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Association between quantitative bacterial culture of bronchoalveolar lavage fluid and antibiotic requirement in dogs with lower respiratory tract signs

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Abstract

Background: Historically, positive bacterial cultures from the lower respiratory tract (LRT) have been considered clinically relevant when quantitative bacterial cultures of bronchoalveolar lavage fluid (BALF) were >1700 colony forming units (cfu)/mL. However, this threshold might not accurately predict a requirement for antibiotics.

Objectives: To study whether quantitative BALF bacterial culture results were predictive of antibiotic requirement in dogs with LRT signs.

Animals: Thirty-three client-owned dogs.

Methods: Cross-sectional study. Dogs with positive quantitative bacterial culture of BALF were included. Dogs were divided into 2 groups, depending on whether they had a LRT infection requiring antibiotics (LRTI-RA) or LRT disease not requiring antibiotics (LRTD-NRA), based on thoracic imaging features, presence of intracellular bacteria on BALF cytology, and response to treatment. Predictive effect of cfu/mL and BALF total nucleated cell count (TNCC) on antibiotic requirement, adjusting for ongoing or prior antibiotic therapy and age, were studied using logistic regression.

Results: Twenty-two and 11 dogs were included in the LRTI-RA and LRTD-NRA groups, respectively. The cfu/mL was not significantly predictive of antibiotic requirement, independent of ongoing or prior antibiotic treatment and age (LRTI-RA: median, 10 000 cfu/mL; range, 10-3 $\times$ 10^8; LRTD-NRA: median, 10 000 cfu/mL; range, 250-1.3 $\times$ 10^9; $P = .27$). The TNCC was not significantly predictive of antibiotic requirement when only dogs with bronchial disease were considered (LRTI-RA: median, 470 cells/μL; range, 240-2260; LRTD-NRA: median, 455 cells/μL; range, 80-4990; $P = .57$).

Abbreviations: AP, aspiration pneumonia; BAL, bronchoalveolar lavage; BALF, bonchoalveolar lavage fluid; CAP, community acquired pneumonia; CFU, colony forming unit; CRP, C-reactive protein; CT, computed tomography; LRT, lower respiratory tract; LRTD-NRA, lower respiratory tract disease not requiring antibiotics; LRTI, lower respiratory tract infection; LRTI-RA, lower respiratory tract infection requiring antibiotics; NRA, not requiring antibiotic; TNCC, total nucleated cell count.

Preliminary results were presented at the 2021 European College of Veterinary Internal Medicine virtual congress.

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Conclusion and Clinical Importance: The cfu/mL is an inappropriate measure for determining whether antibiotics are of benefit in dogs with LRT signs.

KEYWORDS
BAL, bronchitis, canine, colony forming unit, pneumonia

1 | INTRODUCTION

Lower respiratory tract infection (LRTI) is an important differential diagnosis in dogs with respiratory signs, fever, or sepsis because it can be life-threatening when involving the lungs, or impact quality of life if inadequately treated.[1] Classification of LRTI usually is based on anatomic location (lung parenchyma or bronchi) or underlying mechanisms associated with infection: aspiration pneumonia (AP), community-acquired pneumonia (CAP), hematogenous spread of bacteria, foreign body, or immunodeficiency. Aspiration pneumonia and CAP appear to be the most common causes of LRTI in dogs in clinical practice.[2] Imaging, cytological examination, and bacterial culture of bronchoalveolar lavage fluid (BALF) have been widely adopted as clinically useful for diagnosis of LRTI in dogs.[1,3,4] Evidence of infection includes positive bacterial culture of BALF, neutrophilic inflammation and visualization of intracellular bacteria on BALF cytology, and supportive thoracic imaging features (eg, alveolar pattern).[1,3] A previous study suggested a quantitative threshold of >1700 colony forming units (cfu)/mL within BALF, above which clinically relevant bacterial growth can be defined.[3] According to this study, a yield of >1700 cfu/mL provided a sensitivity of 86% and a specificity of 100% in diagnosing infection.[3] However, bacterial colonization has been reported in airways of both healthy dogs and humans with a cfu/mL often as high as those found for infected airways.[5-8] A recent study on AP in dogs also questioned the use of this threshold to diagnose bacterial infection.[9]

Indeed, the lower respiratory tract (LRT) does not appear to be sterile, as previously thought.[10,11] Culture-independent molecular techniques have suggested commensal microbial populations in healthy lungs, likely resulting from extension from upper airway communities, repeated microaspiration, and inhalation of bacteria.[12] Although the interaction between commensal pathogens, natural host defenses, and the immune system is complex, growing evidence suggests a protective role of these commensal flora against LRTI.[1,9-11] Destruction of commensal flora populations with antibiotics therefore could be detrimental were a misdiagnosis of LRTI to be made, although most changes seem to normalize within 2 weeks after discontinuation of antibiotics.[13] Furthermore, antibiotic overuse is a leading factor in development of multidrug resistance and therefore is increasingly recognized as a major public health issue.[14] Relying on a cfu/mL threshold for LRTI diagnosis therefore might be inappropriate if it leads to using antibiotics in dogs that do not require them.

The primary aims of our cross-sectional study were to assess whether BALF quantitative bacterial culture results could be predictive of antibiotic requirement in dogs with LRT disease, and explore the clinical relevance of the previously established threshold of 1700 cfu/mL regarding antibiotic usage. A secondary aim was to investigate whether the total nucleated cell count (TNCC) on BALF analysis also could be predictive of antibiotic requirement in dogs with LRT disease.

We hypothesized that BALF quantitative bacterial culture results would be poorly predictive of LRTI requiring antibiotics (LRTI-RA) in dogs. We also hypothesized that antibiotic therapeutic decisions should not be based on a BALF quantitative bacterial culture threshold.

