Received: 18 April 2020 | Accepted: 20 January 2021

DOI: 10.1111/jvim.16052

STANDARD ARTICLE

Journal of Veterinary Internal Medicine Open Access

ACVIM American College of Veterinary Internal Medicine

Factors associated with clinical interpretation of tracheal wash fluid from dogs with respiratory disease: 281 cases (2012-2017)

Amber M. Graham[1] | Karen M. Tefft[1] | Devorah M. Stowe[2] | Megan E. Jacob[2] | James B. Robertson[3] | Eleanor C. Hawkins[1]

[1]Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina

[2]Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina

[3]College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina

Correspondence
Karen M. Tefft, Department of Clinical Sciences, North Carolina State University, 1052 William Moore Drive, Raleigh, NC 27607.
Email: ktefft@ncsu.edu

Present address
Amber M. Graham, Department of Small Animal Clinical Sciences, College of Veterinary Medicine, Texas A & M University, College Station, Texas

Abstract

Background: Clinicians face several dilemmas regarding tracheal washes (TWs) for the diagnosis of respiratory disease, including method and prediction of bacterial growth from cytology results.

Objective: To compare cytology and culture of endotracheal and transtracheal washes and identify factors associated with discordancy and bacterial growth.

Animals: Two hundred forty-five dogs with respiratory disease.

Methods: Retrospective study. Tracheal wash submissions were included if cellularity was sufficient for cytologic interpretation and aerobic cultures were performed. Collection technique, cytology, bacterial growth, and antibiotic history were analyzed.

Results: Fewer transtracheal specimens (9/144, 6.3%) were excluded for hypocellularity than endotracheal (28/174, 16.1%); otherwise, results were similar and were combined. Of 281 specimens with cellularity sufficient for interpretation, 97 (34.5%) had bacteria on cytology and 191 (68.0%) had bacterial growth. Cytology positive/culture negative discordancy was uncommon (8/97, 8%). Cytology negative/culture positive discordancy was frequent (102/184, 55.4%), but occurred less often (28/184, 14.2%) when only 1+ growth or greater was considered positive. Oropharyngeal contamination was associated with bacterial growth, but not discordancy. No association was found between antibiotic administration and bacterial growth.

Conclusions and Clinical Importance: Endotracheal wash fluid, in particular, should be screened for gross mucus or turbidity to maximize the likelihood of an adequate specimen. Otherwise, endotracheal and transtracheal specimens were similar. Presence of bacteria on cytology was a good predictor of any growth, while their absence was a good predictor of the absence of growth of 1+ or more. Recent antibiotic usage should not discourage TW culture if there is compelling reason to avoid delay.

Abbreviations: BAL, bronchoalveolar lavage; ETW, endotracheal wash; TTW, transtracheal wash; TW, tracheal wash.

KEYWORDS
aerobic culture, antibiotics, endotracheal, transtracheal

1 | INTRODUCTION

Tracheal wash (TW) is a minimally invasive technique for collecting specimens from dogs with respiratory disease. Fluid analysis can provide important diagnostic information and guide therapy. Transtracheal wash (TTW) and endotracheal wash (ETW) techniques are easily mastered, are relatively inexpensive compared with bronchoscopy, and can be performed with light sedation (TTW) or brief anesthesia (ETW). Endotracheal wash can be conveniently performed during anesthesia for another procedure.[1]

Tracheal wash predominantly samples from the trachea and larger airways, but can identify disease of lower airways and alveoli. Mechanisms, including the cough reflex and mucociliary clearance, move cells and organisms proximally into the trachea.[2] As a dog is anesthetized during an ETW procedure, the cough reflex is suppressed. Multiple resources cite this as a potential cause of decreased diagnostic yield in ETW specimens as compared to TTW specimens[1,3,4]; however, this is an a priori assumption. Another concern frequently expressed about ETW is that oropharyngeal contamination might be introduced into the trachea during intubation.

