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Endoscopic Assessment of Airway Inflammation in Horses

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Background: Comprehensive endoscopic scoring of the upper and lower airways for inflammation has not been critically assessed among a large population of horses. The relationship between upper and lower airways described in humans by the "one airway, one disease" concept might also apply to horses.

Hypothesis/Objectives: To evaluate if an association exists between endoscopic inflammatory scores and mucus scores of upper and lower airways and to investigate if endoscopic findings correlate with the lower airway inflammation measured by bronchoalveolar lavage (BAL) cytology.

Methods: Prospective field study. Pharyngitis, pharyngeal mucus, tracheal mucus, tracheal septum thickness, and bronchial mucus were scored using new and previously described scoring systems on a convenience sample of 128 horses with and without lung inflammation. Based on BAL fluid cytology, horses were categorized as having normal, moderate, or severe inflammation of the lower airways.

Results: All 5 endoscopy scores showed excellent interobserver agreement. Tracheal mucus ($P < .001$), tracheal septum thickness ($P = .036$), and bronchial mucus ($P = .037$) were significantly increased in horses with severe inflammation BALs and were correlated among themselves but not with upper airways scores. BAL neutrophils percentage was correlated with tracheal mucus ($r_s = 0.41$, $P < .001$), bronchial mucus ($r_s = 0.27$, $P = .003$), and had a weak negative correlation with pharyngitis ($r_s = -0.25$, $P = .004$).

Conclusions and Clinical Importance: Lower airway endoscopy scores are reflective of lower airway inflammation; however, upper and lower airways are independent in terms of severity of inflammation. Therefore, observing upper airway inflammation is not an indication to test for lower airway inflammation.

Key words: Bronchi; Bronchoalveolar lavage; Heaves; Pharynx; Recurrent airway obstruction; Trachea.

The "one airway, one disease" concept is based on the fact that the upper and lower airways are integral parts of the respiratory tract. Upper and lower respiratory tract issues often coexist. For instance, there is evidence that allergic rhinitis and sinusitis coexist in a high proportion of patients with asthma and chronic obstructive pulmonary disease (COPD).[1,2] This evidence led the World Health Organization (WHO) to publish recommendations for practitioners to assess the lower airway conditions in patients with rhinitis and, conversely, to perform upper airway evaluations in patients suffering from asthma.[3] The "one airway, one disease" concept has yet to be demonstrated in equine medicine. In fact there is even evidence that pharyngeal inflammation is not associated with changes in tracheal cytology[4] or bronchoalveolar lavage (BAL) cytology.[5] However, tracheal cytology is not the best measure of lung inflammation and the conclusions using the BAL cytology are based on a small number of 18-month-old healthy horses with low mucus scores.[5] Upper and lower airway endoscopic examination provides information on the severity of airway inflammation. Endoscopic scores are described for pharyngitis,[6] accumulation of mucus in the trachea,[7] and tracheal septum thickness.[7,8] While these scores are commonly used, they have not been combined altogether in one study to establish a comprehensive endoscopic score of both upper and lower airways. Since upper airway endoscopy is more readily available to equine practitioners and lung inflammation is reliably assessed using a BAL cytology, it is important to know if the presence of upper airway inflammation also suggests the presence of lower airway inflammation in a horse, thus representing an indication for a BAL procedure.

Abbreviations:

BAL	bronchoalveolar lavage
BALF	bronchoalveolar lavage fluid
CI	confidence interval
COPD	chronic obstructive pulmonary disease
IAD	inflammatory airway disease
RAO	recurrent airway obstruction
WHO	World Health Organization

The main objective of this study was therefore to determine if the "one airway, one disease" concept is applicable to horses by establishing if there is an association between endoscopic findings (upper and lower airway inflammation scores as well as mucus scores) with the severity of lower airway inflammation measured using BAL cytology. Another objective was to evaluate if there is an association between endoscopic scores for airway inflammation and septum thickness with the scores for mucus accumulation.

To address these objectives, we scored the upper and lower airway mucus and inflammation and performed a BAL on 128 horses with and without

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lung inflammation. Furthermore, since no endoscopic scoring system was available to assess the amount of mucus in the pharynx and in the lower bronchi, the equivalent of the "bronchitis index" performed in human medicine to visually assess lower airway inflammation was used.[10] In addition to this index, we proposed two new scores to assess theses parameters. In conjunction with the previously published endoscopic scores, these new scores are used in a comprehensive manner to assess the amount of mucus and severity of inflammation in the upper and lower airways.

Materials and Methods

Horses

Videoendoscopy and BAL examinations were performed in the field by the authors on a convenience sample of 128 horses from the Calgary area (Alberta, Canada). Horses were sedated with 0.6–1.0 mg/kg xylazine[a] IV and 20–30 μg/kg butorphanol[b] IV before the procedures. The recruitment of horse owners was done through veterinarians, advertisement at horse shows, and a website. Inclusion criteria were limited to the absence of infectious disease detected after a complete physical examination, as well as the absence of any health condition that would present a risk for the neuroleptanalgesia and BAL procedure. The horses were of various age (Table 1), breeds, and both sexes. Horses enrolled in the study were used for pleasure, performance, or racing. All BALs were performed in the morning on farms and no horses were transported or exercised for at least 12 hours prior to the procedure. We recorded the housing conditions as indoor (ie, indoor at night, but some horses might be kept outdoor during the day) or outdoor (ie, outdoor day and night), as well as if they were fed with round or square hay bales at the time of the study. This study was approved by the Animal Care Committee of the Health Science Centre at the University of Calgary and all owners signed a consent form.