2 | MATERIALS AND METHODS

2.1 | Case selection

To determine the minimum number of cases required for inclusion, a sample size calculation was performed before the study using an online calculator (https://epitools.ausvet.com.au/twomeanstwo). Considering previous data, mean quantitative bacterial culture results of 30 000 ± 28 300 cfu/mL in dogs requiring antibiotics and 300 ± 300 cfu/mL in dogs not requiring antibiotics and an enrollment ratio of 2:1 were used for calculation.[3] A minimum sample size of 20 dogs requiring antibiotics and 10 dogs not requiring antibiotics was obtained to achieve an alpha of .05 and a power of 90%.

Medical records for client-owned-dogs presented to Centre Hospitalier Vétérinaire Frégis (Arcueil, France) between July 2016 and February 2021 that had positive BALF quantitative bacterial culture results were identified and retrospectively reviewed. Samples before this timepoint were not included because quantitative bacterial cultures were not performed at our laboratory before this date. For each dog, demographic data, relevant history including recent antimicrobial use (ie, ongoing or <2 weeks before), physical examination findings, radiographic or computed tomography (CT) findings, and BALF cytology and culture with PCR results if available were extracted from medical records. Dogs with evidence of oropharyngeal contamination (ie, presence of squamous epithelial cells or *Simonsiella* sp. on BALF cytology)[3] were excluded.

Included dogs were divided into 2 groups: a group of dogs with LRTI-RA and a group of dogs with LRT disease not requiring antibiotics (LRTD-NRA) despite positive BALF culture results. A positive BALF culture was defined as any bacterial growth reported by the laboratory, regardless of the cfu/mL count. Dogs were allocated to the LRTI-RA group if they had (a) a positive BALF bacterial culture with neutrophilic inflammation (ie, ≥12% neutrophils)[15] on BALF cytology and (b) radiographic or CT findings consistent with an infectious process according to a board-certified radiologist, or presence of

intracellular bacteria on BALF cytology, or an unambiguous response to antibiotic treatment, without concomitant corticosteroid use. Because neither imaging nor presence of intracellular bacteria is pathognomonic for bacterial infection, an unequivocal response to antibiotics also was required for allocation to the LRTI-RA group.

Dogs were allocated to the LRTD-NRA group if they had (a) a positive BALF bacterial culture with or without neutrophilic inflammation on BALF cytology, (b) no radiographic or CT findings consistent with an infectious process according to a board-certified radiologist, (c) no intracellular bacteria on BALF cytology, and (d) no response to an appropriate antibiotic course based on susceptibility profile or an unambiguous response to an alternative treatment (eg, corticosteroids). Dogs that could not be allocated to 1 of these 2 groups based on these criteria (eg, because of concomitant use of antibiotics and corticosteroids) were excluded.

2.2 | Diagnostic imaging

Thoracic radiographs or CT images were evaluated in a blinded fashion by a single board-certified radiologist (EG). Thoracic CT was performed under general anesthesia, after lung inflation, and using a 16-slice helical CT scanner (Brivo CT385, GE Medicals system, Buc, France). Findings consistent with an infectious process mainly were based on the location of the lesion: lobar, ventral, and asymmetric alveolar or interstitial lesion.[1]

2.3 | Bronchoscopic procedure

Bronchoscopy was performed using a 5.0-mm diameter flexible pediatric videobronchoscope (Olympus, Andover, Massachusetts). Before each use, the bronchoscope was sterilized by immersion in an activated solution of peracetic acid (Anioxyde 1000, Lille-Hellemmes, France) for 30 minutes, and then thoroughly rinsed with 0.9% sterile saline solution. After each use, the bronchoscope was cleaned with an enzymatic solution (Aseptiline Instrument +, CEVA, Libourne, France) for 30 minutes, rinsed with tap water for 10 minutes, sterilized by immersion in an activated solution of peracetic acid for 15 minutes, and rinsed with 0.9% sterile saline solution. Finally, the bronchoscope was dried with pulsed air for 5 minutes before being hung for storage.

For each procedure, anesthesia was induced with propofol (2-5 mg/kg IV) after premedication (butorphanol [0.3 mg/kg IV], midazolam [0.25 mg/kg IV], dexmedetomidine [1-3 μg/kg IV]) or some combination of these) and was maintained with propofol boluses. No animals were intubated and 100% oxygen was delivered via the biopsy channel. Bronchoscopy was performed with the dog in sternal recumbency. The right and left principal and all lobar bronchi were examined and 2 bronchoalveolar lavage (BAL) sites were selected on the basis of imaging and gross bronchoscopic findings. Where gross lesions had not been identified, the right and left caudal lung lobes (RB4 and LB2) were chosen. A 0.9% sterile saline solution was used for the BAL. Although bronchoscopy was performed by different clinicians, a standardized institutional protocol was followed, and the BAL volume used per site was 1 mL/kg with a minimum of 4 mL in small dogs. After gently wedging the tip of the bronchoscope in a distal bronchus, the sterile saline was rapidly instilled through the biopsy channel using a syringe, followed by 4 mL of air to clear the channel. The sample then was immediately collected into the same syringe by gentle pulsatile aspiration. Where necessary, the same syringe was emptied of air to continue aspiration. Aliquots of BALF from the 2 selected lobes were combined for bacterial culture analysis. Aliquots were analyzed separately for cytological examination. At completion of the procedure, all animals were intubated to receive supplemental oxygen during anesthesia recovery.