The canine respiratory tract supports a resident, nonpathogenic microbiome.[5,6] Nevertheless, culture of TW specimens can be used to effectively diagnose respiratory infections.[7-12] A diagnosis of bacterial infection is further supported by the concurrent identification of bacteria by cytology. The Antimicrobial Guidelines Working Group of the International Society for Companion Animal Infectious Diseases recommends culture of TW or bronchoalveolar lavage (BAL) fluid to guide antibiotic therapy in dogs suspected of having bacterial bronchitis or pneumonia.[13]

A clinician faces several dilemmas when interpreting results from TW specimens. Cytology results are usually available prior to culture results. Clinicians must assess the relative risk of bacterial infection based upon cytology results and weigh that against proper antibiotic stewardship when deciding on initial treatment. Septic neutrophilic inflammation is associated with bacterial growth in BAL fluid[14]; however, bacteria are more frequently identified on BAL fluid than on TW specimens.[15] When interpreting tracheal wash cytology, the clinician must consider that the culture results might be discordant. Infection might be present despite no bacteria visualized on cytology (cytology negative/culture positive discordancy). Conversely, infection might not be present despite visualization of bacteria (cytology positive/culture negative discordancy). Oropharyngeal contamination of the sample is 1 consideration for cytology negative/culture positive discordancy. Alternatively, the clinician could consider the impact of recent history of antibiotic use as a cause for cytology positive/culture negative discordancy.

The purposes of this study were to determine if cytologic and bacteriologic results of ETW and TTW are comparable, to determine frequency of discordant results and factors associated with discordancy, and to identify associations between antibiotic use and bacterial growth. We hypothesized that oropharyngeal contamination would be more common in specimens collected by ETW, that cytology negative/culture positive discordancy would occur more in specimens with oropharyngeal contamination or when bacterial growth was relatively low, and that bacterial growth would be negatively affected by recent antibiotic use.

2 | MATERIALS AND METHODS

2.1 | Criteria for selection of cases

The North Carolina State Veterinary Hospital Clinical Pathology Database was searched for canine TW submissions between January 2012 and July 2017. Medical records from dogs were reviewed to confirm clinical evidence of respiratory disease. Tracheal washes were included in the main study if an aerobic culture was performed and if the cytology report concluded that cellularity was sufficient for interpretation. The washes excluded for low cellularity were investigated for association with collection method and body weight.

2.2 | Procedures

Data retrieved from medical records included: body weight, antibiotic use in the previous 30 days, TW technique, TW fluid cytology findings, and aerobic culture results. All antibiotics were recorded regardless of treatment intent. Duration of administration was not recorded, as this information could not be reliably retrieved. The last day an antibiotic was reported to be given relative to the performance of the TW was recorded and grouped into subjectively determined antibiotic-free time intervals of clinical interest. These groups were: antibiotic-free interval of 0 days (date of last antibiotic given being the same date as the TW was performed); antibiotic-free interval of 1 to 7 days (date of last antibiotic given within a week of TW); antibiotic-free interval of 30 days or less; and, antibiotic-free intervals of greater than 30 days.

The decision to perform ETW or TTW was based on the clinical judgment of the primary clinician on each case. The ETW and TTW procedures were performed according to previously described techniques.[3] Specific information related to sedation protocol, catheter size, volume of fluid instilled and retrieved, and number of boluses was not available. The TW specimens were submitted for processing by the North Carolina State Veterinary Hospital Diagnostic Laboratory immediately after collection if the procedure was performed during regular business hours. Specimens collected after hours were held in refrigeration (4°C) until the next business day.

TABLE 1 Cytology and culture results of tracheal wash (TW) specimens

Variable	All TW (n = 281)	ETW (n = 146)	TTW (n = 135)	*P*-value
Inflammation				
Neutrophilic	225 (80.1%)	118 (80.8%)	107 (79.3%)	.86
Eosinophilic	40 (14.2%)	18 (12.3%)	22 (16.3%)	.44
None	16 (5.7%)	10 (6.8%)	6 (4.4%)	.54
Bacteria identified	97 (34.5%)	55 (37.7%)	42 (31.1%)	.30
Oropharyngeal contamination	71 (25.3%)	42 (28.8%)	29 (21.5%)	.21
Discordant results	61 (21.7%)	34 (23.3%)	27 (20.0%)	.6
Positive aerobic growth	191 (68.0%)	107 (73.3%)	84 (62.2%)	.063
Enrichment broth only	99 (35.2%)	60 (41.1%)	39 (28.9%)	.044
Growth of 1+ or greater	92 (32.7%)	47 (32.2%)	45 (33.3%)	.94

Abbreviations: ETW, endotracheal wash; TTW, transtracheal wash.