Airway Scoring

The 128 endoscopic examinations were performed using a flexible 3.2 m long videoendoscope[c] that was passed through the left nostril, pharynx, into the trachea, and directed into the left lung until wedged in the main bronchus. Coughing during the procedure was recorded. Each endoscopic procedure was recorded digitally in a high quality format and special attention was paid to the examination of the pharynx, trachea, and carina to facilitate the scoring process. Each video was reviewed and scored by two of the authors (KK, RL) at a later date in a randomized order. The reviewers performed the scoring independently and were blinded to the identification of the horses and the BAL results. The digital video clips were viewed on personal computer screens and could be paused or played in slow motion until the reviewer felt comfortable with the scores assigned. The upper and lower airways scoring system used was as described below (Fig 1).

Upper Airway. Pharyngitis and pharyngeal mucus accumulation were scored first, using views of the soft palate, the nasopharynx, including the dorsal pharyngeal recess, and the larynx. Pharyngitis was scored using a grade 1–4 scale[6] (Fig 1).

To score pharyngeal mucus accumulation, we created a scoring system using a 0–2 scale as follows (Fig 1): Score 0: no mucus at all, Score 1: little blobs of mucus ventrally and abaxially to the epiglottis, Score 2: confluent or large amount of mucus ventrally as well as dorsally to the epiglottis, occasionally mucus accumulating in the ventricles and corniculate processes.

Lower Airway. Three parameters were scored: (1) mucus accumulation in the trachea was scored as[7] (Fig 1) 0: none (clean singular), 1: little (multiple small blobs), 2: moderate (larger blobs), 3: marked (confluent, stream-forming), 4 large: (pool-forming) and 5 extreme: (profuse amounts), (2) tracheal septum thickness was scored using a 0–4 scale[8] (Fig 1), and (3) bronchial mucus accumulation scored using the sequence of the videoendoscope being advanced from the tracheal septum into the left lung until wedged. We adapted the tracheal mucus scoring system to the bronchi using the same scale.

Bronchoalveolar Lavage

Once the videoendoscope was wedged into a bronchus in the left lung, a BAL was performed on each of the 128 horses following a standardized procedure as described previously.[11] The head of the horses was kept in a natural position and at approximately the same height level for all horses (chest level of one of the authors). Then 250 mL boluses of sterile 0.9% sodium chloride were alternatively instilled into the bronchus and aspirated via the endoscope biopsy channel using a suction pump. The BAL fluid was collected and 2 slides were prepared within 6 hours of the BAL using a cytocentrifuge[d] then an automatic stainer[e] with a Modified Wright Giemsa solution for better visualization of the mast cells.[12] Total cell count was performed on

Table 1. Endoscopic scores for pharyngitis, pharyngeal mucus, tracheal mucus, tracheal septum thickness, and bronchial mucus in 128 horses with bronchoalveolar lavage (BAL) cytology categorized as normal BAL, moderate, and severe inflammation BAL.

	Age	Pharyngitis	Pharyngeal Mucus	Tracheal Mucus	Tracheal Septum Thickness	Bronchial Mucus
		Scores for all horses (n = 128)				
Mean	11.5 (±7)	1.98	0.47	1.39	1.29	0.30
		Scores for horses with normal BAL (n = 33)				
Mean	11.5 (±6.8)	2.06	0.44	1.12	1.31	0.28
		Scores for horses with moderate inflammation BAL (n = 71)				
Mean	11 (±6.4)	1.97	0.38	1.12	1.07	0.15
		Scores for horses with severe inflammation BAL (n = 24)				
Mean	13.1 (±8.8)	1.86	0.97[b]	2.50[a,b]	1.91[a,b]	0.75[a,b]

[a]Indicates different from normal BAL.
[b]Indicates different from moderate inflammation BAL.