2.4 | BALF cytology

Samples of BALF were delivered to the clinical pathology laboratory (Vebio Laboratory, Arcueil, France) within 10 minutes of collection. Total nucleated cell count was determined using an automated cell counter (ADVIA 2120 Siemens, Saint-Denis, France), and then was verified manually. Direct smear slides were prepared by cytocentrifugation (Cytospin 4 thermo Shandon, France) and stained with May-Grünwald Giemsa stain. The slides were examined and reported by a clinical pathologist at the time of diagnosis and were not re-examined for the purpose of this study. Differential cell counts were performed by counting 100 to 300 nucleated cells with an oil-immersion objective lens at 500× magnification. Slides were carefully examined for type of inflammation, cellular morphology, presence of intracellular bacteria, squamous epithelial cells, and *Simonsiella* spp. Neutrophilic (suppurative) inflammation was defined as >12% neutrophils.[15]

2.5 | Bacterial culture

The BALF samples were quantitatively cultured for bacteria by inoculation of 100 μL onto a medium for the growth of fastidious organisms with clearly visible hemolytic reactions (Thermo Scientific Columbia agar plate with 5% sheep blood^{PLUS}, Wesel, Germany). Subsequently, samples were serially diluted in sterile water to 10^{-1}, 10^{-3}, and 10^{-5}. One hundred microliter of each dilution then was inoculated onto a Columbia agar plate with 5% sheep blood^{PLUS} (Thermo Scientific, Wesel, Germany). Plates were incubated at 37°C for at least 24 hours. Plates were examined for growth after both 24 and 48 hours of incubation. When growth was observed, the number of colony forming units (cfu/mL) was calculated by multiplying the number of colonies by 10, and applying the dilution factor. Bacterial identification and antibiotic susceptibility testing also were performed (VITEK-2 analyzer, Biomerieux, Craponne, France). At the discretion of the clinician, quantitative polymerase chain reaction (qPCR) was performed for *Mycoplasma* sp. detection. Specific mycoplasma cultures were not performed. Finally, when >1 bacterial species were retrieved from BALF culture, the species with the highest cfu/mL was used for statistical analysis.

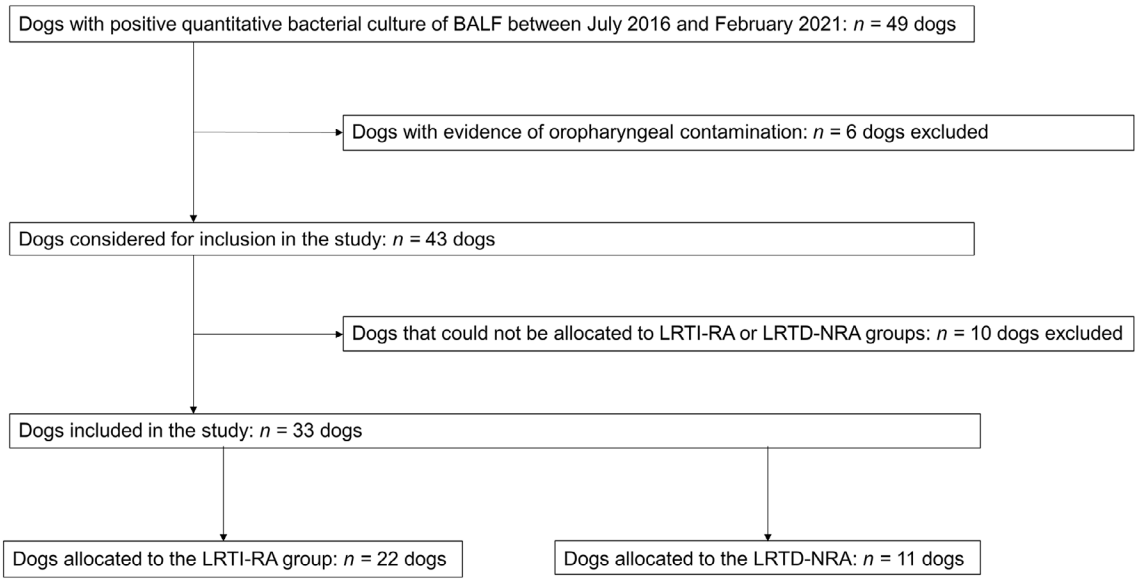

FIGURE 1 Flow chart showing the process of case inclusion. BALF, bronchoalveolar lavage fluid; LRTD-NRA, lower respiratory tract disease not requiring antibiotics; LRTI-RA, lower respiratory tract infection requiring antibiotics

2.6 | Statistical analysis

Statistical analyses were performed using STATA version 14.2 software (StataCorp LLC, College Station, Texas). Demographic continuous data (age and weight) were assessed for Gaussian distribution using histogram evaluation and the Shapiro-Wilk test (Gaussian if $P > .2$).[16] Because none of the continuous variables were normally distributed, a Mann-Whitney test was used to compare them between LRTI-RA and LRTD-NRA dogs. A 2-tailed Fisher exact test was used to compare the 2 groups in terms of sex.

First, the number of cfu/mL was compared between the 2 groups using a Mann-Whitney test.

Then, a logistic regression model was used, with the dependent variable being group (ie, LRTI-RA or LRTD-NRA) and the independent variables being the logarithm of the number of cfu/mL, the logarithm of the BALF TNCC, recent antibiotic treatment, and age. The number of cfu/mL and BALF TNCC were log-transformed to improve specification of the model. The model was applied to the entire population and to a subpopulation of dogs without radiographic or CT evidence of pneumonia, to make the 2 groups more comparable in terms of underlying disease process. Specification errors and goodness-of-fit of the logistic regression were assessed using the linktest and fit functions of the statistical software.

Finally, the number of dogs correctly and incorrectly classified as LRTI-RA or LRTD-NRA according to the previous cfu/mL threshold (1700 cfu/mL) was calculated, with and without considering recent antibiotic treatment. Dogs were considered misclassified when the cfu/mL value was <1700 despite LRTI-RA, or the cfu/mL value was ≥1700 despite LRTD-NRA.

