Coughing, mucus accumulation, airway obstruction, and airway inflammation in control horses and horses affected with recurrent airway obstruction

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Objective—To investigate relationships between cough frequency and mucus accumulation, airway obstruction, and airway inflammation and to determine effects of dexamethasone on coughing and mucus score.

Animals—13 horses with recurrent airway obstruction(RAO and 6 control horses.

Procedure—6 RAO-affected and 6 control horses were stabled for 3 days. Coughing was counted for 4 hours before and on each day horses were stabled. Before and on day 3 of stabling, tracheal mucus accumulation was scored, airway obstruction was assessed via maximal change in pleural pressure (ΔPpl_{max}), and airway inflammation was evaluated by use of cytologic examination of bronchoalveolar lavage fluid (BALF). Effects of dexamethasone (0.1 mg/kg, IV, q 24 h for 7 days) were determined in 12 RAO-affected horses.

Results—To assess frequency, coughing had to be counted for 1 hour. In RAO-affected horses, stabling was associated with increases in cough frequency, mucus score, and ΔPpl_{max}. Control horses coughed transiently when first stabled. In RAO-affected horses, coughing was correlated with ΔPpl_{max}, mucus score, and airway inflammation and was a sensitive and specific indicator of $\Delta Ppl_{max} > 6$ cm H_2O, mucus score > 1.0, and > 100 neutrophils/µL and > 20% neutrophils in BALF. Dexamethasone reduced cough frequency, mucus score, and ΔPpl_{max}, but BALF neutrophil count remained increased.

Conclusions and Clinical Relevance—Because of its sporadic nature, coughing cannot be assessed accurately by counting during brief periods. In RAO-affected horses, coughing is an indicator of airway inflammation and obstruction. Corticosteroid treatment reduces cough frequency concurrently with reductions in ΔPpl_{max} and mucus accumulation in RAO-affected horses. (*Am J Vet Res*2003;64:550–557)

Coughing is a nonspecific defense mechanism of the respiratory system that aids in clearance of material from the lumen of the larger airways and is triggered by activation of receptors in the airway mucosa.[1] Sensitivity of the cough reflex is increased when there is inflammation[2,3]; hence, coughing is frequently a sign of airway inflammation. In horses, coughing is a common and specific, but not extremely sensitive, sign of respiratory tract disease.[4] In 2 large studies[5,6] that involved young racehorses, coughing was strongly associated with airway inflammation as judged by accumulated airway secretions with an increased number of neutrophils; however, many horses with such evidence of airway inflammation did not cough.

Despite its frequency as a clinical finding in horses with **recurrent airway obstruction (RAO)**, coughing has never been quantified, and its association with airway inflammation, airway obstruction, and mucus accumulation has not been investigated. The objectives of the study reported here were to determine the minimum period necessary when counting the number of coughs to accurately quantify cough frequency in RAO-susceptible horses, to investigate the effect of exposure to organic dust in a stable on cough frequency in RAO-susceptible and control horses, to evaluate the association between coughing and mucus accumulation, airway obstruction, and airway inflammation in RAO-affected horses, and to determine whether cough frequency and mucus accumulation decrease concurrently with airway obstruction during dexamethasone treatment of RAO-affected horses.

Materials and Methods

Animals—Thirteen RAO-affected horses (mean ± SD, 19.9 ± 3.9 years old) and 6 control horses (7.3 ± 2.1 years old) were used in the study. The RAO-affected horses were from a herd of horses maintained by the Pulmonary Laboratory at the College of Veterinary Medicine, Michigan State University, and met the criteria for such animals as defined by the International Workshop on Equine Chronic Airway Disease[7] (ie, they developed airway obstruction and inflammation when exposed to organic dust in a stable). Control horses were selected on the basis that they did not have a history of RAO and did not develop airway obstruction when stabled. The study was approved by the All-University Committee on Animal Use and Care at Michigan State University.