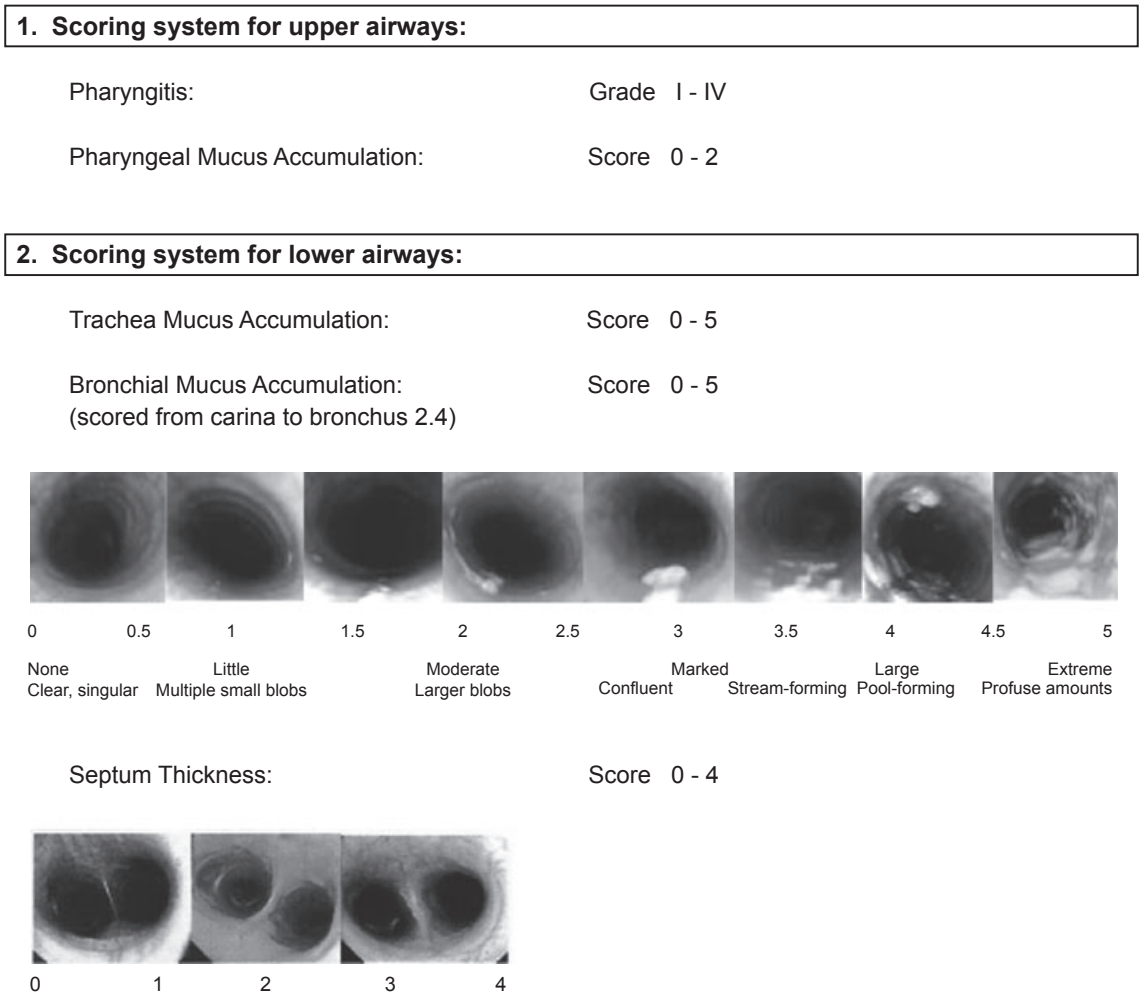

Fig 1. Scoring sheet for mucus and inflammation in the upper and lower airways (modified from[6,7,8]).

10 μL of BAL fluid. Differential counts were performed on 400 nucleated cells by one of the authors (AW). Epithelial cells were not included in the differential count.

Lower Airway Inflammation Categories

Based on the results of the BAL cytology, horses were first put into one of three categories[13]: (1) normal BAL: ≤2% mast cells, ≤1% eosinophils, and <10% neutrophils, (2) moderate inflammation BAL: >2% mast cells and/or >1% eosinophils and/or 10–25% neutrophils, and (3) severe inflammation BAL: >25% neutrophils.

Statistical Analysis

Bronchoalveolar lavage cell percentages and endoscopic scores are reported as mean ± SD with 95% confidence interval (CI).[14] Interobserver agreement for the endoscopic scores was analyzed using an absolute single measure interclass correlation (ICC) analysis for which reliability was tested with a Cronbach's Alpha test. Analysis involving endoscopic scores was repeated separately for the scores obtained from the 2 readers (KK and RL). Differences in each endoscopic parameter scored, as well as age, housing, and feeding conditions between BAL categories (normal BAL, moderate and severe inflammation BAL) were analyzed using an analysis of variance and posthoc Tukey analysis when indicated. Exploratory analysis of correlations between endoscopic scores and horses' age or housing conditions was performed using a Spearman rank order test. Exploratory analysis of correlations between endoscopic scores themselves, as well as between endoscopic scores and BAL fluid cell percentages was performed with a correlation matrix using Spearman rank order test. A P-value <.05 was considered statistically significant for all results.

Results

Interobserver Agreement on Upper and Lower Airways Endoscopic Scores

Upper Airways. The interobserver agreement on pharyngitis and pharyngeal mucus was excellent (ICC = 0.89 [CI 0.85–0.92] and ICC = 0.76 [CI 0.65–0.83], respectively) (Fig 2).

Lower Airways. The interobserver agreement for the tracheal mucus (ICC = 0.87; CI 0.81–0.9), tracheal septum thickness (ICC = 0.78; CI 0.7–0.84), and bronchial mucus (ICC = 0.9; CI 0.85–0.93) scores was excellent (Fig 2).