Statistical significance was set at $P < .05$ for all analyses. Clinical relevance was interpreted using regression coefficients and their 95% confidence intervals (CI).

3 | RESULTS

3.1 | Study population

The process of case inclusion is shown in Figure 1. Thirty-three dogs were included in the study; 22 dogs were allocated to the LRTI-RA group and 11 dogs to the LRTD-NRA group.

Demographic data for the dogs in each group are presented in Table 1. No statistically significant differences were found in age ($P = .33$), body weight ($P = .97$), and sex ($P = .63$) between LRTI-RA and LRTD-NRA groups. In the LRTI-RA group, the most represented breed was the French bulldog (n = 4), followed by the Jack Russell terrier (n = 2), mixed breed dog (n = 2), Yorkshire terrier (n = 2), and 1 each of the following breeds: Boxer, British spaniel, Dachshund, Dobermann, English Setter, Eurasier, German Spitz, Irish Wolfhound, Labrador retriever, Samoyed, Springer spaniels, and White shepherd. In the LRTD-NRA group, breeds included Australian shepherds (n = 2), Labrador retrievers (n = 2), and 1 each of the following breeds: Bichon, Chihuahua, German Spitz, French bulldog, Shetland, Shi-Tzu, and Springer spaniel.

In the LRTI-RA group, 15/22 (68%) dogs were presented for cough, 4/22 (16%) for fever and 4/22 (16%) for respiratory distress. One dog was presented for both cough and fever. In the LRTD-NRA group, 10/11 (91%) dogs were evaluated for cough and 1/11 (9%) was evaluated for exercise intolerance. Twenty-four dogs of 33 (73%) had thoracic radiography performed and 9/33 (27%) had thoracic CT. In 1 dog in the LRTI-RA group and 2 dogs in the LRTD-NRA group, thoracic radiographs were not available for review and the radiologist's report was used. Ongoing antibiotic treatment was reported in 8/22 (36%) dogs from the LRTI-RA group and in no dogs from the LRTD-NRA group. Antibiotics used were amoxicillin-clavulanate in 5 dogs, doxycycline in 2 dogs, and, clindamycin in

TABLE 1 Demographic data and bronchoalveolar lavage fluid culture results in the LRTI-RA and LRTD-NRA groups

Variable	LRTI-RA group (n = 22)	LTRD-NRA group (n = 11)	*P* value
Age (in years)	2 (0.25-11)	6 (0.45-15)	0.33
Body weight (in kg)	12.1 (1.7-50)	14 (1.38-34)	0.97
Sex			
Intact male	9 (41%)	5 (45%)	0.63
Neutered male	4 (18%)	0	
Intact female	5 (23%)	3 (27.5%)	
Neutered female	4 (18%)	3 (27.5%)	
cfu/mL (including dogs with prior/ongoing antibiotherapy)	10 000 (10-3 x 10^8)	10 000 (250-1.3 x 10^9)	0.70
cfu/mL (excluding dogs with prior/ongoing antibiotherapy)	10 200 (60-3 x 10^8)	10 000 (250-1.3 x 10^9)	0.86

Note: Table entries represent median values (minimum-maximum) for continuous variables and number of dogs (percent of dogs) for categorical variables.
Abbreviations: cfu, colony forming unit; LRTI-RA, lower respiratory tract infection requiring antibiotics; LRTD-NRA, lower respiratory tract disease not requiring antibiotics.

1 dog. Median duration of ongoing antibiotic treatment was 5 days (range, 1-10 days). In 1 dog from the LRTI-NRA group, a course of doxycycline had been completed 5 days before BALF collection.

Angiostrongylus vasorum antigenemia (AngioDetect rapid assay, Idexx Laboratories) was assessed in 5/22 (23%) dogs with LRTI-RA and 6/11 (55%) dogs in the LRTD-NRA group. All tests were negative. Fecal Baermann concentration tests were performed in 2/22 (9%) dogs with LRTI-RA and all were negative.

Mycoplasma sp. qPCR was performed on the BALF of 12/22 (56%) dogs with LRTI-RA, 3 of which had positive results. In the LRTD-NRA group, *Mycoplasma* sp. qPCR was performed on the BALF of 8/11 (73%) dogs, all of which had negative results.

The likely cause of the LRTI-RA was determined in 16/22 (73%) dogs and included CAP in 8/16 (50%) dogs, AP in 6/16 (37%) dogs, and chronic bronchitis with bacterial superinfection in 2/16 (13%) dogs. In dogs with LRTD-NRA, final diagnosis included chronic bronchitis in 7/11 (64%) dogs, gastro-esophageal reflux disease (GERD) in 2/11 (18%) dogs (based on history and increased bile acids concentration in BALF), and eosinophilic bronchitis in 1 dog. A definitive diagnosis was not achieved for 1 dog.

The outcome was favorable in 18/22 (82%) dogs with LRTI-RA with infection cleared after antibiotics. Relapse, despite initial improvement, was seen in 2 of 22 (9%) dogs. Finally, 1 dog with LRTI-RA died suddenly at home few days after hospital discharge, and 1 dog was lost to follow-up (unknown outcome). The outcome was favorable in all 11 dogs with LRTD-NRA. Treatments in this group included: inhaled corticosteroids (n = 1), inhaled and PO corticosteroids (n = 1), PO corticosteroids (n = 5), fenbendazole (n = 3), diet change (n = 2), and codeine (n = 1). Two dogs received no treatment and the cough resolved spontaneously. Finally, doxycycline was prescribed in 2 dogs but was not associated with improvement in the reported clinical signs. *Bordetella bronchiseptica* was isolated in 3 dogs of the LRTD-NRA groups. Two (2.8×10^5 and 3×10^7 cfu/mL) were treated with corticosteroids and 1 (2.4×10^6 cfu/mL) recovered spontaneously without treatment.

