Co-infections with Respiratory Viruses in Dogs with Bacterial Pneumonia

Viitanen, S. J.

2015


http://hdl.handle.net/10138/166587
https://doi.org/10.1111/jvim.12553

Downloaded from Helda, University of Helsinki institutional repository.

This is an electronic reprint of the original article.

This reprint may differ from the original in pagination and typographic detail.

Please cite the original version.
Co-infections with Respiratory Viruses in Dogs with Bacterial Pneumonia

S.J. Viitanen, A. Lappalainen, and M.M. Rajamäki

Background: Bacterial pneumonia (BP) is an inflammation of the lower airways and lung parenchyma secondary to bacterial infection. Because BP is difficult to induce experimentally in healthy animals, the pathogenesis is considered complex and involves several underlying mechanisms. Possible predisposing factors for the development of BP, such as diseases leading to aspiration, decreased ciliary function, or immunodeficiency, have been described in Dogs. 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. It is highly contagious and affects the larynx, trachea, bronchi, and nasal mucosa, causing acute respiratory signs, mainly cough, which usually are self-limiting. Because of the contagious nature of CIRD, it is most prevalent in dense dog populations such as in kennels and rehoming centers. 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. 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. Canine herpes virus (CHV) also was detected in dogs with respiratory signs decades ago, but its role in CIRD remains controversial. 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. Subsequently, evidence of contagious tracheobronchitis caused by CRCoV has been reported worldwide. Canine influenza virus (CIV) was first described in a racing Greyhound population in

Abbreviations:
- BAL: bronchoalveolar lavage
- BALF: bronchoalveolar lavage fluid
- BBTB: tracheobronchitis caused by Bordetella bronchiseptica
- 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
- CRP: C-reactive protein
- CRCoV: canine respiratory coronavirus
- CnPnV: canine Pneumovirus
- CPIV: canine parainfluenza virus
- TTW: transtracheal wash
Viral Co-infections in Dogs with Pneumonia

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 (lateralateral 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 cytokine (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, 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). Transtracheal wash (TTW) sampling was used in dogs with BP in which anesthesia was considered unsafe because of the severity of the disease.

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. A bacterial origin was proved by cytological confirmation of bacterial infection in respiratory samples (>2 intra-cellular bacteria/oil immersion field), positive bacterial culture (>10^5 colony-forming units/mL in airway samples), 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* (>10^5 colony-forming units/mL) in bronchoalveolar lavage fluid (BAL) 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. Semiquantitative bacterial culture of BALF and TTW fluid was performed as follows: A 10-μL volume of the specimen was streaked onto agar. 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°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. *CnPV* was analyzed by RT-PCR at the laboratory responsible for the discovery of this pathogen.

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 was also 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.

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.

<table>
<thead>
<tr>
<th></th>
<th>Negative Respiratory Virus PCR (n = 12)</th>
<th>Positive Respiratory Virus PCR (n = 8)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Male 5/12</td>
<td>Male 5/8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Female 7/12</td>
<td>Female 3/8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>25.0 ± 16.4</td>
<td>41.7 ± 15.7</td>
<td>.037</td>
</tr>
<tr>
<td>Body temperature (°C)</td>
<td>39.6 ± 0.9</td>
<td>39.2 ± 0.8</td>
<td>.31</td>
</tr>
<tr>
<td>Respiratory rate (breaths/min)</td>
<td>59 ± 21</td>
<td>45 ± 20</td>
<td>.15</td>
</tr>
<tr>
<td>Arterial paO₂ (mmHg)</td>
<td>74.9 ± 11.1</td>
<td>79.1 ± 14.1</td>
<td>.49</td>
</tr>
<tr>
<td>Arterial paCO₂ (mmHg)</td>
<td>29.3 ± 3.9</td>
<td>30.9 ± 1.1</td>
<td>.19</td>
</tr>
<tr>
<td>Alveolar-arterial O₂ gradient</td>
<td>41.3 ± 11.8</td>
<td>33.5 ± 14.4</td>
<td>.23</td>
</tr>
<tr>
<td></td>
<td>Median (IQR)</td>
<td>Median (IQR)</td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>5.8 (1.3–8.4)</td>
<td>0.9 (0.7–1.4)</td>
<td>.057</td>
</tr>
<tr>
<td>Duration of clinical signs (days)</td>
<td>1.0 (1–3.5)</td>
<td>3.0 (1–5.3)</td>
<td>.48</td>
</tr>
<tr>
<td>Duration of hospitalization (days)</td>
<td>1.8 (0–4.8)</td>
<td>1.0 (0.3–1.5)</td>
<td>.43</td>
</tr>
<tr>
<td>Serum C-reactive protein (mg/L)</td>
<td>140 (84–192)</td>
<td>63 (52–178)</td>
<td>.98</td>
</tr>
<tr>
<td>Blood leukocyte count (10⁹/L)</td>
<td>14.6 (12.5–22.0)</td>
<td>10.6 (7.4–22.9)</td>
<td>.34</td>
</tr>
<tr>
<td>Segmented neutrophil count (10⁹/L)</td>
<td>11.7 (9.1–15.6)</td>
<td>7.8 (5.2–17.5)</td>
<td>.27</td>
</tr>
<tr>
<td>Band neutrophil count (10⁹/L)</td>
<td>0.5 (0.08–2.0)</td>
<td>0.3 (0.0–0.9)</td>
<td>.48</td>
</tr>
<tr>
<td>Lymphocyte count (10⁹/L)</td>
<td>0.7 (0.4–1.7)</td>
<td>1.5 (0.4–3.6)</td>
<td>.52</td>
</tr>
<tr>
<td>Eosinophil count (10⁹/L)</td>
<td>0.3 (0.0–0.6)</td>
<td>0.2 (0.0–0.6)</td>
<td>.91</td>
</tr>
<tr>
<td>Monocyte count (10⁹/L)</td>
<td>1.0 (0.3–1.7)</td>
<td>0.7 (0.5–1.5)</td>
<td>.97</td>
</tr>
<tr>
<td>Basophil count (10⁹/L)</td>
<td>0.0 (0–0.0)</td>
<td>0.0 (0–0.0)</td>
<td>.68</td>
</tr>
</tbody>
</table>
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, 0.0–14.7; \( P = 0.008 \)), 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. \(^{47}\) 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. \(^{47}\) 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