TABLE 2 Comparison between cytologic presence of bacteria and culture outcomes in tracheal wash specimens

	Cytologic presence of bacteria		
Culture outcome	Positive (n = 97)	Negative (n = 184)	*P*-value
Positive growth	89 (91.8%)	102 (55.4%)	<.001
Enrichment broth only	25 (25.8%)	74 (40.2%)	.023
Growth of 1+ or greater	64 (66.0%)	28 (14.2%)	<.001
Negative growth	8 (8.2%)	82 (44.6%)	<.001

A direct smear, a concentrated direct smear, 2 cytocentrifugation preparations and 2 squash preparations of mucus (when present) from each TW fluid specimen were stained with Wright-Giemsa. A board-certified veterinary clinical pathologist interpreted each specimen. Differential cell counts based on 100 or 200 cells were performed on cytocentrifugation preparations. The type of inflammation recorded for each specimen was based on the percentage of neutrophils and eosinophils in the sample. Samples were categorized as no inflammation if the neutrophils were equal to or less than 10% and the eosinophils equal to or less than 5% of the total cellularity. Samples were categorized as neutrophilic inflammation if the neutrophils were greater than 10% and the eosinophils equal to or less than 5% of the total cellularity. Samples were categorized as eosinophilic inflammation if the eosinophils were greater than 5% of the total cellularity regardless of the neutrophil percentage. Cytology was considered positive for bacteria whether bacteria were seen intracellularly or extracellularly. Oropharyngeal contamination was defined as the presence of squamous epithelial cells or *Simonsiella* spp. bacteria.

An aliquot of TW fluid was placed into a universal transport medium (A.C.T. I, Remel, Lenexa, KS) and submitted for aerobic culture. A 10 μL sterile loop was used to inoculate Columbia sheep blood agar and MacConkey agar. The plates were struck for bacterial isolation using a 4 quadrant streak method. The remaining volume of fluid from the transport media was inoculated into 10 mL of thioglycollate enrichment broth (Thermo Scientific, Remel, Lenexa, Kansas). Plates and enrichment broth were incubated at 37°C for 48 to 72 hours in either 5% CO_2 or ambient air (MacConkey agar, enrichment tube). Plates were evaluated and quantified on a 1-4+ scale based on the number of quadrants in which growth was reported, or as growth in enrichment broth only. For specimens with growth of more than 1 organism, the quadrant count from the organism with the greatest growth was used for analysis. Any degree of growth of organisms was considered positive for growth, except where otherwise stated. All isolates were identified by MALDI-TOF mass spectrometry, biochemical or cytological appearance, and Gram stain.

2.3 | Statistics

All categorical variables were summarized as frequencies and continuous data were summarized as medians and ranges. Weights were aggregated into categories by 5 kg increments. Proportions were compared among groups using a chi-square test with Yates correction. Proportional analyses were only performed when there were at least 5 occurrences for comparison. The M^2 statistic for linear regression was used to investigate if there was a relationship between antibiotic-free interval and the degree of aerobic growth. A cumulative logit model was designed to examine the influence of the number of days since an antibiotic was last administered and the class of antibiotic used with culture outcome. Dogs who received multiple classes of antibiotics were excluded. Backwards selection with the Akaike Information Criteria (AIC) as the selector was used to reduce models to the useful factors.

Variable	Oropharyngeal contamination		
	Positive (n = 71)	Negative (n = 210)	*P*-value
Bacteria on cytology	46 (64.8%)	51 (24.3%)	<.001
Positive growth	58 (81.7%)	133 (63.3%)	.007
Enrichment broth only	25 (35.2%)	74 (35.2%)	1
Growth of 1+ or greater	33 (46.5%)	59 (28.1%)	.007

TABLE 3 Comparison between the presence of oropharyngeal contamination and identification of bacteria by cytology or aerobic culture in tracheal wash specimens