3.2 | Quantitative bacterial culture of BALF

In the LRTI-RA group, 21/22 (95%) dogs had a single bacterial species isolated on BALF culture and 1 had 2 bacterial species isolated. Organisms identified in each group are summarized in Table 2 and included *Bordetella bronchiseptica*, *Brevundimonas* sp., *Enterococcus* sp., *Klebsiella* sp., *Pasteurella* sp., *Pseudomonas* sp., *Sphingomonas* sp., and *Staphylococcus* sp. in the LRTI-RA group. The dog with 2 bacteria retrieved had *Pasteurella* sp. and *Aerococcus* sp. In the LRTD-NRA group, all cultures yielded growth of a single organism, which included *Achromobacter* sp., *Bordetella bronchiseptica*, *Enterobacter* sp., *Neisseria* sp., *Pseudomonas* sp., and *Sphingomonas* sp.

The outputs of the logistic models assessing the predictive effects of the logarithm of BALF cfu/mL and TNCC on antibiotic requirement are presented in Tables 3 and 4. When the entire population was considered, no significant difference in cfu/mL was detected between the LRTI-RA and the LRTD-NRA groups (LRTI-RA group: median, 10 000 cfu/mL; range, 10-3 $\times$ 10^8; LRTD-NRA: median, 10 000 cfu/mL; range, 250-1.3 $\times$ 10^9; $P = .27$), independent of prior or ongoing antibiotic treatment ($P = .16$; Figure 2A,B) and age ($P = .14$). Similar results were obtained when dogs from the LRTI-RA group with radiographic evidence of pneumonia were excluded: LRTI-RA group ($n = 9$): median, 10 400 cfu/mL; range, 1000-3.2 $\times$ 10^5; LRTD-NRA (n = 11): median, 10 000 cfu/mL; range, 250-1.3 $\times$ 10^9 ($P = .30$).

3.3 | BALF cytology

In the LRTI-RA group, BALF neutrophilia was present in all dogs, with intracellular bacteria observed in 7/22 (32%) dogs. Degenerate neutrophils were described in 10/22 (45%) dogs. The TNCC was determined in 13/22 (59%) dogs (median, 2260 cells/μL; range, 240-45 140). In the LRTD-NRA group, BALF neutrophilia was present in 8/11 (73%) dogs. No degenerate neutrophils were described in any dogs. The TNCC was determined in 8/11 (73%) dogs (median, 455 cells/μL; range, 80-4990).

TABLE 2 Distribution of bacteria retrieved from cultures of bronchoalveolar lavage fluid in the LRTI-RA and LRTD-NRA groups

Bacteria	LRTI-RA group (n = 22)	LTRD-NRA group (n = 11)	Total (n = 33)
Achromobacter sp.	0	1 (9%)	1 (3%)
Bordetella sp.	7 (31%)	3 (27%)	10 (31%)
Brevundimonas sp.	1 (5%)	0	1 (3%)
Enterobacter sp.	0	2 (19%)	2 (6%)
Enterococcus sp.	2 (9%)	0	2 (6%)
Klebsiella sp.	1 (5%)	0	1 (3%)
Neisseria sp.	0	1 (9%)	1 (3%)
Pasteurella sp.	6 (27%)	0	6 (18%)
Pseudomonas sp.	3 (13%)	3 (27%)	6 (18%)
Sphingomonas sp.	1 (5%)	1 (9%)	2 (6%)
Staphylococcus sp.	1 (5%)	0	1 (3%)

Note: Table entries represent number of dogs (percent of dogs).
Abbreviations: LRTI-RA, lower respiratory tract infection requiring antibiotics; LRTD-NRA, lower respiratory tract disease not requiring antibiotics.

TABLE 3 Output of the logistic model assessing the predictive effect of the logarithm of bronchoalveolar lavage fluid cfu/mL and total nucleated cell count on antibiotic requirement, and adjusting for the potential confounding effects of prior antibiotherapy and age, in the entire population of included dogs

	Odds ratio	95%CI	*P* value
Log cfu/mL	0.8	(0.6-1.2)	0.27
Log BALF TNCC	2.6	(1.0-7.0)	0.05
Prior/ongoing antibiotherapy (reference group: no prior antibiotherapy)	19.2	(0.3-1158)	0.16
Age (in years)	0.7	(0.4-1.1)	0.14

Abbreviations: Log BALF TNCC, logarithm of bronchoalveolar lavage fluid total nucleated cell count; Log cfu, logarithm of colony forming unit; CI, confidence interval; LRTD-NRA, lower respiratory tract disease not requiring antibiotics; LRTI-RA, lower respiratory tract infection requiring antibiotics; LRTI-RA (n = 22) was coded 1 and LRTD-NRA (n = 11) was coded 0.

TABLE 4 Output of the logistic model assessing the predictive effect of the logarithm of bronchoalveolar lavage fluid cfu/mL and total nucleated cell count on antibiotic requirement, and adjusting for the potential confounding effects of prior antibiotherapy and age, in the subpopulation of dogs with bronchial disease

	Odds ratio	95%CI	*P* value
Log cfu/mL	0.8	(0.5-1.2)	0.30
Log BALF TNCC	1.6	(0.3-8.2)	0.57
Prior/ongoing antibiotherapy (reference group: no prior antibiotherapy)	256	(0.1-444 629)	0.15
Age (in years)	0.5	(0.2-1.2)	0.11

Abbreviations: Log BALF TNCC, logarithm of bronchoalveolar lavage fluid total nucleated cell count; Log cfu, logarithm of colony forming unit; CI, confidence interval; LRTD-NRA, lower respiratory tract disease not requiring antibiotics; LRTI-RA, lower respiratory tract infection requiring antibiotics LRTI-RA (n = 9) was coded 1 and LRTD-NRA (n = 11) was coded 0.