Experimental design—The study comprised 2 experiments. In experiment 1, the effect of stabling on cough frequency, mucus score, lung function, and airway inflammation was determined in 6 RAO-affected and 6 control horses. All horses had been on pasture for at least 3 weeks before the

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start of the experiment. They were allocated into 2 groups of 6 horses, with each group comprising 3 control and 3 RAO-affected horses. On day 0 (baseline), horses were transported from the pasture to the laboratory (distance of 1.6 km), where they were placed into 2 adjacent outdoor paddocks. They were allowed to recover from transport for 30 minutes, and then the number of coughs was counted for 4 hours. After the number of coughs was counted, horses were placed in a stock for measurement of **maximal change in pleural pressure (ΔPpl_{max})**, endoscopy, and bronchoalveolar lavage. Horses were then placed in separate stalls in a 9-stall stable, bedded on straw, and fed poor-quality, dusty hay. On days 1, 2, and 3 of stabling, the number of coughs was counted for 4 hours. After the number of coughs was counted on day 3, measurement of ΔPpl_{max}, endoscopy, and bronchoalveolar lavage were repeated.

In experiment 2, the effect of dexamethasone administration on cough frequency, mucus score, and lung function was determined in 12 RAO-affected horses investigated in 2 groups (6 horses/group). These horses included 5 of the 6 RAO-affected horses used in experiment 1. Horses were moved from pasture to the stable; they were then fed hay. Three days later, the number of coughs was counted, ΔPpl_{max} was measured, and endoscopy and bronchoalveolar lavage were conducted. In addition, each horse was given a clinical score on the basis of its abdominal effort to breathe and the degree of nasal flaring.[8] Each of these components was scored from 1 to 4, and the values for the 2 variables were summed. Horses were then treated once daily with dexamethasone (0.1 mg/kg, IV, q 24 h for 7 days). On day 3 of treatment, the number of coughs was counted, and on day 7, the number of coughs was counted, ΔPpl_{max} was measured, and endoscopy and bronchoalveolar lavage were conducted. Experiment 2 did not incorporate a control group for 2 reasons. First, we have documented that RAO-affected horses that remain untreated for 7 days do not improve.[9] Second, the animal use committee would not approve having RAO-affected horses remain untreated for 7 days.

Cough frequency—The number of coughs was counted during a 4-hour period by 1 of the authors (CB). On day 0, horses were in an outdoor paddock (ie, not dusty), and counting began 30 minutes after the horses had been transported from pasture. The number of coughs was counted for 4 hours. Once horses were stabled, the number of coughs was counted for 15-minute intervals throughout the 4-hour period to determine the amount of time necessary to accurately assess cough frequency. When horses were stabled, counting of the number of coughs began in the morning at least 30 minutes after provision of feed and cleaning of stalls.

Measurement of lung function—Lung function was evaluated by measurement of ΔPpl_{max}. An esophageal balloon[a] on the end of a 240-cm polyethylene catheter[b] was passed via the nares into the middle third of the esophagus. The catheter was secured in position by taping it to the horse's halter. The catheter was connected via a pressure transducer[c] to a portable physiograph.[d] Fluctuations in esophageal pressure, which are an accurate indication of pleural pressure,[10] were recorded for 20 consecutive breaths. The ΔPpl_{max} (ie, difference between peak inspiratory and peak expiratory pressure) was calculated for each breath. Swallows and sighs were not included. The transducer and physiograph were calibrated by use of a water manometer before each measurement period.

Endoscopy and mucus scoring—Xylazine hydrochloride (100 mg, IV) was administered to each horse. Approximately 5 minutes later, an endoscope connected to a videotape recorder was passed via the nasal cavity and pharynx into the trachea. A recording was made as the endoscope was passed down the trachea and into a bronchus. The endoscope was then wedged in a peripheral bronchus, and bronchoalveolar lavage was performed.