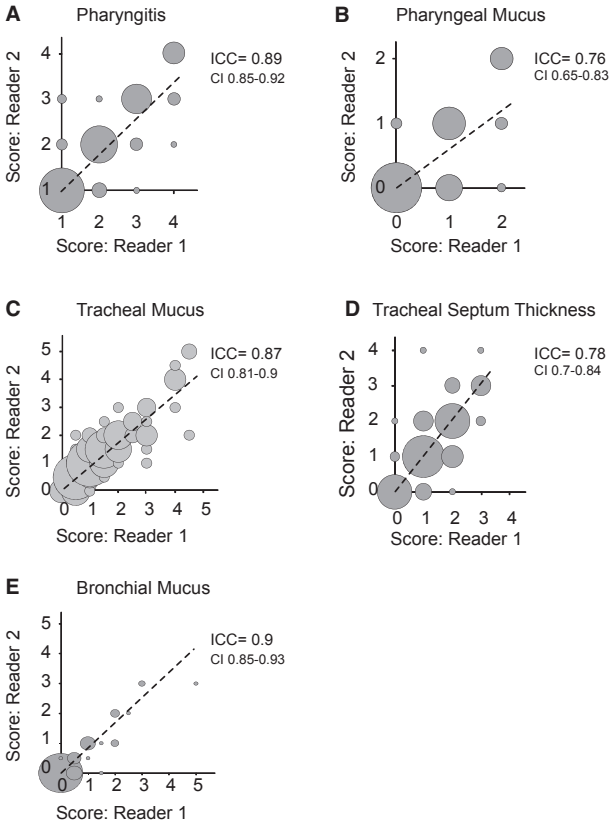

Fig 2. Inter-readers agreement for the upper and lower airway endoscopic scores used in the study: Bubble size is proportional to the number of points with the same coordinates. Inter-observer agreement is indicated on each figure (ICC value with 95% confidence interval, CI). Pharyngitis (**A**), pharyngeal mucus (**B**), tracheal mucus (**C**), tracheal septum thickness (**D**) and bronchial mucus (**E**) were scored independently by reader 1 and reader 2.

Although we performed the analysis for the 2 readers, the scores from only one of the authors (KK) were reported in the study because the agreement between readers was excellent and because similar conclusions were obtained in both cases.

Description of the Endoscopic Scores and BAL Cytologies (Tables 1 and 2, Fig 3)

Using the criteria described above to categorize the horses based on the cytology of their BALs, we found 33 horses had a normal BAL, 71 had moderate inflammation BAL, and 24 had severe inflammation BAL (Table 2).

Endoscopic Scores. Twelve horses coughed evidently during the procedure (5 with normal BAL, 6 with moderate inflammation BAL, and 1 with severe inflammation BAL).

When analyzing the differences for each endoscopic score between the normal BAL and the severe inflammation BAL categories, tracheal mucus ($P < .001$), tracheal septum thickness ($P = .036$), and bronchial mucus ($P = .037$) were significantly increased in horses with the severe inflammation BALs (Table 1). These are endoscopic parameters exclusively describing lower airways condition. The pharyngeal mucus ($P = .012$), tracheal mucus ($P < .001$), tracheal septum thickness ($P < .001$), and bronchial mucus ($P < .001$) scores were significantly different between the moderate inflammation BAL and the severe inflammation BAL category (Table 1). No difference was detected for any endoscopic parameter between horses with normal BAL and those with moderate inflammation BAL.

Bronchoalveolar Lavage. There were significant differences in cell type percentage between the BAL categories (Table 2). Notably, horses with severe inflammation BAL had greater neutrophil percentages than horses from the normal BAL and moderate inflammation BAL category and horses from the moderate inflammation category had greater mast cell percentages than horses from the normal BAL category.

Effect of Age

There was no difference in age ($P = .44$) between the BAL categories (normal BAL, moderate and severe inflammation BAL) (Table 1). The pharyngitis score was negatively correlated with the age ($r_s = -0.72$, $P < .001$). The bronchial mucus as well as the tracheal mucus scores showed a significant, but weak, correlation with age ($r_s = 0.34$, $P < .001$ and $r_s = 0.24$, $P = .006$, respectively).

Effect of Housing and Feeding Conditions

There was no difference in housing conditions ($P = .252$) reported (inside versus outside) between BAL categories. Hay feeding conditions (round hay bale versus square hay bale) were different ($P = .007$) between horses from the moderate inflammation and severe inflammation BAL categories. There was no correlation between housing conditions (inside versus outside) or feeding conditions (round hay bales versus square hay bales) and any endoscopic score, as assessed by the Spearman rank order test (all P values between .142 and .966).

Correlations between the Endoscopic Scores

Out of the 5 endoscopic parameters scored, only the scores for lower airways showed significant correlation between them as follows: tracheal mucus scores were well correlated with bronchial mucus scores ($r_s = 0.52$, $P < .001$) (Fig 4) and weakly correlated with tracheal septum thickness scores ($r_s = 0.18$, $P = .043$) (Fig 4). Tracheal septum thickness scores were weakly correlated with bronchial mucus scores ($r_s = 0.32$, $P < .001$) (Fig 4).

