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Bacterial infection in dogs with aspiration pneumonia at 2 tertiary referral practices

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Abstract

Background: In dogs, antimicrobial drugs are widely prescribed for aspiration pneumonia (AP) despite poor documentation of bacterial infection in AP (b-AP) using bronchoalveolar lavage fluid (BALF) analysis. Interpretating discordant cytology and culture results is challenging, contributing to lack of a criterion standard, and highlighting differences between veterinary and human medical criteria for b-AP.

Objectives: Determine how many dogs with AP had BALF collection and differences in diagnosis of b-AP using veterinary vs human medical criteria. Report findings of noninvasive markers (e.g. fever, band neutrophilia, radiographic severity score) in dogs with and without b-AP.

Animals: Retrospective cohort study of client-owned dogs (n = 429) with AP at 2 university veterinary hospitals. Twenty-four dogs met enrollment criteria.

Methods: Inclusion criteria were radiographic diagnosis of AP, ≥1 risk factor, CBC findings, and BALF cytology and culture results. Veterinary medical b-AP criteria were cytology findings compatible with sepsis with or without positive culture, or cytology findings not consistent with sepsis and positive culture (≥1.7×10^3 cfu/mL). Human medical b-AP criteria required culture with ≥10^4 cfu/mL or > 7% cells with intracellular bacteria on cytology.

Results: Only 24/429 dogs met all enrollment criteria; 379/429 dogs lacked BALF collection. Diagnosis of b-AP differed using veterinary (79%) vs human (29%) medical criteria. Fever, band neutrophils and high radiographic scores were noted in dogs with and without b-AP.

Conclusions and Clinical Importance: Lack of routine BALF collection hampers definitive recognition of bacterial infection in AP. Differences in dogs meeting veterinary vs human medical definitions for b-AP and usefulness of noninvasive markers warrant further study to improve understanding of the role of bacteria in AP.

Abbreviations: AP, aspiration pneumonia; APn, aspiration pneumonitis; AU-VTH, Auburn University Veterinary Teaching Hospital; BALF, bronchoalveolar lavage fluid; b-AP, bacterial aspiration pneumonia; cfu, colony forming units; MU-VHC, University of Missouri Veterinary Health Center.

KEYWORDS
antimicrobial, bacterial culture, bronchoalveolar lavage fluid, pneumonitis, thoracic radiography

1 | INTRODUCTION

Although aspiration is recognized as an important cause of secondary bacterial pneumonia, not all patients with aspiration pneumonia (AP) develop secondary bacterial infection (b-AP) and require antimicrobial treatment.[1] In people, b-AP implies inhalation of oropharyngeal secretions with resident bacteria resulting in lung infection, whereas aspiration pneumonitis (APn) refers to aspiration of gastric acid and enzymes causing inflammation without clinically relevant infection.[2] Treatment differs in people: b-AP is treated using antimicrobial drugs, whereas APn typically is managed supportively.[3] Antimicrobial drugs are controversial in people with APn because they can promote development of antimicrobial resistance.[3,4] In dogs, the term AP is used to describe both b-AP and APn, likely because no clear clinical distinction is made between them.[5,6] Furthermore, despite lack of confirmation of bacterial infection, routine empirical use of antimicrobial drugs for AP in dogs is widespread,[6,7] conflicting with antimicrobial use guidelines.[1]

Documenting b-AP in dogs ideally relies on evaluation of bronchoalveolar lavage fluid (BALF) for cytologic examination and culture.[1,8,9] However, presence of bacteria does not always imply causation because the lungs are not sterile but harbor a rich and diverse microbiota.[10] There are challenges in interpreting BALF cytology and culture results, especially when changes are subtle or discordant. Aspiration would be expected to bring commensal bacteria from the gastrointestinal tract, oropharynx and upper airways into the lungs, calling into question if cytologic or microbiologic detection represents contamination or infection. When aspirated material enters the lower respiratory tract, cells of the host immune system should engulf bacteria and promote clearance. Thus, cytologic presence of intracellular bacteria in low numbers is not synonymous with b-AP after aspiration. Similarly, presence of bacteria, including aspirated commensal organisms, may lead to degenerative changes in neutrophil morphology that may not be pathognomonic for infection. In practice, definitive diagnosis of bacterial pneumonia in dogs is made using noninvasive clinicopathologic features (eg, fever, peripheral neutrophilia or neutropenia, and radiographic infiltrates) with BALF cytology reflecting septic suppurative inflammation, and a positive culture.[11] More specifically, identification of >2 intracellular bacteria in any of 50 high-powered fields using gram stained cytospin material and clinically relevant bacterial growth from semiquantitative BALF cultures of ≥1.7×10^3 cfu/mL have been recommended for diagnosis of bacterial pneumonia in dogs.[12] These criteria contrast with recommendations in people where cytology is either not a criterion for diagnosis[13,14] or where intracellular bacteria must be present in much higher quantities (eg, cutoff of 7%[15] or 25%[16] of airway lavage cells with intracellular organisms). In people, quantitative BALF cultures most frequently are used, with ≥10^4 cfu/mL recommended as a diagnostic threshold.[13,17,18] To promote better antimicrobial stewardship, reevaluation of criteria used in dogs compared to humans to diagnose b-AP is warranted.

Our first objective was to determine how many dogs from 2 tertiary referral university hospitals had diagnostic testing (specifically BALF cytology and culture) to document presence of bacteria in the lungs. For the second objective, using the subpopulation of dogs with BALF collection, veterinary and human medical criteria for diagnosis of b-AP were applied to determine if clinically relevant differences may exist in definitions between species. A third objective was to determine if noninvasive markers (eg, fever, band neutrophilia, radiographic severity score) would be different in dogs with b-AP and APn, thus guiding future studies to investigate which dogs would benefit most from airway lavage for culture and susceptibility testing to guide antimicrobial treatment.