TABLE 4 Antibiotics administered within 30 days of tracheal wash

Antibiotic	n
Penicillin class	52
Amoxicillin/clavulanic acid	42
Amoxicillin	5
Ampicillin/sulbactam	4
Piperacillin/tazobactam	1
Fluoroquinolone class	44
Enrofloxacin	29
Ciprofloxacin	8
Marbofloxacin	5
Orbifloxicin	2
Tetracycline class	20
Doxycycline	19
Minocycline	1
Miscellaneous class	38
Trimethoprim/sulfadiazine	6
Clindamycin	6
Azithromycin	5
Meropenem	5
Cephalexin	4
Chloramphenicol	4
Cefovecin	2
Cefpodoxime	2
Amikacin	1
Metronidazole	1
Nitrofurantoin	1
Tylosin	1

Regression analysis and modeling was performed using R scientific statistical software (R Foundation for Statistical Computing; version 3.6.1). The remainder of the statistical analysis was performed using GraphPad (GraphPad Software, Inc, San Diego, California). Statistical significance was defined as $P < .05$.

3 | RESULTS

During the study period, 333 TW specimens were submitted for cytologic review. Of those specimens, 14 were not submitted for aerobic culture and 37 were excluded for low cellularity. One additional specimen was excluded because it was the only specimen obtained from a dog receiving ventilator support at the time of TW and was not considered typical of the study sample. The 281 specimens included in the study were from 245 dogs. Twenty-seven dogs had more than 1 TW performed, most often to guide antibiotic treatment for recurrent pneumonia in dogs with underlying esophageal dysmotility.

3.1 | Comparison of endotracheal and transtracheal wash techniques

Of the 281 TW specimens in the main study sample, 146 were collected by ETW and 135 by TTW. Specimens collected by ETW were over-represented among those specimens that were excluded for low cellularity ($P = .011$). Of 174 total specimens collected by ETW, 28 (16.1%) were excluded, compared with 9 of 144 (6.3%) collected by TTW. While dogs that had ETW performed were significantly smaller (7.3 kg; 1.8-48 kg) than dogs that had TTW (24.2 kg; 3.68-50.6 kg; $P < .001$), there was no association between exclusion for low cellularity and body weight in either the ETW ($P = .42$) or TTW groups ($P = 1.0$).

Within the main study sample, no significant differences were found between ETW and TTW for type of inflammation, cytologic evidence of bacteria, oropharyngeal contamination or discordant results (Table 1). When only specimens with 1+ growth or greater were considered positive, there was no different between ETW and TTW for aerobic growth. Subsequently, data from all TW specimens were combined for further analysis.

3.2 | Relationships between cytology and culture results

Tracheal wash specimens with bacteria noted on cytology were more likely to have aerobic growth ($P < .001$). Cytology positive specimens were discordant (had no growth) less than 10% of the time (Table 2). However, specimens without bacteria noted on cytology were discordant (had any degree of growth) more than half the time. Raising the threshold for considering a culture as being positive to those with growth of 1+ or greater markedly decreased the discordancy in cytology negative specimens (15.2%). However, this change in threshold increased the discordancy in cytology positive specimens to 34%.

Specimens with oropharyngeal contamination noted on cytology were more likely to have bacteria noted ($P < .001$) and to have bacterial

TABLE 5 Comparison of antibiotic-free interval and degree of growth of aerobic organisms in tracheal wash specimens

		Degree of growth					
Antibiotic-free interval (days)	n	Enrichment broth	1+	2+	3+	4+	*P*-value
0	74	27 (36%)	8 (11%)	6 (8%)	3 (4%)	5 (7%)	.70
1-7	26	9 (35%)	4 (15%)	2 (8%)	0 (0%)	2 (8%)	.74
≤30	126	45 (35.7%)	14 (11.1%)	9 (7.1%)	4 (3.2%)	11 (8.7%)	.77
>30	155	54 (34.8%)	19 (12.3%)	20 (12.9%)	6 (3.9%)	9 (5.8%)	NA

Notes: P values were calculated from the M^2 statistic for linear regression.
Abbreviation: NA, test not applicable due to inclusion criteria.

growth (P = .007) than those without (Table 3). However, the presence of oropharyngeal contamination did not allow for the prediction of discordancy. That is to say, the presence of oropharyngeal contamination was not associated with cytology negative/culture positive discordancy when considering any growth as positive by culture (P = .25).