Correlation between Endoscopic Scores and BAL Cell Types

Bronchoalveolar lavage neutrophil percentage showed the best correlation with tracheal mucus

Table 2. BAL volumes, total and differential cell counts in 128 horses categorized as normal BAL, moderate, and severe inflammation BAL.

	BAL Volume (ml)	Total Cell Count (/mm^3)	Neutrophils (%)	Eosinophils (%)	Mast Cells (%)	Lymphocytes (%)	Macrophages (%)
All 128 horses							
Mean (±SD)	334 (±70.2)	56.6 (±37.5)	14 (±18.9)	0.2 (±0.7)	2.6 (±2)	40.7 (±13.4)	42.6 (±16.4)
First quartile			2.4	0	1.1		
Median			5.4	0	2.1		
Third quartile			16	0.1	3.5		
Horses with normal BAL (n = 33)							
Mean (±SD)	343.2 (±61.2)	59.6 (±31)	4.3 (±2.6)	0.1 (±0.2)	1.4 (±0.4)	46.3 (±11.6)	47.9 (±12)
First quartile			2.1	0	1.0		
Median			4.1	0	1.4		
Third quartile			6.4	0	1.8		
Horses with moderate inflammation BAL (n = 71)							
Mean (±SD)	347.9 (±62)	47.2 (±27.2)	6.7 (±6.3)[a]	0.2 (±0.4)	3.7 (±2.1)[a]	42.3 (±12.4)[a]	47.2 (±14.3)[a]
First quartile		1.5	0	2.3			
Median		4	0	3			
Third quartile		11.7	0.3	4.7			
Horses with severe inflammation BAL (n = 24)							
Mean (±SD)	280.2 (±80.7)	79.8 (±57.7)	48.7 (±17.1)[a,b]	0.4 (±1.5)	1.2 (±0.9)[a,b]	28.3 (±10.8)[a,b]	21.4 (±9.2)[a,b]
First quartile		37	0	0.5			
Median		45.6	0	0.9			
Third quartile		56.9	0.1	1.5			

[a]Different from normal BAL.
[b]Different from moderate inflammation BAL.

($r_s = 0.41$, $P < .001$), then bronchial mucus ($r_s = 0.27$, $P = .003$), and finally a weak negative correlation with pharyngitis ($r_s = -0.25$, $P = .004$) (Fig 5A).

Bronchoalveolar lavage mast cell percentage showed weak negative correlation with tracheal mucus ($r_s = -0.26$, $P = .003$), bronchial mucus ($r_s = -0.23$, $P = .011$), and tracheal septum thickness ($r_s = -0.23$, $P = .009$) (Fig 5B).

Bronchoalveolar lavage macrophage percentage showed negative correlation with the tracheal mucus score ($r_s = -0.41$, $P < .001$) (Fig 5C), the bronchial mucus ($r_s = -0.29$, $P = .001$) (Fig 5C), and the tracheal septum thickness ($r_s = -0.21$, $P = .02$) as well as a weak positive correlation with the pharyngitis score ($r_s = 0.25$, $P = .005$).

The eosinophil and lymphocyte percentages of the BAL fluid did not show any correlation with any endoscopic parameter scored.

Pharyngeal mucus was the only endoscopic parameter that did not show any correlation with any BAL cell type percentage.

Discussion

Assuming that the visual aspect of the upper airways reflects the severity of inflammation in the tissues in this area, the results of this study do not support the "one airway, one disease" concept in a population of horses of various ages housed in different conditions.

The endoscopic scores described in this study combine scoring systems available in the literature with 2 new scores (pharyngeal mucus and bronchial mucus), thus allowing a comprehensive scoring of the airways from pharynx to bronchi during endoscopic examinations. However, we did not assess the guttural pouches' inflammation like previously described,[5] because our endoscope was too long and too large of a diameter. The interobserver agreement was excellent for all 5 endoscopic scores (Fig 2) which indicated that all 5 scores were reproducible and were reliable to exchange information between clinicians. The single measure interclass correlation (ICC) analysis used herein to measure interobserver agreement in the endoscopic scoring is conservative. This statistical analysis was chosen because endoscopies are often rated by a single individual (clinician) in practice.