2 | MATERIALS AND METHODS

2.1 | Case selection

For this retrospective cohort study, medical records for dogs presented to veterinary teaching hospitals at the University of Missouri Veterinary Health Center and Auburn University Veterinary Teaching

TABLE 1 Clinical inclusion criteria for dogs with aspiration pneumonia

Clinical criteria for aspiration	
Risk factors for aspiration	Witnessed or suspected vomiting or regurgitation event, dysphagia, neurologic disorders, impaired gag reflex, laryngeal paralysis, brachycephaly
Anesthesia	Anesthetic event within the preceding 24 hours
Clinicopathologic evidence of inflammation[a]	Inflammatory leukogram, presence of bands on CBC
Physical examination evidence of inflammation[b]	Fever, tachycardia, tachypnea

Note: In addition to radiographic evidence of aspiration pneumonia, patients had to have any 1 of the criteria from any of the categories listed below.

[a]Reference ranges at the University of Missouri and Auburn University, respectively for neutrophils were (2.49-9.28 × 10^3/μL and 2.60-10.40 × 10^3/μL) and for bands (0.00-0.110 × 10^3/μL and 0.00-0.300 × 10^3/μL).

[b]Fever was defined as rectal temperature >39.1°C obtained at least twice; tachycardia was defined as heart rate >90, >120, and >160 in large breed, medium breed and small/toy breed dogs, respectively.

Hospital between January 1, 2010 and December 31, 2019 were retrospectively reviewed to identify dogs with a clinical diagnosis of AP or thoracic radiography diagnostic of AP. Records then were evaluated for inclusion criteria: a diagnosis of AP on thoracic radiographs (minimum of 2 orthogonal views; alveolar or interstitial pattern in ventrally-dependent lungs),[6] CBC, and BALF culture and cytology. Radiographs and CBC must have been performed within 48 hours of airway sampling. Dogs also must have met at least 1 of the following criteria (Table 1): identifiable risk factor for aspiration, recent anesthesia, or evidence of inflammation on CBC or physical examination. Dogs that received cefovecin or >1 dose of other antimicrobial drugs before BALF collection within the previous 7 days were excluded.

Demographic data, risk factors for aspiration, and clinicopathologic data (eg, physical examination findings, CBC, BALF cytology and culture), antimicrobial use, and radiographic findings were extracted from medical records.

2.2 | Radiography

Thoracic radiographs were evaluated in a blinded fashion by a single board-certified radiologist (Greg Almond). A modified scoring system (0-14 points) was used to assess radiographic severity.[19] The aforementioned scoring system in dogs was adapted from a study of radiographic severity in people with pulmonary edema. Radiographic scores were assigned 1 point for an interstitial pattern and 2 points for an alveolar pattern in each lobe. Lobes with both lung patterns were assigned 2 points. Unaffected lobes were assigned 0 points. Maximum radiographic score was 14.

2.3 | Airway lavage

Bronchoalveolar lavage fluid was collected in a sterile fashion using a 20 to 25 mL aliquot of warm sterile saline under endoscopic guidance or using a blind technique in a sterile fashion. The blind technique used a sterile 8 Fr red rubber catheter passed through a sterile endotracheal tube until wedged. Samples of BALF were placed on ice and delivered to the clinical pathology and diagnostic laboratories at the University of Missouri Veterinary Health Center and Auburn University Veterinary Teaching Hospital for cytology and culture. Total nucleated cell count was recorded. A 100 to 500 cell count differential was performed on Wright's-stained cytospin material. The type of inflammation, cellular morphology, and presence of intracellular bacteria were reported. In cases with reported cytologic evidence of intracellular bacteria, slides having more than a rare bacterium reported were rereviewed by a board-certified clinical pathologist to determine the percentage of cells containing bacteria using a 200 cell count. Reference ranges for BALF cytology were total nucleated cell count <500 cells/μL, ≥78% macrophages, ≤7% lymphocytes, ≤5% neutrophils, ≤6% eosinophils, ≤1% mast cells, and ≤ 1% epithelial cells.[8]

Samples were plated on MacConkey and blood agar for aerobic, and chocolate agar for capnophilic, cultures. Dogs that had received antimicrobial drugs recently (1 dose in preceding 7 days) at the University of Missouri Veterinary Health Center had BALF inoculated into a growth medium with antimicrobial removal devices. Organisms were identified and reported semiquantitatively.

Diagnosis of b-AP differed using veterinary or human medical criteria. Veterinary medical criteria resulted in diagnosis of b-AP if there was (1) BALF cytologic evidence of septic inflammation (ie, degenerative neutrophils, intracellular bacteria or both) with or without positive culture or (2) a nonseptic exudate with positive bacterial culture. Positive bacterial cultures were defined quantitatively using a threshold for clinically relevant bacterial growth of $\geq 1.7 \times 10^3$ cfu/mL.[12] Diagnosis of b-AP using human medical criteria was made if there was (1) cytology indicating >7% cells with intracellular bacteria with or without positive culture or (2) positive culture with $\geq 10^4$ cfu/mL. Dogs not fulfilling the criteria for b-AP were classified as APn.

2.4 | Statistics

Statistical analysis was performed using commercial statistical software (SigmaPlot data analysis software, version 12.0). Descriptive statistics were performed where appropriate. All data (patient demographics, risk factors for aspiration, clinicopathologic features, antimicrobial drug use and radiographic scores) were found to be nonnormally distributed using a Shapiro-Wilk test. Data were evaluated nonparametrically with results presented as median and interquartile ranges (IQR). McNemar's test was used to compare a veterinary medical (and separately, a human medical) diagnosis of b-AP between dogs receiving a single dose of antimicrobial drugs to those not receiving antimicrobial drugs before BALF collection. A Cohen's kappa coefficient was used to evaluate for agreement between BALF cytology and culture in veterinary and human medical diagnosis of b-AP as well as agreement between diagnosis of b-AP utilizing veterinary vs human criteria. For all analyses, significance was defined as $P < .05$.