3.3 | Relationship between antibiotic use and culture results

Antibiotics were given to dogs within 30 days of TW in 126 (44.8%) of the specimens. The antibiotic-free interval was 0 days in 74 (26.3% of all specimens) and 1 to 7 days in 26 (9.2% of all specimens). Penicillins, fluoroquinolones, tetracyclines, and a variety of other classes of antibiotics (referred to as "miscellaneous class" in the remainder of the analysis) were administered in the study sample (Table 4). More than 1 antibiotic was administered within 30 days of 29 (10.3%) of the specimens.

No significant relationships were found between usage of an antibiotic prior to TW and bacterial culture outcome. No negative effect on bacterial growth was found for antibiotic-free interval of 30 days or less (P = .58), 1 to 7 days (P = .83) or 0 days (P = .71). Similarly, the M^2 statistic for linear regression did not identify a relationship between degree of growth and the antibiotic-free interval when compared to 30 days or less, 1 to 7 days or 0 days (Table 5). There were insufficient cytology positive/culture negative discordant specimens to determine if antibiotics contributed to this discordancy. Only 2 specimens with this discordancy were from dogs that had received antibiotics. In these dogs, the antibiotic-free intervals were 16 and 25 days.

Classes of antibiotics were also investigated individually. No negative effect on growth was found when considering the time since an antibiotic was given or if a penicillin, fluoroquinolone or tetracycline class antibiotic was administered. However, a negative effect on growth was noted if a miscellaneous class antibiotic was administered in the previous 6 days.

4 | DISCUSSION

Endotracheal wash and TTW provide comparable cytology and culture results when an adequately cellular sample is retrieved. Oropharyngeal contamination occurred with similar frequency in ETW and TTW specimens, failing to support the hypothesis that specimens collected by ETW would be contaminated more often. Even specimens collected by TTW were contaminated approximately 20% of the time. Since there was no difference in the rate of contamination compared with ETW, oropharyngeal contents could populate the larger airways to a greater extent than generally thought. This finding could be a result of microaspiration during anesthesia or sedation, impaired mucociliary clearance with airway disease, or a variation of normal. Our findings are consistent with a previous study in which oropharyngeal contamination was noted in 16% of bronchoscopically collected BAL specimens.[16]

Endotracheal wash more commonly resulted in a nondiagnostic cytology specimen than TTW, although the rate was still relatively low (16.1%). Clinicians should be particularly careful to evaluate fluid retrieved during ETW to ensure the fluid is turbid and/or has visible mucus strands before concluding the procedure. This finding was outside our initial study design, and the medical records do not contain information that could allow us to identify commonalities between cases with nondiagnostic cytology. Poor cellularity can result if attention is not paid to the length of the wash catheter relative to the length of the endotracheal tube. Therefore, we investigated whether larger dogs more often had nondiagnostic specimens by using body weight as a marker for size, but no associations were found. One previously proposed explanation for the increased rate of nondiagnostic cytology in ETW specimens is that the lack of cough reflex under anesthesia impairs delivery of fluid from smaller airways into the trachea.[1,3,4] Another possibility is that ETW was more often performed in dogs with mild clinical signs or interstitial lung disease than TTW since it is an inexpensive, low risk procedure that can be readily performed in association with unrelated diagnostics or procedures such as computed tomography, surgery, or dental procedures.

We did confirm our hypothesis that cytology negative/culture positive discordancy would occur with relatively low levels of bacterial growth, but did not find an association with oropharyngeal contamination. While almost half of the negative cytology specimens had some degree of growth, the majority of this growth (72.5%) was limited to enrichment broth only. Given this low level of growth, it is likely that many of these discordant specimens reflect the presence of commensal organisms or contamination. Therefore, the clinician assessing the relative risk of bacterial infection based upon cytology, prior to receiving final culture results, could reasonably consider withholding antibiotic

therapy in light of aseptic neutrophilic inflammation. There is no gold standard for confirming a diagnosis of lower respiratory bacterial infection, making it impossible to determine the true significance of individual discordant results. It is particularly difficult to confirm the diagnosis in a referral canine population. These dogs often have chronic, multifactorial diseases, waxing and waning of signs and the frequent use of multiple drugs simultaneously for treatment. Furthermore, it is not uncommon for dogs with respiratory disease to be started on combination therapy, making response to any particular medication difficult to capture.