The BALF TNCC was significantly higher in the LRTI-RA group than in the LRTD-NRA group (Figure 3A; $P = .05$), independent of prior or ongoing antibiotic treatment and age. However, the difference was no longer significant when dogs in the LRTI-RA group with radiographic evidence of pneumonia were excluded (Figure 3B): LRTI-RA group: median, 470 cells/μL; range, 240-2260; LRTD-NRA: median, 455 cells/μL; range, 80-4990 ($P = .57$).

3.4 | Predictive value of 1700 cfu/mL threshold for relevant infection requiring antibiotics

Ten of 22 (45%) dogs with LRTI-RA had a cfu/mL value <1700, which would have been interpreted as having no clinically relevant bacterial growth. Conversely, 7/11 (63%) dogs with LRTD-NRA had a cfu/mL value >1700. This result would have resulted in a misdiagnosis of LRTI-RA, leading to unnecessary antibiotic treatment. When dogs with prior or ongoing use of antibiotics were excluded, 6/14 (43%) dogs with LRTI-RA had a cfu/mL value <1700, compatible with an interpretation of no clinically relevant bacterial growth. Conversely, 6/10 (60%) dogs with LRTD-NRA had a cfu/mL value >1700, and therefore would have been misdiagnosed with LRTI-RA, leading to unnecessary antibiotic treatment.

Finally, when dogs with radiographic evidence of pneumonia were excluded from the LRTI-RA group, 4/9 (44%) dogs with LRTI-RA had a cfu/mL value <1700, which would have led to an interpretation of no clinically relevant bacterial growth.

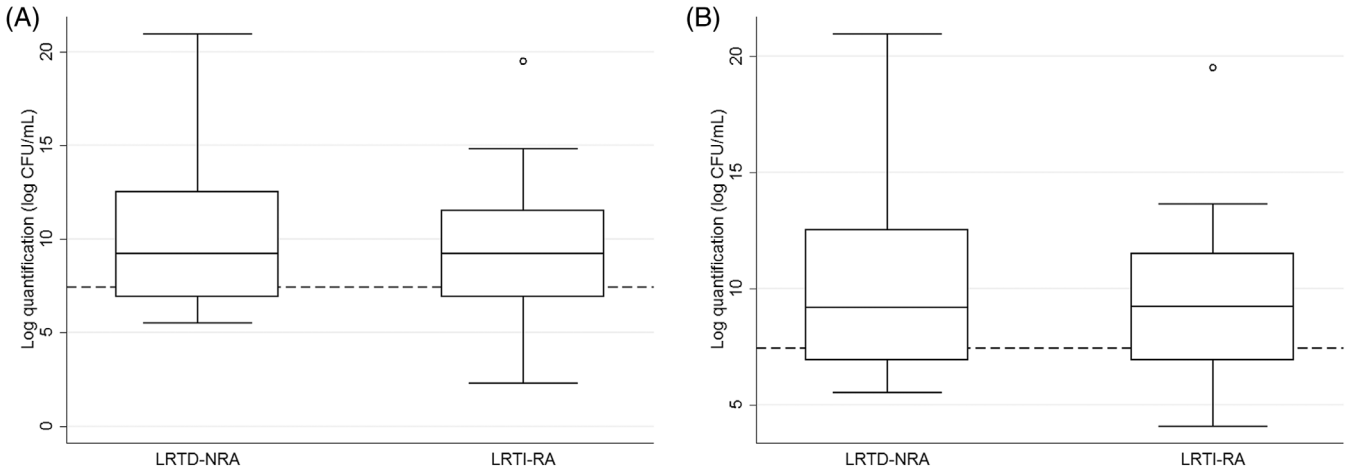

FIGURE 2 Boxplots displaying cfu/mL log-values in the LRTI-RA and LRTD-NRA groups including (A) or excluding (B) dogs with prior antibiotherapy. The box represents the interquartile range (ie, from 1st to 3rd quartile), the line represents the median, the whiskers represent the highest and lowest value within 1.5× the interquartile range, and the circles indicate outliers. Dashed line represent the log-value of the 1700-cfu/mL threshold for clinically relevant bacterial growth. Cfu, colony forming unit; LRTD-NRA, lower respiratory tract disease not requiring antibiotics; LRTI-RA, lower respiratory tract infection requiring antibiotics