3 | RESULTS

3.1 | Animals

Four hundred twenty-nine cases were identified for potential study inclusion over the 10-year period (Figure 1). Of these, 358/429 (83%) were from University of Missouri Veterinary Health Center and 71/429 (17%) were from Auburn University Veterinary Teaching Hospital. Of these, only 47/429 (11%) had BALF collection performed. From these 47 dogs, 22/47 (46%) were excluded for having received >1 dose of an antimicrobial in the preceding 7 days and 1 for incomplete medical records. Twenty-four cases (23/24, [95%] from University of Missouri Veterinary Health Center and 1/24 [5%] from Auburn University Veterinary Teaching Hospital) met all criteria for inclusion. Of these 24 cases, the median (IQR) age was 7.5 (2.0-10.8) years. The median (IQR) weight was 13.6 (7.0-19.4) kg. The median (IQR) body

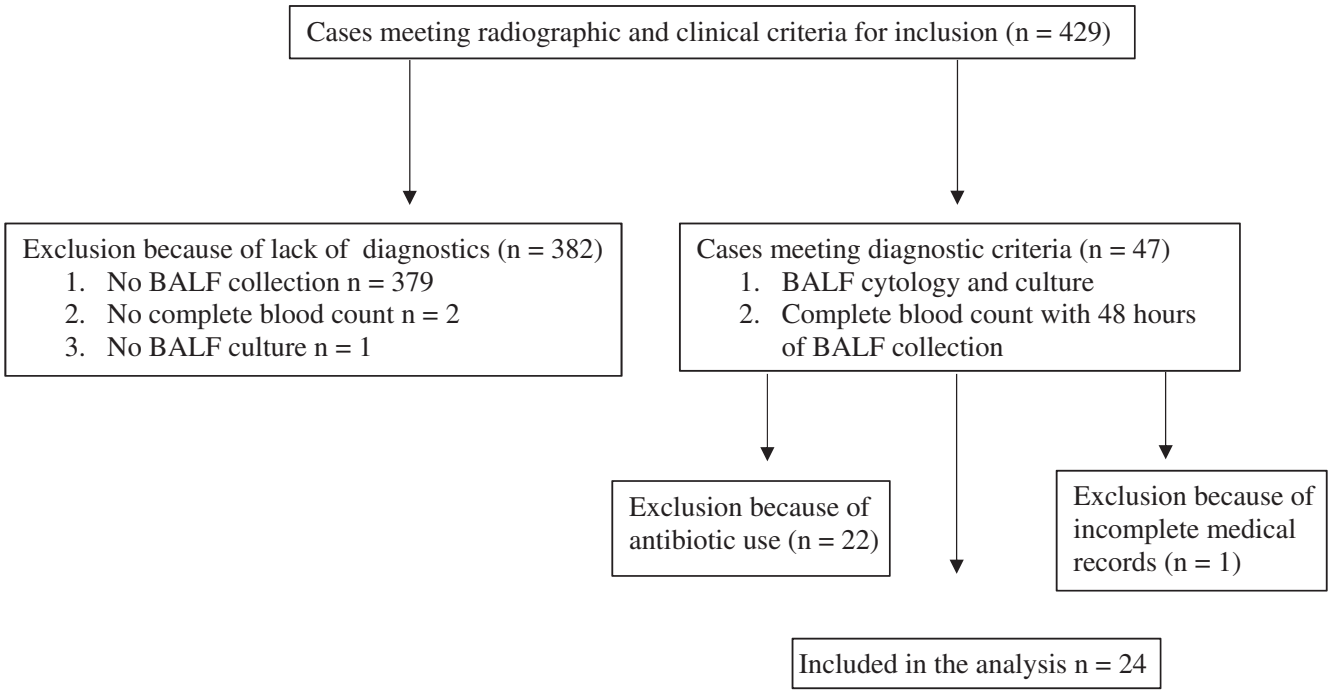

FIGURE 1 Flow chart showing selection of dogs with aspiration pneumonia for study inclusion. Cases were reviewed for radiographic evidence (alveolar or interstitial pattern in dependent lung lobes) of aspiration pneumonia, and 1 additional clinical criterion predisposing to aspiration including historical risk factors, anesthetic event within the previous 24 hours, or clinicopathologic evidence of inflammation on physical examination or CBC. Twenty-four dogs met all entry criteria to be included in the analysis

condition score (recorded in 21/24 [87.5%] dogs) was 5/9 (4-6/9). Eleven dogs (11/24, 45%) were spayed females, (11/24, 45%) were castrated males, 1 dog was an intact female and 1 dog an intact male. Breeds included mixed breed (4/24, 16%), English bulldog (3/24, 12.5%), Boston terrier (3/24, 12.5%), German shepherd dog (3/24, 12.5%), Cavalier King Charles spaniel (2/24, 8%), and 1 each of Golden retriever, French bulldog, Brittany, Yorkshire terrier, Labrador retriever, Miniature Pinscher, Goldendoodle, Standard Poodle, and Shih Tzu. Head confirmation included brachycephalic (10/24, 41%), mesencephalic (6/24, 25%), dolichocephalic (4/24, 16%), and unidentified (4/24, 16%).