Videotape recordings of the trachea were subsequently assessed for the amount of mucus. The investigator who performed the assessment (SE) was familiar with endoscopy of airways in horses and was unaware of the horse's group or treatment. Mucus was scored by use of a scale (**Appendix**). The scale was similar to that used in other studies.[11,e]

Bronchoalveolar lavage—After the bronchoscope was wedged in an airway, three 100-mL aliquots of PBS solution were instilled into the airway and then aspirated. Aspirates were pooled and mixed. Total number of cells in the **bronchoalveolar lavage fluid (BALF)** was counted by use of a hemacytometer. Differential cell counts were made on 200 cells of a cytocentrifuge preparation stained with Wright-Giemsa. Total neutrophil counts were calculated from total and differential cell counts.

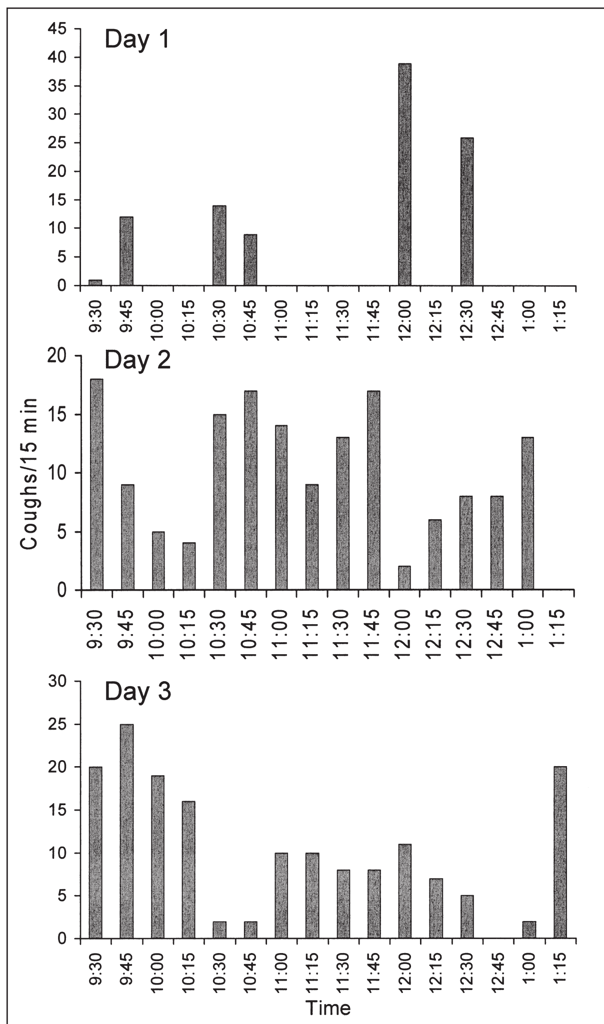

Figure 1—Number of coughs per 15-minute period in the horse with recurrent airway obstruction (RAO) that had the highest cough frequency during 3 days of stabling. Notice that coughing is paroxysmal on day 1 of stabling but becomes more regular on days 2 and 3.

Statistical analysis—Coefficient of variation for the number of coughs was calculated on the basis of counting periods of 15 minutes and 1 hour. Effects of stabling on cough frequency, mucus score, ΔPpl_{max}, and cytologic content of BALF were determined by use of a 2-way ANOVA with group and treatment as main effects. When main effects were significant ($P < 0.05$), means were compared by use of the Student-Newman-Keuls procedure. Relationships among mucus score, coughing, lung function, and BALF cells were evaluated by the Pearson product-moment or Spearman rank-order correlation, as appropriate. Effects of dexamethasone treatment were determined by use of a paired *t*-test or the Wilcoxon paired-sample test, as appropriate. Sensitivity and specificity of coughing as a clinical sign of mucus accumulation were also calculated. Statistical analysis was performed by use of a statistical computer program.[f]