Surprisingly, we did not find that bacterial growth was negatively impacted by recent antibiotic use, nor was there an association with discordancy. There are a number of limitations of the study design, discussed later, which might have obscured the identification of a negative effect of antibiotic administration. Nevertheless, the percentage of positive cultures was nearly identical between washes with an antibiotic-free interval of greater than 30 days and washes within each of the groups with shorter antibiotic-free intervals.

The failure to find a negative effect of antibiotic use on bacterial growth is counter to numerous studies in people and conventional belief.[17-22] Studies suggesting that antibiotic use does not interfere with growth do exist, and it has been proposed that the effect is not seen in the face of superinfection that develops during antibiotic administration, but after the initiation of a new antibiotic.[18,19] If this explanation applies to dogs, it might influence the findings of studies like the current one that have been carried out at referral rather than primary practice. The study of bronchoscopically collected BAL specimens was also carried out in an academic institution.[16] Of 7 dogs that were receiving antibiotics at the time of BAL, 5 samples resulted in sufficient growth to be considered significant.

Failing to find a relationship between antibiotic administration and bacterial growth does not eliminate the possibility of a negative effect on growth for individual dogs, specific antibiotics, specific dosing regimens, specific organisms, or various combinations of these variables. In fact, when analyzing the data for specific classes of antibiotics, a negative effect on aerobic growth was noted if a miscellaneous class antibiotic had been administered in the previous 6 days. As this is a very heterogenous group of antibiotics, some of which were not even prescribed for respiratory disease, it is challenging to understand the clinical impact.

We investigated a large database of TW results to identify factors related to discordancy and degree of bacterial growth that could provide context to interpret discordant results and the results of cytology prior to the completion of aerobic culture. The major limitations to this study were the absence of a gold standard to identify dogs with true infection, and the retrospective study design. There was not a rigid protocol for performing TW and there will have been variations to technique between clinicians as well as the time intervals between specimen collection and processing. Sedation protocol, location of the catheter during the wash, positioning of the dog, number of saline aliquots used during the wash, and cooperation of the dog might all be factors contributing to the quality of the specimen. Specimens with only extracellular bacteria seen were included because we were investigating discordancy within individual TW, and growth would be expected from such specimens.

As a predominantly referral hospital, dogs in this study likely had more chronic and multifactorial diseases than in a primary practice population. It is likely that they more often had received prior treatments, and that dogs that responded readily to conventional treatments were never referred. The duration of time a dog was administered antibiotics was unable to be reliably determined since most treatments would have been prescribed prior to referral.

In conclusion, our findings suggest that there is little difference between results from endotracheal and transtracheal wash with the exception that endotracheal washes are more often hypocellular. The hypocellularity might be mitigated in part by visual screening of specimens before concluding the procedure. Further study is needed to investigate factors that could maximize obtaining diagnostic specimens. It is reasonable to predict bacterial growth from tracheal wash specimens based on cytology, considering a specimen that is positive for bacteria by cytology to have bacterial growth at least in enrichment broth and for a specimen that is negative by cytology to have no growth greater than 1+. Importantly, recent antibiotic usage should not discourage the analysis of TW fluid in situations where there is a compelling reason to avoid delay such as failure to respond to current therapy or worsening of clinical signs.

ACKNOWLEDGMENT

No funding was received for this study. The authors thank Dr. John Bonagura for his advice during manuscript preparation.

CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Due to the retrospective nature of the study, label usage of antibiotics could not be determined in all dogs; however, no antibiotics are currently labeled for treatment of lower respiratory tract infections in dogs in the United States.

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.

ORCID

Karen M. Tefft https://orcid.org/0000-0003-4523-9763
Devorah M. Stowe https://orcid.org/0000-0002-4058-2995
James B. Robertson https://orcid.org/0000-0002-6115-042X
Eleanor C. Hawkins https://orcid.org/0000-0002-4006-184X

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How to cite this article: Graham AM, Tefft KM, Stowe DM, Jacob ME, Robertson JB, Hawkins EC. Factors associated with clinical interpretation of tracheal wash fluid from dogs with respiratory disease: 281 cases (2012-2017). *J Vet Intern Med*. 2021;35:1073–1079. https://doi.org/10.1111/jvim.16052