Risk factors for aspiration included regurgitation (12/24 [50%] including 7/24 [29%] with megaesophagus), vomiting (9/24, 37.5%), recent anesthetic events (2/24, 8%), esophagitis (2/24, 8%), laryngeal paralysis (2/24, 8%), epilepsy or seizure activity (2/24, 8%), and brachycephalic obstructive airway syndrome (2/24, 8%). Twelve dogs (12/24, 50%) had chronic risk factors (present >2 weeks). More than 1 risk factor was identified in 13/24 (54%) dogs.

Six dogs (6/24, 25%) were febrile. Median (IQR) rectal temperature was 37.8 (37.9-39.3°C). Median (IQR) heart rate and respiratory rate were 120 (100-138) beats per minute and 41 (24-50) breaths per minute, respectively. Nine dogs (9/24, 37.5%) had tachycardia and 6 dogs (6/24, 25%) were tachypneic.

On CBC, peripheral neutrophilia was present in 18/24 dogs (75%), band neutrophilia in 14/24 dogs (58%), and neutropenia in 2/24 dogs (8%). One dog had a normal leukogram. In the 18 dogs with neutrophilia, median (IQR) segmented neutrophils and band neutrophils were $12 \times 10^3/\mu L$ (12×10^3-$17 \times 10^3/\mu L$) and $0.270 \times 10^3/\mu L$ (0.010×10^3-$0.570 \times 10^3/\mu L$), respectively.

Median (IQR) thoracic radiographic score was 7 (4–10). An alveolar or interstitial pattern was present in at least 1 lung lobe in 22/24 (92%) and 2/24 dogs (8%), respectively. Fourteen dogs (14/24, 58%) had both interstitial and alveolar patterns in >1 lung lobe. Twenty-three dogs (23/24, 95%) had >1 lung lobe affected. Affected lobes included the right middle (21/24 87.5%), right cranial (17/24, 70%), caudal subsegment of the left cranial (17/24, 70%), cranial subsegment of the left cranial (16/24, 66%), right caudal (15/24, 62.5%), left caudal (11/24, 45%), and accessory (9/24, 37.5%).

Collection of BALF was performed using bronchoscopic guidance in 18/24 (75%) of dogs and by blind technique in 6/24 (25%) of dogs. A standardized bronchoscopic report was available for review in 10 dogs. Abnormalities included hyperemic and edematous mucosa of the trachea or mainstem bronchi or both (6/24, 25%), hemorrhage (3/24, 12.5%), bronchiectasis (2/24, 8%), bronchomalacia (2/24, 8%) and exudate or mucus accumulation (2/24, 8%). Sites sampled or percentage of saline aliquot retrieved were not consistently recorded.

Median (IQR) total nucleated cell count of BALF was 3740/μL (259-6821/μL). Median (IQR) percentage neutrophils was 75% (54%-90%) with 23/24 dogs (95%) having airway neutrophilia. Three dogs (3/24, 12.5%) had a BALF lymphocytosis (19%, 17%, 12%); remaining

dogs had a median (IQR) of 2% lymphocytes (1%-4%). Three dogs (3/24, 12.5%) had BALF eosinophilia (45%, 37%, 26%) with remaining dogs having a median (IQR) of 1% (0%-3%) eosinophils. Aerobic bacterial organisms isolated on culture included *Escherichia coli*, *Klebsiella pneumonia*, *Pasteurella multocida*, *Streptococcus canis*, *Klebsiella oxytoca*, *Acinetobacter* spp., *Neisseria weaver*, and *Frederikenia canicola*. The only organism recovered on capnophilic culture was *Pasteurella dagmatis*. Table S1 provides details on the species and frequency of cultured organisms.

3.2 | Veterinary vs human medical definitions of b-AP

Nineteen of 24 dogs (79%; 95% confidence interval [CI], 0.579-0.929) met the veterinary medical criteria for diagnosis of b-AP, with 5 (21%; 95% CI, 0.071-0.425) having APn. In contrast, only 7/24, (29%; 95% CI, 0.126-0.510) dogs met the human medical criteria for diagnosis of b-AP, with 17/24 (71%; CI, 0.489-0.873) having APn. Table S2 provides individual patient data for dogs with b-AP and APn