Results

Amount of time for counting coughs—In RAO-affected horses, coughing was paroxysmal. Number of coughs per 15 minutes in the RAO-affected horse that coughed the most was determined (**Fig 1**). On the first day of stabling, that horse did not cough for long periods, and then it coughed in paroxysms. After 2 days in the stable, coughing became more evenly distributed over time. However, analysis revealed that on the first day of stabling, counting the number of coughs for periods of < 1 hour would not adequately evaluate cough frequency in such a horse. This was borne out by calculations of the coefficients of variation for cough frequency. The coefficient of variation was significantly greater when the number of coughs was counted for 15 minutes than when counted for 1 hour. Coefficient of variation for the number of coughs was the same when counted for 1 hour and 4 hours, and the number of coughs per hour did not differ significantly when calculated on the basis of counting for 1, 2, 3, or 4 hours (**Fig 2**).

In all control horses except 1, coughing was so infrequent that it would have been missed with any counting duration < 4 hours. In the control horse that coughed 40 times in 4 hours on day 1 of stabling, 27 coughs were during the first hour, and 13 were during one 15-minute period of the third hour. The horse never coughed during hours 2 or 4.

Effect of stabling on cough frequency, mucus score, ΔPpl_{max}, and cytologic content of BALF—In each RAO-affected horse, cough frequency increased with duration of stabling, but there was considerable variability in cough frequency among horses (**Fig 3**). One control horse coughed once per hour when initially transported from pasture, but coughing subsided in that horse when it was stabled. Four other control horses had an increase in cough frequency on day 1 of stabling, but in all but 1 of these horses, coughing subsided by day 3. In 1 of these horses, cough frequency was 10 coughs/h on day 1, whereas it was ≤ 5 coughs/h for days 2 and 3. When cough frequency was analyzed

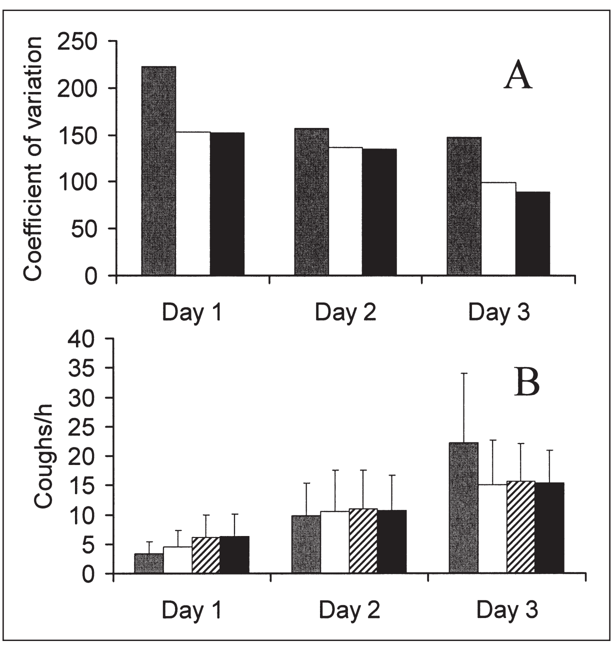

Figure 2—Coefficient of variation of cough frequency based on counting the number of coughs during 15-minute (dark gray bars), 1-hour (white bars), and 4-hour intervals (black bars; A) and mean ± SEM cough frequency of RAO-affected horses on days 1, 2, and 3 of stabling based on the number of coughs counted during 1 (dark gray bars), 2 (white bars), 3 (diagonal bars), or 4 (black bars) hours (B). We did not detect significant differences among the amount of time used for counting on each day.

