>50–52</sup>

Nosocomial infections with respiratory viruses also have been reported in dogs. An outbreak of CPIV was described in an animal hospital and an outbreak of CHV was reported in immunocompromised dogs.<sup>53,54</sup> Because co-infections with CIRD viruses are shown to be common in dogs with BP, the infection risk needs to be taken into account when treating BP patients in the same premises (eg, intensive care units) with immunocompromised patients.

Dogs with viral co-infections were significantly heavier than those without virus infection. This finding might be influenced by the structure of the virus-negative group: All 4 dogs with another predisposing factor for the development of BP were <20 kg (West Highland White Terrier, Dachshund, Spanish Water Dog, and Schnauzer). Dogs with viral co-infections also were younger than those without viral co-infection, although this did not reach statistical significance. This finding is not unexpected, because young animals might have insufficient acquired immunity against CIRD viruses.<sup>2</sup>

Clinical findings, arterial blood gas analysis, and hematology, as well as respiratory sample cytology in both groups were in accordance with previously reported findings in BP and did not differ between virus-negative and virus-positive groups.<sup>42,43,48</sup> On thoracic radiographs, an alveolar pattern in the cranial and the middle lobes was predominant in both groups without group predisposition. Radiographic findings in dogs with BP have been thoroughly reported previously for cases of aspiration etiology.<sup>55,56</sup> In our study, radiographic findings in dogs with BP caused by other etiologies were similar to those reported for aspiration pneumonia. Aspiration etiology was considered unlikely, because none of the dogs with BP had a history of vomiting, regurgitation, recent anesthesia or signs compatible with laryngeal paralysis. Our findings could indicate that an alveolar pattern in cranial and middle lung lobes may be typical for pneumonia, regardless of etiology. On the other hand, aspiration pneumonia might have played a role in some dogs but could not be

confirmed or denied based on available history, examination findings, or imaging. We were unable to identify clinical variables to reliably distinguish dogs with BP and viral co-infection, and PCR testing therefore appears to be required to identify viral respiratory infections in dogs with BP. A similar finding was reported in humans.<sup>57</sup>

There were no significant differences in the duration of hospitalization ( $P = .427$ ) or partial pressures of arterial oxygen at presentation ( $P = .343$ ) between BP dogs with and without viral co-infection, indicating that viral co-infections do not appear to cause a more severe course of BP. In dogs, limited information is available on the severity of BP of different etiological origins, and in humans the reports are contradictory. Some studies have shown that mixed infections with viruses and bacteria induce a more severe clinical disease, whereas others have been unable to demonstrate significant differences in disease severity.<sup>49,58–61</sup>

Previous studies reporting microbiological findings in dogs with pneumonia have found growth of a single species of bacteria in 40–74% of cases.<sup>42,43</sup> All of these studies used TTW as a sampling method. Factors that might have influenced the finding of primarily a single species of bacteria in our study may be the use of BAL as a sampling method in majority of cases, compared to previous studies where TTW was used and the widespread use of prior antimicrobial treatments in these dogs.

Novel molecular methods have allowed the rapid testing of several respiratory pathogens simultaneously. Lower respiratory tract samples are considered ideal when diagnosing viral respiratory infections in humans with CAP, and it has been shown that virus-positive PCR in BALF is associated with respiratory symptoms in humans.<sup>62,63</sup> Naturally, the invasiveness of retrieving BALF, compared to upper respiratory sampling, limits the usefulness of this accurate sample. However, especially when using molecular methods, virus recovery from the upper respiratory tract may be suggestive of virus exposure rather than indicative of an active viral infection.<sup>6</sup> We chose lower respiratory tract samples in order to decrease the number of false-positive results, but a comparison of PCR findings between upper and lower respiratory tract samples would be useful. Underestimation of virus-positive PCR results may have occurred in our dogs in cases in which viral infection preceded BP and sampling was performed outside of the viral shedding period.

*Bordetella bronchiseptica* and *Mycoplasma* spp. were tested using both PCR and conventional culture methods. Polymerase chain reaction was, as expected, able to reliably demonstrate both pathogens in dogs with positive culture results. Additionally, *Mycoplasma* PCR was positive in 3 dogs with negative culture results. The clinical relevance of these positive results is difficult to interpret, because *Mycoplasma* spp. are also encountered in the respiratory tract of healthy dogs.<sup>64</sup> On the other hand, because *Mycoplasma* requires special culture methods (and in this study also shipping to an outside laboratory), there might have been dogs in which *Mycoplasma* culture was falsely negative. Quantitative

PCR might have aided in assessing the clinical relevance of these PCR findings.

Respiratory viruses were not detected in control dogs with prolonged BBTB. *Bordetella bronchiseptica* commonly accompanies CIRD viruses in acute respiratory infections and signs usually are self-limiting.<sup>6,19,28,29</sup> Dogs with prolonged BBTB were considered more likely than those of the general dog population to have been exposed to CIRD viruses previously. Infections with CIRD viruses are self-limiting within the first weeks, and because all BBTB dogs had prolonged clinical signs, an active viral infection therefore was considered unlikely.<sup>7,9,11,65</sup> Consequently, the negative results in the BBTB group are considered to increase the reliability of positive virus PCR findings in dogs with BP.

The most important limitation in this study was the small number of dogs in each group. This decreases statistical power (ie, the possibility of detecting a true difference between groups or reporting a difference that does not truly exist). Additionally, because this study was performed in Northern Europe in household dogs with low infection pressure, the results may not be applicable in all situations.

In conclusion, respiratory viruses, primarily CPIV, were found frequently in lower respiratory samples of dogs with BP and may play an important role in the etiology and pathogenesis of BP. Additionally, clinical variables and disease severity did not differ between BP dogs with and without viral co-infection.

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## Footnotes

- <sup>a</sup> Olympus GIF N180, Olympus Medical Systems Europa GmbH, Hamburg, Germany  
<sup>b</sup> IDEXX GmbH, Mörkestraße 28/3, D-71636 Ludwigsburg, Germany  
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<sup>d</sup> PASW Statistics 18, SPSS Inc, 233 South Wacker Drive, Chicago, IL
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*Conflict of Interest Declaration:* Authors disclose no conflict of interest.

*Off-label Antimicrobial Declaration:* Authors declare no off-label use of antimicrobials.

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## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Table S1.** Respiratory sampling technique, information on prior antimicrobial treatment as well as the Gram stain, culture and PCR results for individual dogs (n = 20) with bacterial pneumonia.