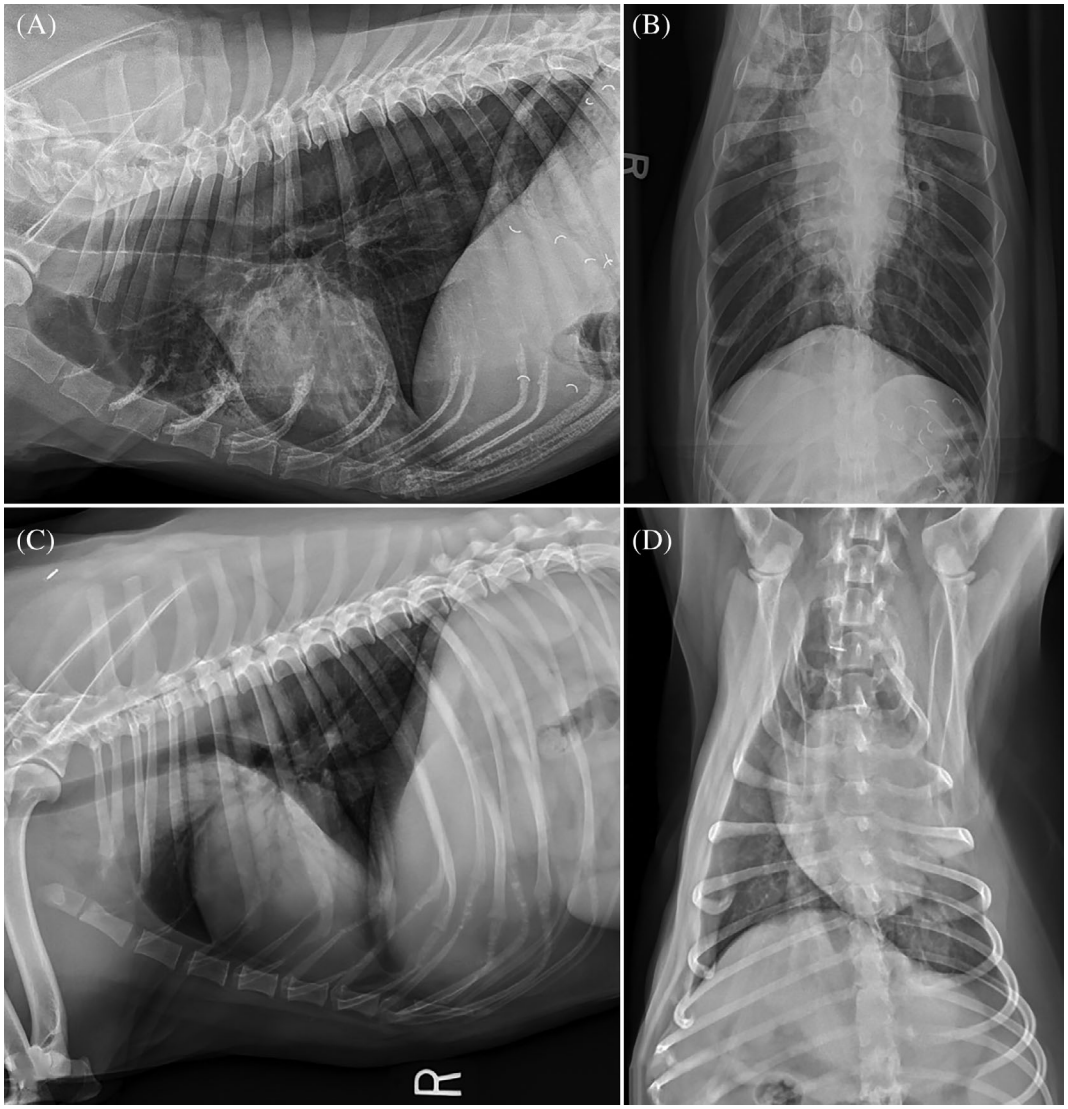

FIGURE 2 Two dogs meeting the veterinary criteria for (A, B) b-AP or (C, D) APn having identical radiographic scores of 9 out of a total of 14 points. (A, B) An 11-year-old German Shepherd dog with fever and mild band neutrophilia. There is an alveolar pattern in the right cranial lung lobe visible in both projections. Superimposed over the heart in the lateral projection there is an alveolar pattern in the right middle lung lobe. There are small patchy areas of alveolar pattern in the cranial and caudal subsegments of the left cranial lung lobe. When applying the human medical criteria, this dog was reclassified as having APn. (C, D) An 8-year-old MC Brittany lacking fever but with mild band neutrophilia showed a pronounced alveolar pattern in the caudal subsegment of the left cranial lung lobe characterized by air bronchograms and a lobar sign visible in both projections. APn, aspiration pneumonitis; bAP, bacterial aspiration pneumonia

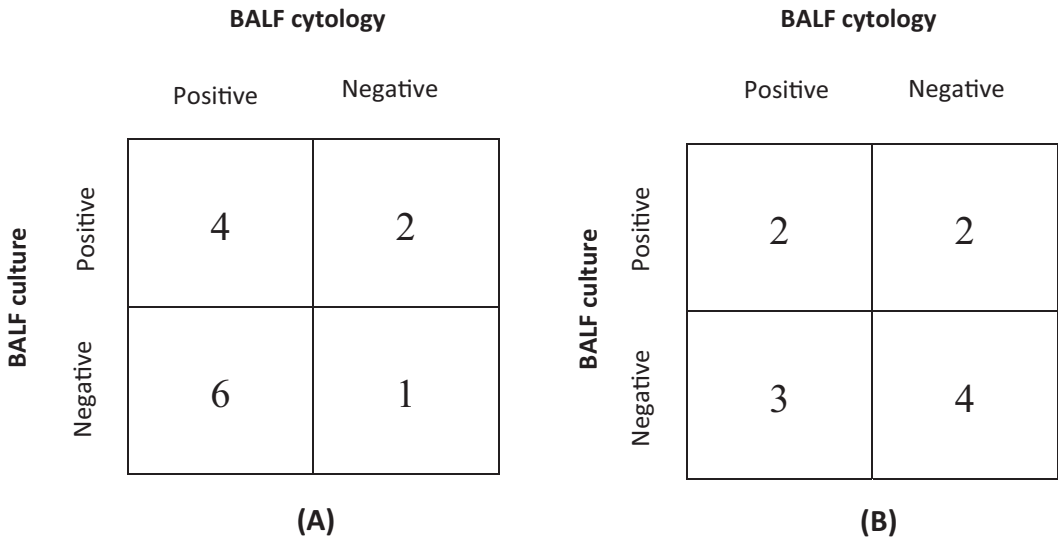

FIGURE 3 In a study of 24 dogs with aspiration pneumonia, 2 × 2 boxes demonstrate no agreement between cytology and culture using (A) veterinary (Cohen's Kappa = −0.154) or (B) human (Cohen's Kappa = −0.077) criteria for b-AP. Diagnosis of b-AP using veterinary criteria was made if there was (1) BALF cytologic evidence of septic inflammation with or without positive culture or (2) no cytologic evidence of sepsis with positive bacterial culture. Positive bacterial cultures were defined quantitatively using a threshold for clinically relevant bacterial growth ≥1.7×10^3 cfu/mL. Using this definition, 19 dogs total had b-AP. Diagnosis of b-AP using human medical criteria was made if there was (1) cytology reflecting >7% cells with intracellular bacteria with or without positive culture or (2) positive culture with ≥10^4 cfu/mL. Seven dogs fit the human medical criteria for b-AP. Dogs not fulfilling the criteria for b-AP were classified as APn. APn, aspiration pneumonitis; bAP, bacterial aspiration pneumonia