Upper Airways

The inflammatory status of the upper airways was evaluated using endoscopy to grade the pharyngeal lymphoid hyperplasia as well as the mucus quantity in the pharynx. Pharyngeal lymphoid hyperplasia, which is accepted as an indicator of pharyngitis, was evaluated using a visual scoring system previously described and widely used in practice as well as in research.[4,6,9,15–18] However, the agreement between observers was never reported for this pharyngitis score[6,18] and we found that it was excellent (ICC = 0.89; CI 0.85–0.92). In accordance with results published previously in racehorses,[4,15,17] we found a strong negative correlation between the pharyngitis score and the age of the horses ($r_s = -0.72$, $P < .001$). These results are even more meaningful considering

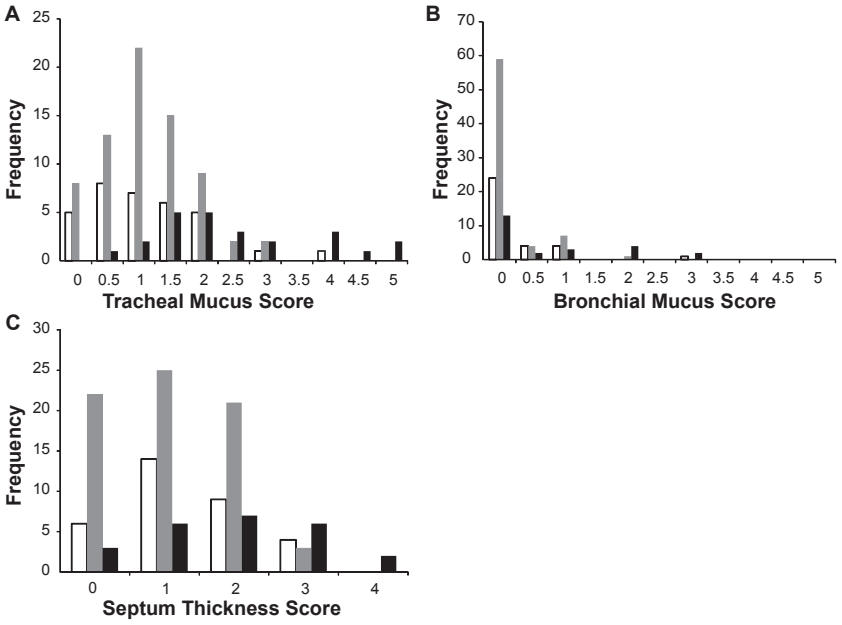

Fig 3. Histograms showing the distribution of tracheal mucus (**A**), bronchial mucus (**B**) and tracheal septum thickness (**C**) in a population of 128 horses categorized by normal BAL (white bar), moderate (gray bar), or severe (black bar) inflammation BAL (frequency = number of horses).

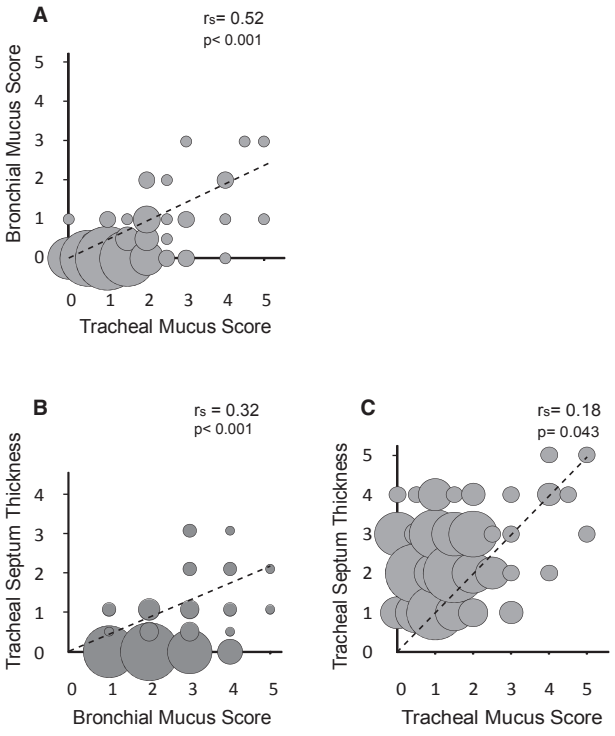

Fig 4. Correlation between endoscopic scores in 128 horses. Only the scores with significant correlation are shown herein (all are lower airways scores).

that our horse population had a broader age range and was older (age 11.4 ± 6.7) than the racehorses used in previous studies.

We proposed a simple three grade scoring system for pharyngeal mucus accumulation (1: No mucus at all, 2: Little blobs, 3: Confluent or large amount) and found that the agreement between the 2 observers was excellent for this score (ICC = 0.76 (CI 0.65–0.83)). Interestingly, the pharyngeal mucus score was not correlated with the pharyngitis score, which suggests that inflammation of the pharynx did not produce local mucus accumulation in the oropharynx. The pharyngeal mucus score was not correlated with the tracheal mucus score either, which was surprising because we thought that the pharyngeal mucus might arise from the trachea. This result was different from a previous study in racehorses where an increase in the pharyngitis score is associated with an increase in the tracheal mucus score.[4] One explanation for our findings could be that horses swallowed some mucus that accumulated in the pharynx. It could also have reflected the independence between upper and lower airway inflammation, thus invalidating the "one airway, one disease" concept. In this sense, only lower airway scores were significantly correlated between themselves and none were correlated with any upper airway score.