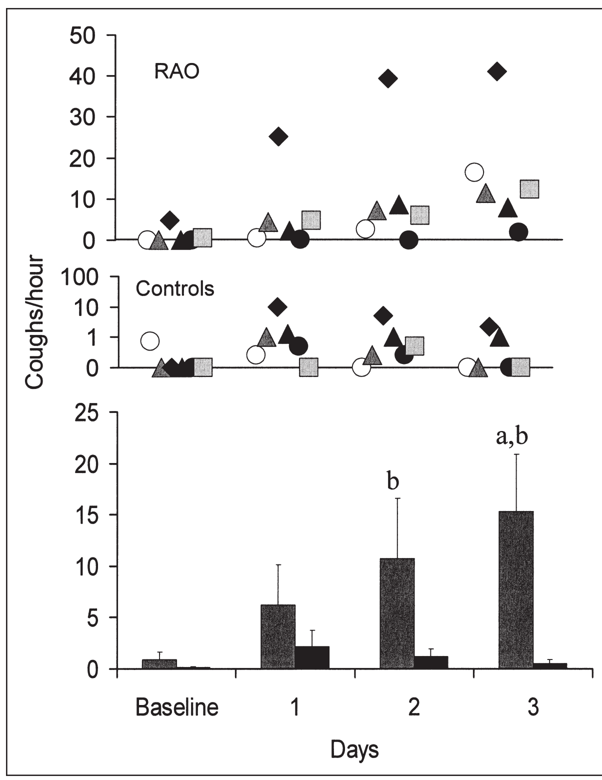

Figure 3—Cough frequency for individual horses 30 minutes after they had been transported from pasture (baseline) and after 1, 2, and 3 days of stabling for RAO-affected (top) and control horses (middle) as well as mean ± SEM values for 6 RAO-affected (gray bars) and 6 control (black bars) horses(bottom). In the top and middle graphs, each symbol represents a specific horse. Notice that data from control horses are plotted on a logarithmic scale because of the small increase in cough frequency in most of these horses. [a]Mean value for RAO-affected horses differs significantly ($P < 0.05$) from mean value for control horses during same time period. [b]Within RAO-affected horses, mean value differs significantly ($P < 0.05$) from mean baseline value.

by use of an ANOVA, there were significant effects of time and group. In RAO-affected horses, cough frequency was significantly increased from baseline on mdays 1, 2, and 3 of stabling. The RAO-affected horses coughed significantly more than control horses only on day 3.

Values for ΔPpl_{max}; cough frequency; and total cells, percentage of neutrophils, and total neutrophils in BALF were significantly affected by group and time after stabling. At baseline, these variables did not differ significantly between groups of horses. Values for all variables increased significantly during stabling in RAO-affected horses but not in control horses (**Table 1**). In the case of mucus score, there was a significant difference between groups, with RAO-affected horses having a higher score than control horses; however, there was not a significant effect of time or a significant interaction of time and group.

Relationships among cough frequency, mucus score, ΔPpl_{max}, and inflammation in RAO-affected horses—In RAO-affected horses in experiment 1, ΔPpl_{max}, mucus score, number of coughs per hour, total number of cells, total number of neutrophils, and percentage of neutrophils in BALF were all significantly correlated, except for ΔPpl_{max} and mucus score, which were correlated but not significantly ($P = 0.08$; **Table 2**). When data from control and RAO-affected horses were combined for experiment 1, mucus score, number of coughs per hour, and ΔPpl_{max} were significantly correlated with each other and with total number of cells, total number of neutrophils, and percentage of neutrophils in BALF.

Relationships among cough frequency, mucus score, and ΔPpl_{max} were determined (**Fig 4**). To increase the sample size, we included data from RAO-affected horses in experiment 1 and the pretreatment data from the horses in experiment 2. Coughing was always associated with an increase in mucus score, but the reverse was not true. Some horses with mucus scores up to 3 did not cough or coughed little. Each mucus score was associated with a wide range of cough frequencies, but the maximal number of coughs recorded became higher with each increase in mucus score. For example, horses with a mucus score of 3.0 had up to 15 coughs/h, whereas horses with a mucus score of 4 had up to 25 coughs/h. The ΔPpl_{max} began to increase once mucus score exceeded 1.0. Each mucus score was associated with a wide range of ΔPpl_{max}, but the greatest ΔPpl_{max} observed became higher with each increase in mucus score. However, some horses with mucus scores up to 3.0 did not have particularly high values for ΔPpl_{max}. There was considerable scatter in the relationship between coughing and ΔPpl_{max}.