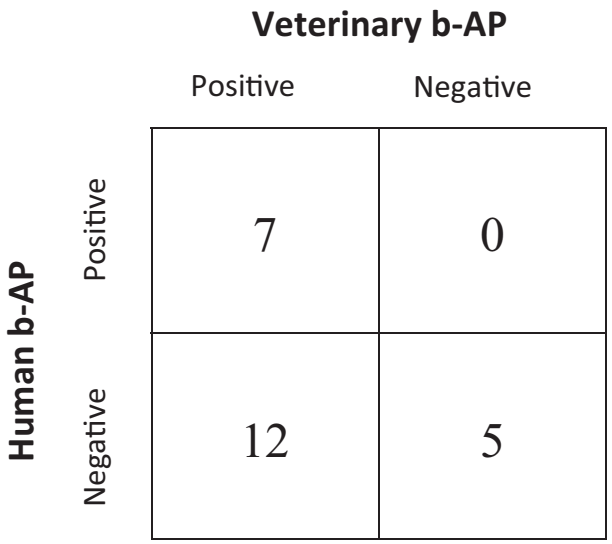

FIGURE 4 In a study of 24 dogs with aspiration pneumonia, 2 × 2 boxes demonstrating slight agreement (Cohen's Kappa = 0.196) between those meeting the veterinary vs human medical criterion of b-AP. Of the 24 dogs included in analysis, using veterinary criteria, 19 were classified as b-AP and five as APn. Using human medical criteria, only 7 dogs had b-AP with 17 having APn. APn, aspiration pneumonitis; bAP, bacterial aspiration pneumonia

using veterinary and human medical criteria, respectively. An example of radiographs taken in 2 dogs with identical radiographic scores, 1 with b-AP and 1 with APn when using veterinary medical criteria is shown in Figure 2A-D. Thirteen dogs (13/24, 54%) received a single dose of antimicrobial drugs <7 days before BALF collection and 11 dogs (11/24, 45%) had no antimicrobial drugs administered. No significant difference was found in diagnosis of b-AP using veterinary ($P = .06$) or human ($P = .43$) medical criteria between dogs receiving and not receiving a single dose of an antimicrobial drug.

When comparing dogs with a veterinary medical diagnosis of b-AP and APn, agreement was not noted between BALF cytology and culture ($\kappa = -0.154$; Figure 3A). Similarly, when comparing dogs with a human medical diagnosis of b-AP and APn, agreement was not noted between BALF cytology and culture ($\kappa = -0.077$; Figure 3B). Only slight agreement (defined as κ between 0.1 and 0.2) was found between dogs diagnosed as having b-AP using the veterinary and human medical criteria ($\kappa = 0.196$; Figure 4).

4 | DISCUSSION

Although lower airway sampling is recommended to document bacterial pneumonia and select optimal treatment,[1] our study highlights challenges in diagnosing b-AP, including few dogs having airway lavage and unclear optimal criteria to define active bacterial infection after aspiration. In our study, poor agreement was found between veterinary and human medical criteria for b-AP, highlighted by the difference in diagnosis of b-AP using the veterinary (19/24 [79%] dogs) and human (7/24 [29%] dogs) medical criteria to define the conditions. These results could cause markedly different treatment recommendations, with antimicrobial drugs being justified in either a majority or minority of dogs using the veterinary or human medical criteria for b-AP,

respectively. Depending on future studies, these findings call into question the routine practice of empirically prescribing antimicrobial drugs for all dogs with suspected AP. Judicious antimicrobial use is especially important in dogs with recurrent AP associated with underlying disorders such as megaesophagus because antimicrobial resistance is a likely outcome with repetitive use. To better inform clinical decision making, we described results of clinically applicable, noninvasive markers (eg, fever, bands, radiographic score) that could be used as surrogates for bacterial infection. However, because of small sample sizes, statistical comparisons were not performed to assess if any of the markers were significantly different between dogs with b-AP and APn using either veterinary or human medical criteria. Although our study suggests that airway lavage is the most useful diagnostic tool to identify bacterial infection, larger studies are needed to further evaluate the usefulness of noninvasive markers in predicting b-AP.

In contrast to published antimicrobial use guidelines in respiratory tract disease in dogs that advocate airway sampling when there is clinical suspicion of bacterial pneumonia,[1] 379/429 (88%) dogs with radiographic evidence of AP at 2 university veterinary hospitals lacked BALF collection. Perception of anesthetic and procedural risk in dogs with respiratory disease is a common reason to omit lower airway lavage. However, advanced diagnostic testing including BALF collection in dogs with respiratory disease has been shown to carry low risk of complications, regardless of disease severity.[20] Anecdotally, antimicrobial drug use for AP in dogs is widespread and occurs without confirmation of secondary bacterial infection in most cases. Clinical practice should proceed with consideration of how antimicrobial drug use may negatively impact the patient and contribute to poor antimicrobial stewardship. These considerations are important because our study indicated that, like people, dogs develop both b-AP and APn in response to aspiration. Antimicrobial drug administration in humans with APn is controversial because it does not offer clinical benefit and promotes antimicrobial drug resistance.[4] Evaluation of antimicrobial treatment in dogs with bacterial pneumonia, including aspiration as an etiology, is hampered by lack of definitive confirmation of bacterial infection.[21] Although it is recognized that delays in antimicrobial treatment can be detrimental, especially in patients with concern for sepsis, and that airway sampling is not feasible in all cases, our study emphasizes the importance of sampling when possible. Not only will BALF cytology and culture be useful to guide antimicrobial treatment, in particular when there is substantial antimicrobial drug resistance, but in future studies it also may be used to refine diagnostic criteria for b-AP in dogs.