Lower Airways

The endoscopic evaluation of the lower airways included the following three scores: tracheal mucus, tracheal septum thickness, and bronchial mucus. The tracheal mucus accumulation score used herein has been very well validated in a previous study[7] and we applied the same scale to the bronchial mucus score. A previous study used a mucus score at the level of the carina in horses[19] but based on a human study on a

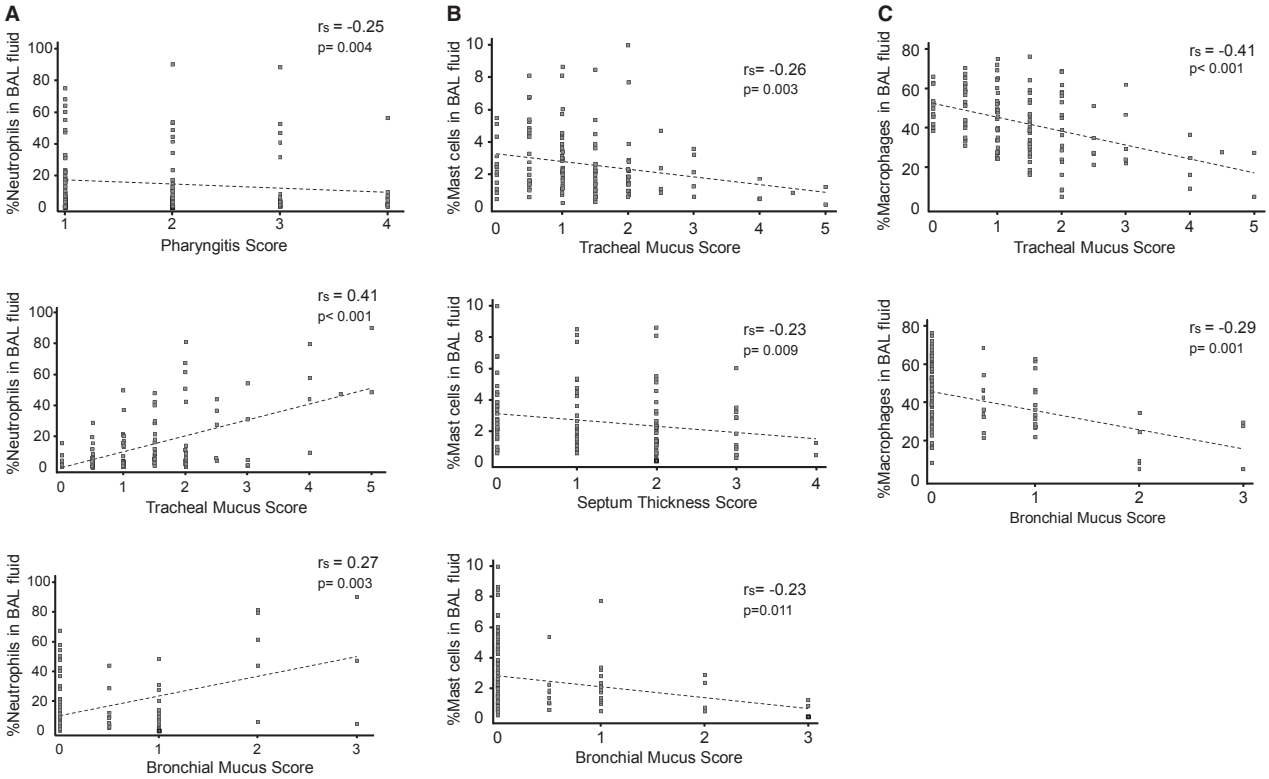

Fig 5. Correlation between upper and lower airway endoscopic parameters and bronchoalveolar lavage (BAL) cell types (dashed lines indicate the correlation): (**A**) Correlation between upper and lower airway endoscopic parameters and BAL neutrophil percentage. Significant correlations are shown. (**B**) Correlation between lower airway endoscopic parameters and BAL mast cell percentage. Significant correlations are shown. (**C**) Correlation between lower airway endoscopic parameters and BAL macrophage percentage. Significant correlations are shown.

bronchitis index,[10] we opted to score the mucus in more peripheral airways at the bronchial level to differentiate it better from the tracheal mucus accumulation. The interobserver agreement for the bronchial mucus score was excellent (ICC = 0.9, CI 0.85–0.93) (Fig 2). We found that bronchial mucus scores were lower on average for the horses overall (1.39 versus 0.30) as well as when horses were categorized by degree of lower airway inflammation (normal: 1.12 versus 0.28; moderate: 1.13 versus 0.15; severe: 2.5 versus 0.75) (Table 1). This was probably because the ratio of lung area drained versus airway diameter was larger for the trachea than for a bronchus. We also found a good correlation between tracheal mucus and bronchial mucus scores ($r_s = 0.52$, $P < .001$) (Fig 4), which confirmed the assumptions previously made in other studies that tracheal mucus accumulation is representative of bronchial mucus accumulation.[4]