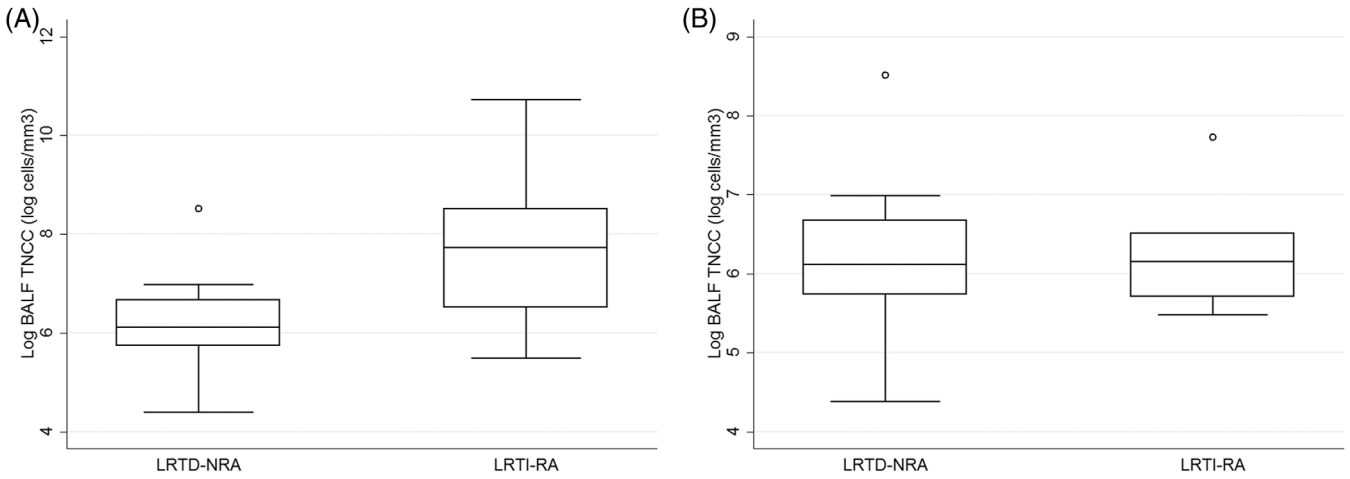

FIGURE 3 Boxplots displaying bronchoalveolar lavage fluid total nucleated cell count log-values in the LRTI-RA and LRTD-NRA groups including (A) or excluding (B) dogs with radiographic evidence of infection. The box represents the interquartile range (ie, from 1st to 3rd quartile), the line represents the median, the whiskers represent the highest and lowest value within 1.5× the interquartile range, and the circles indicate outliers. BALF, bronchoalveolar lavage fluid; LRTD-NRA, lower respiratory tract disease not requiring antibiotics; LRTI-RA, lower respiratory tract infection requiring antibiotics; TNCC, total number cell count

4 | DISCUSSION

As hypothesized, our results showed that the BALF number of cfu/mL was poorly predictive of antibiotic requirement in the population of dogs evaluated at our hospital. Results remained similar even when excluding dogs with radiographic or CT evidence of pneumonia from the LRTI-RA group in order to make the groups more comparable for underlying disease process. The BALF TNCC was higher in dogs with LRTI-RA than in dogs with LRTD-NRA. However, this finding was likely because of the difference in respiratory diseases, because when dogs with pneumonia were excluded from the analysis, the BALF TNCC was no longer different between these 2 groups. Finally, the threshold of 1700 cfu/mL for interpretation of BALF quantitative culture was misleading. If antibiotic treatment had been decided based on this threshold, nearly half of the dogs with LRTI-RA in our study would have not received an appropriate course of antimicrobials. This treatment error could have prevented a positive outcome for those dogs. Moreover, nearly two thirds of the dogs with LRTD-NRA would have received antibiotics, leading to antimicrobial overuse and increased risk of multidrug-resistant bacteria.

Use of a threshold for considering relevant LRTI and to aid therapeutic decision-making already has been questioned in dogs and humans.[4,8] It should be emphasized that the respiratory tract is not a sterile environment, as evidenced by next generation sequencing

techniques showing the LRT of healthy dogs to contain a rich and diverse microbiota.[12] A previous study found mean bacterial counts of BALF quantitative cultures in healthy dogs as high as 25 610 cfu/mL, and notably higher than the 1700-cfu/mL threshold usually considered indicative of relevant infection. This finding was despite using a laryngeal mask airway to avoid oropharyngeal contamination.[8]

Composition of commensal respiratory microbiota is likely to be influenced by factors such as hormonal environment, ecological disturbances, and antimicrobial use.[17] The resident microbiome includes potentially pathogenic microorganisms, making interpretation of BALF culture especially challenging.[8] Bacteria species isolated from BALF cultures in our study were similar to those of previous reports.[3,4,8,18] *Bordetella bronchiseptica*, *Pasteurella* sp., *Pseudomonas* sp., and *Staphylococcus* sp. frequently are isolated from BALF of dogs suffering from LRTI or other LRT diseases, but also from healthy dogs.[3,4,8,18-20] Thus, therapeutic decisions should not be based solely on bacterial culture results but should integrate clinical, imaging, and cytological findings.

Criteria based on BALF cytology, thoracic imaging findings and response to treatment were used in our study to distinguish LRTI that likely required antibiotics from other LRT diseases that did not. Although response to treatment cannot be used for initial therapeutic decision-making, it is undoubtedly the best criterion to assess antimicrobial requirement.[1] To be allocated to the LRTI-RA group, dogs had to have responded strictly to antibiotic treatment without concomitant corticosteroid use. Whereas, for allocation to the LRTD-NRA group, antibiotic treatment was permitted if dogs were still showing LRT signs despite treatment. However, for dogs in this group, a positive treatment response was defined as that achieved using treatments other than antibiotics. Dogs with concomitant use of antibiotics and corticosteroids therefore were excluded, because of inability to determine which treatment resulted in the clinical improvement. Nevertheless, because cases were collected retrospectively, these therapeutic decisions had been made at the time of diagnosis based on clinical presentation, BALF cytology, and thoracic imaging findings.

Considering BALF cytology findings, intracellular bacteria were identified in 32% of dogs with LRTI-RA in our study. This finding is in contrast with those of previous studies where intracellular bacteria were observed on BALF cytology in 71% to 79% of dogs with LRTI.[3,4] This difference could be explained by previous treatment. However, only 5 of 15 dogs with LRTI-RA, but without intracellular bacteria observed on BALF cytology, had already received prior antimicrobials. Alternatively, infection with *Bordetella bronchiseptica* could be an explanation, because *Bordetella* sp. typically adheres to the cilia of epithelial cells rather than being inside the cytoplasm, and was cultured in 7 dogs with LRTI-RA.[21]

The BALF TNCC also was considered a potentially useful criterion for therapeutic decision-making. Although BALF TNCC in dogs with LRTI has been described as being markedly higher than in dogs with chronic bronchitis or other respiratory diseases, overlap is reported.[3] Similar results were obtained in our study, with an overall higher BALF TNCC in dogs with LRTI-RA when dogs with and without radiographic signs of pneumonia were included in the analysis. When the analysis was restricted to dogs with bronchial disease, no significant difference was found between the LRTI-RA and LRTD-NRA groups.