In human medicine, criteria for diagnosis of b-AP differ from those used in veterinary medicine. Diagnosis in human medicine relies largely on quantitative BALF culture to diagnose b-AP, with $>10^4$ cfu/mL[13,17,18] considered supportive of the diagnosis, contrasting with the lower standard of 1.7×10^3 cfu/ml previously described in veterinary medicine.[12] In the latter veterinary study, diagnosis of bacterial infection was made based on meeting 3 of 5 criteria (history, physical findings, CBC, thoracic radiography, and bronchoscopic findings).[12] Although providing the cutoff for clinically important infection in dogs, the study was retrospective and has not, to date, been validated prospectively. Bronchoalveolar lavage fluid cytology is rarely used or, when utilized, more numerous intracellular bacteria must be visualized to support b-AP.[13,14] Despite these obstacles, more stringent criteria for diagnosis of b-AP in people raises concern for overdiagnosis of b-AP in dogs and subsequent overuse of antimicrobial drugs. Differences between veterinary and human medical diagnostic criteria for b-AP are especially important to consider with aspiration, where bacteria may be brought into the lung by contact with bacterial communities residing in the oral cavity and upper airways or via aspirated contents which themselves contain bacteria. Thus, presence of bacteria in BALF does not necessarily imply a pathogenic role (ie, b-AP). In fact, healthy adult humans commonly have microaspiration without clinical consequence,[22,23] with inhaled bacteria appropriately being cleared by defense mechanisms of the lung without causing infection. How to recognize the threshold between nonpathogenic and pathogenic bacterial presence is highlighted by the different diagnostic criteria in veterinary and human medicine. Even use of quantitative cultures can pose challenges including the dilutional effect of different volumes of lavage fluid instilled and retrieved and, in humans, evidence that bacterial burdens may be cleared depending on the course of infection in some patients.[13,14] Disparate results of BALF cytology and culture noted in the dogs of our study also have been observed in humans.[24] Additional studies refining diagnostic criteria for b-AP, including response to supportive care without antimicrobial treatment for dogs with APn, and prospective analysis of microbial cut offs for defining infection in BALF culture would better guide our antimicrobial recommendations and promote better antimicrobial stewardship.

The lung in health is not sterile as evidenced by a recent study using next generation sequencing and showing the lower airways of the dog to contain a rich and diverse microbiota.[25] Deviation from this healthy microbiota (dysbiosis) occurs in bacterial pneumonia.[10,25] Pathogens identified by culture may reflect taxa that disrupt the healthy respiratory microbiota, further contributing to pathogenicity. Amoxicillin/clavulanic acid (an antimicrobial drug recommended by the International Society of Companion Animal Infectious Disease guidelines) administered in healthy dogs altered the relative abundance and diversity of lung microbial communities.[26] Disruption of the microbial environment of the lung can dysregulate pulmonary immune development, promote allergic sensitization, and diminish antiviral immunity.[27] Collectively, these studies underscore the importance of distinguishing pathogens from commensals and understanding the impact that antimicrobial treatment has on both populations of microbes. Future studies may help determine if antimicrobial drugs in dogs with AP without pathogenic bacterial infection can be harmful by inducing respiratory dysbiosis.

Limitations of our study include its retrospective nature and, despite the large number of initial cases with clinical and radiographic criteria of AP, the ultimately small sample size of dogs having BALF cytology and culture, which may have contributed to bias. Because it was a retrospective study, it is unclear if a bias existed against including more severely affected dogs that would be at higher risk for decompensation under general anesthesia, or if a bias existed against very mildly affected dogs because airway lavage might have been considered an overly aggressive diagnostic procedure. Another potential

limitation was that 13/24 dogs received a single dose of antimicrobial within 7 days before to BALF collection, which could have influenced the cytology or culture results. However, all 13 of these dogs were from 1 institution (University of Missouri) with a culture protocol using antimicrobial removal devices before plating BALF. Additionally, when comparing antibiotic use between dogs with b-AP and APn, no significant difference was found between groups (veterinary medical criteria, $P = .06$; human medical criteria, $P = .43$).

In conclusion, our study identified clinically relevant differences that could impact antimicrobial drug recommendations based on veterinary vs human medical diagnostic criteria to identify secondary bacterial infection in dogs with AP. Although current practice, even at 2 tertiary university referral hospitals, underutilized BALF collection to confirm b-AP, empirical use of antimicrobial drugs remains routine. Our study indicates that airway lavage should be considered in cases of AP to confirm the need for antimicrobial drugs and better guide antimicrobial selection. Future prospective studies enrolling dogs with a spectrum (mild to severe) of disease are needed to improve diagnostic criteria for b-AP and better guide antimicrobial drug use. Refining the definition of bacterial infection using BALF cytology and culture will take additional study, ideally a blinded clinical trial to determine if aggressive supportive care (including airway nebulization and airway clearance techniques) vs antimicrobial drugs is needed to resolve AP. Finally, with a clear definition of b-AP, future studies also should investigate the utility of noninvasive markers of b-AP as surrogates for diagnosis, disease resolution, or both.