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

<table>
<thead>
<tr>
<th>Radiographic Pattern</th>
<th>Number of Dogs</th>
<th>Location Number of Dogs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative virus PCR (n = 12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alveolar, single lobe</td>
<td>4/12</td>
<td>Right cranial lobe 1/4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Right caudal lobe 1/4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Left cranial cranial lobe 1/4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Left caudal cranial lobe 1/4</td>
</tr>
<tr>
<td>Alveolar, multiple lobes</td>
<td>5/12</td>
<td>Right cranial lobe 3/5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Right middle lobe 1/5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Right caudal lobe 1/5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Left cranial cranial lobe 3/5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Left caudal cranial lobe 1/5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Patchy alveolar 1/5</td>
</tr>
<tr>
<td>Positive virus PCR (n = 8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alveolar, single lobe</td>
<td>2/8</td>
<td>Right middle lobe 2/2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Right cranial lobe 2/4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Right middle lobe 3/4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Left cranial cranial lobe 2/4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Left caudal cranial lobe 2/4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Patchy alveolar 1/4</td>
</tr>
<tr>
<td>Alveolar, multiple lobes</td>
<td>4/8</td>
<td></td>
</tr>
<tr>
<td>Bronchointerstitial</td>
<td>3/12</td>
<td></td>
</tr>
<tr>
<td>(graded moderate–severe)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patchy alveolar</td>
<td>2/8</td>
<td></td>
</tr>
</tbody>
</table>

**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.

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

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. 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. 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. 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.

Bacterial pneumonia is one of the most serious lower respiratory tract infections in dogs and can lead to significant morbidity and mortality. Therefore, early recognition and appropriate treatment are crucial to improve the outcome of these cases. In our study, the most common respiratory virus detected was CPIV, followed by CAV and CDV. Mycoplasma spp. were detected in a smaller proportion of cases, and CRCoV was detected in a single case.

Our findings suggest that CPIV is a common respiratory virus in dogs with BP, and its prevalence may be underestimated. Further studies are needed to fully understand the role of CPIV in the development of canine pneumonia and to develop effective vaccination strategies against this virus.
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. 57

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. 59, 66–68

Previous studies reporting microbiological findings in dogs with pneumonia have found growth of a single species of bacteria in 40–74% of cases. 42, 43 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. 62, 63 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. 6 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. 64 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. 6, 19, 28, 29 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. 7, 9, 11, 65 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.

### Footnotes

a Olympus GIF N180, Olympus Medical Systems Europa GMBH, Hamburg, Germany
b IDEXX GmbH, Mörkerve 28/3, D-71636 Ludwigsburg, Germany
c Animal Health Diagnostic Centre, College of Veterinary Medicine, Cornell University, 240 Farrier Road, Ithaca, NY 14853
d PASW Statistics 18, SPSS Inc, 233 South Wacker Drive, Chicago, IL

### Acknowledgments

The authors thank Dr Merja Rantala and Prof Satu Sankari for their contribution to laboratory analysis and Laura Parikka for her technical assistance. Jouni Junnila from 4Pharma Ltd is thanked for his contribution to statistical analysis.

Conflict of Interest Declaration: Authors disclose no conflict of interest.

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

### References


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.