Regarding thoracic imaging findings, interstitial-to-alveolar pattern was not mandatory to allocate dogs to the LRTI-RA group, because it is not always present, especially early in the disease process.[1,22] Furthermore, it can be observed in noninfectious respiratory diseases such as pulmonary hemorrhage, lung torsion, sterile AP, atelectasis, pulmonary edema, or neoplasia.[1]

A C-reactive protein (CRP) concentration >55 mg/L is strongly suggestive of bacterial bronchopneumonia in dogs and a concentration <20 mg/L strongly suggests absence of infection.[22-24] However, routine CRP measurement was not performed in our hospital population of dogs with respiratory disease until relatively recently, and therefore was not available for all included cases. The CRP concentration therefore was not used as a criterion to allocate dogs to the LRTI-RA or LRTD-NRA groups.

If none of these cytological and imaging findings were found and if dogs responded to treatments other than antibiotics, they were allocated to the LRTD-NRA group. However, allocation to this group did not necessarily exclude infection as a cause. *Bordetella* spp., a bacteria associated with canine infectious respiratory disease complex (CIRDC), can cause a clinical illness ranging from mild respiratory disease to severe pneumonia and death. However, asymptomatic infection, colonization or carriage is incrementally described.[19,20] Three dogs with LRTD-NRA had *Bordetella bronchiseptica* isolated from BALF culture in our study. Although bacterial counts ranged from 2.8×10^5 to 3×10^7 cfu/mL in these dogs, they all recovered without antibiotics. Two dogs were treated with corticosteroids and 1 dog recovered spontaneously without treatment. These data emphasize that antibiotics are not necessarily warranted in dogs with *Bordetella bronchiseptica*-positive cultures, especially if they are clinically stable and show no alveolar pattern on thoracic imaging. The question of preventive antibiotic treatment in these dogs to limit transmission to other animals, especially in a shelter environment, remains to be elucidated.

Our study had some limitations. Although no significant difference in cfu/mL was found between dogs with LRTI-RA and dogs with LRTD-NRA independent of prior or ongoing antibiotic treatment, use of antibiotics theoretically could lead to underestimation of BALF cfu/mL values. Few data are available in the literature regarding the impact of previous antibiotic treatment on results of quantitative bacterial cultures of BALF.[8,25,26] The impact likely varies depending on treatment duration, the bacteria's sensitivity profile, and the delay between antibiotic discontinuation and BALF collection. In humans, previous antibiotic treatment has no impact on detecting nosocomial infection using BALF culture in ventilated patients.[27] Because dogs were referred to our hospital for persistent LRT clinical signs despite previous or ongoing antibiotic treatment, the bacteria isolated were more likely to be resistant to the previous or ongoing treatment. Therefore, the impact of prior or ongoing antibiotics on BALF culture results is expected to have been limited.

Inappropriate storage and delay of BALF culture can lead to significant overgrowth of some bacteria such as *Escherichia coli* and

Bordetella bronchiseptica, especially if the delay is >24 hours and when the sample is left at room temperature.[28] Considering the close proximity of our hospital and our laboratory, samples were plated within 1 hour of collection. Therefore, bias associated with delayed cultures is very unlikely.

Contamination of the bronchoscope could be another bias for bacterial growth from BALF of dogs with LRTD-NRA. Having saline lavage of the endoscope for culture, alongside the BALF, would have helped interpret the relevance of bacterial isolates and exclude possible environmental contaminants, but this procedure was not performed. Systematic disinfection before and after each use of the bronchoscope however is expected to have limited the risk of instrument contamination. The diversity of the bacteria isolated from LRTD-NRA animals also makes material contamination unlikely.

To our knowledge, no previous studies have evaluated the effect of aliquot volume on bacterial count retrieved from BALF culture. The volume of BAL aliquots is standardized in our hospital so that all dogs were sampled using the same weight-adjusted aliquot volume. The technique used for BALF culture also could influence the cfu/mL values. Our laboratory used an initial volume of 100 μL of BALF for plating followed by serial dilution to accurately determine the cfu/mL value, as recommended in human medicine.[29] However, to establish the 1700-cfu/mL threshold for relevant bacterial infection, a previous study used a 10 μL BALF volume for plating, without dilution.[3] Differences in bacteriology techniques therefore might explain some of the differences between the findings of that study and ours.

One criterion to allocate dogs into the LRTI-RA group was presence of intracellular bacteria cytologically observed in the BALF. Although presence of intracellular bacteria is suggestive of bacterial infection in humans, bacteria must be present in sufficient numbers to be supportive of infection.[30] Quantitative assessment for intracellular bacteria is not routinely performed by our laboratory. However, only dogs with intracellular bacteria in the BALF that then unequivocally responded to antibiotics were included.

Finally, owing to the retrospective nature of case collection, thoracic radiographs were not available for blinded review by our board-certified radiologist in 3 dogs. The radiologist reports were retrieved from the medical record, but the findings or lack of findings could not be verified.

In conclusion, our results indicate that quantitative BALF bacterial culture results should not be used as the primary determinant for LRTI-RA diagnosis. In our opinion, decisions about the use of antibiotic treatment should integrate clinical, imaging, and cytological information. This does not mean that BALF bacterial cultures are useless, and they remain a useful tool to aid in the diagnosis of LRTI-RA and guide antimicrobial selection. Rather, we advocate for refining the definition of bacterial infection. Future prospective studies involving a large number of dogs are warranted to determine the best criteria for LRTI-RA diagnosis.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

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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