In addition, we used a scoring system previously described for tracheal septum thickness.[8] The interobserver agreement for the tracheal septum thickness score (ICC = 0.78, CI 0.7–0.84) was excellent and was in the same range as previously described.[8] Tracheal septum thickness score has been previously shown to be greater in horses older than 10 years of age.[7] However, we did not find any correlation between this score and the horses' age, even after separating the population into 2 age categories ($\geq$10 and <10 year old). This is possibly because of the number of horses used and because of the diversity in their severity of lower airway inflammation. A previous study found that the tracheal septum thickness is not correlated with the BALF cytology or tracheal mucus scores in horses with RAO.[7] Similarly, we found no correlation between this score and the BALF neutrophils percentage and very weak correlations with the tracheal mucus score ($r_s = .18$, $P = .043$) (Fig 4). However, the correlation was better between the tracheal septum thickness score and the bronchial mucus ($r_s = 0.32$, $P < .001$) (Fig 4). We also found that tracheal septum thickness score increased significantly between normal or moderate inflammation BAL and severe inflammation BAL categories. These results suggested that tracheal septum thickness was part of the indicators of lower airway inflammation but the lack of association with other parameters indicated that it should not be given too much weight in the overall assessment of lower airways.

BALF Cytology

Although the transtracheal wash is less invasive and could be done more easily than a BAL in racehorses, our choice for the BAL procedure was based on the

fact that the transtracheal wash cytological interpretation was more challenging and that its results are now accepted as being less representative of the bronchiolar cytology.[20,21] We found a significant correlation between BALF neutrophil percentage and tracheal mucus score as well as bronchial mucus score (Fig 5A). This was in accordance with a previous study which found a very similar value of r_s between neutrophil percentage and endoscopic tracheal mucus score in 9 horses.[7] It was, however, different from a study from the same group that did not find any correlation between tracheal mucus scores and BALF neutrophils counts in a limited number of 18 month old horses[5] and from another study where no correlation was found between tracheal mucus score and the cytology of BALF and transtracheal wash in racehorses with poor performance.[9] Nonetheless, the authors of the first study suggested that the very mild inflammation in the BALF as well as the low mucus scores in most of the horses were probably indicative of the lack of association found between the 2 measures.[5] The racehorses in the second study had an endoscopic examination performed 60 minutes following high intensity exercise on a treadmill, which potentially induced mucus production without prior accumulation of inflammatory cells in the lower airways.[9] The correlations we reported as well as the fact that neutrophil percentage in BALF of normal horses and RAO horses increased only 6 hours after an environmental challenge[22], suggested that neutrophils played an important role in airway mucus accumulation, either through increased mucus production or through decreased clearance.[23,24]

The role of the mast cells in horses' lung diseases was not clear, and although we found a negative correlation between BALF mast cell percentages and lower airway mucus (Fig 5B), it was weak and did not support an important role of mast cells in airway mucus accumulation. Similarly, mast cell counts in transtracheal washes or BALs were not found to be correlated with endoscopic mucus scores in two studies on racehorses.[4,9]

The three lower airways scores (tracheal mucus, tracheal septum thickness, bronchial mucus), but not the upper airways scores (pharyngitis, pharyngeal mucus), were significantly increased in the severe inflammation BAL category, compared to the normal BAL one. This and the negative correlation between BALF neutrophil percentage and the pharyngitis score ($r_s = -0.25$, $P = .004$) illustrated the divergence we found between upper and lower airway inflammation in these horses (Fig 5A). In this sense, a previous study using transtracheal wash in a high number of racehorses did not find a correlation between any cytological parameter and the pharyngeal lymphoid hyperplasia.[4] Similarly, another study did not find any correlation between BALF cytology or tracheal mucus score and upper airway inflammation in normal young horses.[5] Furthermore, pharyngeal mucus was the only endoscopic parameter that did not show any correlation with any BALF cell type percentage in our study. This again showed disconnect between diseases of the upper airways versus lower airways. One limitation of the present study was that the endoscopies were performed at one point in time only. Analysis of the variance of the scores on a day-to-day basis would have strengthened the validation of the new endoscopic scores proposed herein. In addition, even if many of the scores used herein have already been validated using an analysis that included repeatability measurements[7,8] and were currently used at large, many factors inducing variation in theses scores were probably unknown. It is possible that environmental factors such as air quality (dust content, temperature, and more) affected these scores but they were not described in the literature or recorded in the present study. Also, the population studied may not be representative of the horse population of Alberta because the recruitment method could have raised more interest from owners with horses showing some respiratory clinical signs. Since the population studied was still made of horses with a broad variety of endoscopic scores and BAL fluid cytologies, the effects of this bias on the interpretation of our results are likely minor.

Overall, and from a clinical point of view, these results suggested that upper and lower airways endoscopic examination results should be interpreted independently, thus not supporting the "one airway, one disease" concept, and that the observation of a high mucus score in the lower airways probably involved the presence of a lower airways neutrophilic inflammation, while the absence of mucus did not exclude lung inflammation which could have been secondary to mast cell accumulation in the lower airways.

Footnotes

[a] Rompun, Bayer, Toronto, Canada
[b] Torbugesic, Wyeth Animal Health, Guelph, Canada
[c] Olympus CF-Q140L, 12.9 mm tip diameter
[d] Cytospin
[e] Hematek 2000, Bayer

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