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CONFLICT OF INTEREST DECLARATION

Drs. Reinero, Vientos-Plotts, Cohn, and Grobman speak at regional, national, and international conferences on respiratory disease and may receive honoraria and reimbursement for travel and accommodations. These presentations may cover aspiration pneumonia, but compensation is independent of and unrelated to the study. Dr. Reinero (University of Missouri) has received gifts for respiratory research. This study was not funded by those gifts. No other authors have conflicts of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials. Although some dogs were treated with antimicrobials, the selection of antimicrobials was at the discretion of the attending clinician and was not a part of the study.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Authors declare no IACUC or other approval was needed.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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REFERENCES

1. Lappin MR, Blondeau J, Boothe D, et al. Antimicrobial use guidelines for treatment of respiratory tract disease in dogs and cats: antimicrobial guidelines working group of the International Society for Companion Animal Infectious Diseases. *J Vet Intern Med*. 2017;31:279-294.
2. DiBardino DM, Wunderink RG. Aspiration pneumonia: a review of modern trends. *J Crit Care*. 2015;30:40-48.
3. Prather AD, Smith TR, Poletto DM, et al. Aspiration-related lung diseases. *J Thorac Imaging*. 2014;29:304-309.
4. Dragan V, Wei Y, Elligsen M, Kiss A, Walker SAN, Leis JA. Prophylactic antimicrobial therapy for acute aspiration pneumonitis. *Clin Infect Dis*. 2018;67:513-518.
5. Nafe LA, Grobman ME, Masseau I, Reinero CR. Aspiration-related respiratory disorders in dogs. *J Am Vet Med Assoc*. 2018;253:292-300.
6. Sherman R, Karagiannis M. Aspiration pneumonia in the dog: a review. *Top Companion Anim Med*. 2017;32:1-7.
7. Tart KM, Babski DM, Lee JA. Potential risks, prognostic indicators, and diagnostic and treatment modalities affecting survival in dogs with presumptive aspiration pneumonia: 125 cases (2005-2008). *J Vet Emerg Crit Care (San Antonio)*. 2010;20:319-329.
8. Hawkins EC, DeNicola DB, Kuehn NF. Bronchoalveolar lavage in the evaluation of pulmonary disease in the dog and cat. State of the art. *J Vet Intern Med*. 1990;4:267-274.
9. Hawkins EC, DeNicola DB, Plier ML. Cytological analysis of bronchoalveolar lavage fluid in the diagnosis of spontaneous respiratory tract disease in dogs: a retrospective study. *J Vet Intern Med*. 1995;9:386-392.
10. Ericsson AC, Personett AR, Grobman ME, Rindt H, Reinero CR. Composition and predicted metabolic capacity of upper and lower airway microbiota of healthy dogs in relation to the fecal microbiota. *PLoS One*. 2016;11:e0154646.
11. Dear JD. Bacterial pneumonia in dogs and cats: an update. *Vet Clin North Am Small Anim Pract*. 2020;50:447-465.
12. Peeters DE, McKiernan BC, Weisiger RM, et al. Quantitative bacterial cultures and cytological examination of bronchoalveolar lavage specimens in dogs. *J Vet Intern Med*. 2000;14:534-541.
13. Dickson RP, Erb-Downward JR, Prescott HC, et al. Analysis of culture-dependent versus culture-independent techniques for identification of bacteria in clinically obtained bronchoalveolar lavage fluid. *J Clin Microbiol*. 2014;52:3605-3613.
14. Dennis BM, Betzold RD, Patton D, et al. Bacterial burden in critically injured ventilated patients does not correlate with progression to pneumonia. *Surg Infect (Larchmt)*. 2018;19:369-375.
15. Chastre J, Fagon JY, Domart Y, et al. Diagnosis of nosocomial pneumonia in intensive care unit patients. *Eur J Clin Microbiol Infect Dis*. 1989;8:35-39.
16. Chastre J, Fagon JY, Soler P, et al. Diagnosis of nosocomial bacterial pneumonia in intubated patients undergoing ventilation: comparison of the usefulness of bronchoalveolar lavage and the protected specimen brush. *Am J Med*. 1988;85:499-506.
17. Fagon JY. Diagnosis and treatment of ventilator-associated pneumonia: fiberoptic bronchoscopy with bronchoalveolar lavage is essential. *Semin Respir Crit Care Med*. 2006;27:34-44.

18. Baselski V, Klutts JS, Baselski V, et al. Quantitative cultures of bronchoscopically obtained specimens should be performed for optimal management of ventilator-associated pneumonia. *J Clin Microbiol*. 2013;51:740-744.
19. Le Boedec K, Heng HG, Snyder PW, et al. Pulmonary abnormalities in dogs with renal azotemia. *J Vet Intern Med*. 2012;26:1099-1106.
20. Bianco Z, Bukoski A, Masseau I, Reich C, Schultz L, Reinero C. Risk factors and outcomes in dogs with respiratory disease undergoing diagnostic airway lavage. *Front Vet Sci*. 2020;7:165.
21. Wayne A, Davis M, Sinnott VB, Bracker K. Outcomes in dogs with uncomplicated, presumptive bacterial pneumonia treated with short or long course antibiotics. *Can Vet J*. 2017;58:610-613.
22. Molyneux ID, Morice AH. Airway reflux, cough and respiratory disease. *Ther Adv Chronic Dis*. 2011;2:237-248.
23. Smith JA, Houghton LA. The oesophagus and cough: laryngo-pharyngeal reflux, microaspiration and vagal reflexes. *Cough*. 2013;9:12.
24. Allaouchiche B, Jaumain H, Dumontet C, Motin J. Early diagnosis of ventilator-associated pneumonia. Is it possible to define a cutoff value of infected cells in BAL fluid? *Chest*. 1996;110:1558-1565.
25. Vientós-Plotts AI, Ericsson AC, Rindt H, Reinero CR. Respiratory dysbiosis in canine bacterial pneumonia: standard culture vs microbiome sequencing. *Front Vet Sci*. 2019;6:354.
26. Fastrès A, Taminiau B, Vangrinsven E, et al. Effect of an antimicrobial drug on lung microbiota in healthy dogs. *Heliyon*. 2019;5:e02802.
27. Marsland BJ, Yadava K, Nicod LP. The airway microbiome and disease. *Chest*. 2013;144:632-637.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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