Table 1—Mean ± SEM values of lung function, mucus score, cough frequency, and cytologic content of bronchoalveolar lavage fluid (BALF) for 6 horses affected by recurrent airway obstruction (RAO) and 6 control horses before (baseline) and after 3 days of stabling

	RAO-affected		Control	
Variable	Baseline	Day 3	Baseline	Day 3
ΔPpl_{max} (cm H_2O)	8.3 ± 2.3[a]	25.3 ± 6.0[b,c]	5.6 ± 1.1	5.1 ± 0.6[d]
Mucus score*[e]	2.2 ± 0.5	3.5 ± 3.4	1.8 ± 0.4	1.6 ± 0.5
Number of coughs/h	0.9 ± 0.9	15.3 ± 6.1[c]	0.1 ± 0.1	0.5 ± 0.4[d]
Total cells in BALF (log_{10} number of cells/µL)	1.95 ± 0.11[a]	2.63 ± 0.23[b,c]	1.76 ± 0.05	1.72 ± 0.11[d]
Percentage of neutrophils in BALF (%)	13.2 ± 8.7[a]	79.3 ± 12.1[b,c]	11.5 ± 8.9	25.4 ± 6.8[d]
Total number of neutrophils in BALF (log_{10} number of cells/µL)	0.77 ± 0.35[a]	2.49 ± 0.28[b,c]	0.39 ± 0.27	1.05 ± 0.19[d]

*Mucus score was assessed on a scale of 0 (no mucus) to 5 (profuse amounts of mucus).
[a,b]Within RAO-affected horses, mean values with different superscript letters differ significantly ($P < 0.05$) between time periods. [c,d]Within day 3, mean values with different superscript letters differ significantly ($P < 0.05$) between RAO-affected and control horses. [e,f]Mucus score differed significantly between group, but there was no significant effect of time.
ΔPpl_{max} = Maximal change in pleural pressure during tidal breathing.

Table 2—Correlations among ΔPpl_{max}, mucus score, cough frequency, and cytologic content of BALF

	Variable					
Variable	ΔPpl_{max} (cm H_2O)	Mucus score	Number of coughs/h	Total number of cells in BALF (log_{10} number of cells/µL)	Percentage of neutrophils in BALF (%)	Total number of neutrophils in BALF (log_{10} number of cells/µL)
ΔPpl_{max} (cm H_2O)	NA	0.52 (0.08)	0.85 (< 0.001)	0.74 (0.009)	0.79 (0.004)	0.78 (0.005)
	NA	0.55 (0.005)	0.80 (< 0.001)	0.80 (< 0.001)	0.76 (< 0.001)	0.86 (< 0.001)
Mucus score	0.52 (0.08)	NA	0.77 (0.002)	0.74 (0.006)	0.76 (0.005)	0.73 (0.009)
	0.55 (0.005)	NA	0.78 (0.005)	0.82 (< 0.001)	0.56 (0.007)	0.64 (0.001)
No.of coughs/h	0.85 (< 0.001)	0.77 (0.002)	NA	0.86 (< 0.001)	0.83 (< 0.001)	0.86 (< 0.001)
	0.80 (< 0.001)	0.78 (0.005)	NA	0.75 (< 0.001)	0.70 (< 0.001)	0.74 (< 0.001)

Values in the top line of each box represent data for RAO-affected horses at baseline and after 3 days of stabling, whereas values in the bottom line of each box represent data for RAO-affected and control horses at baseline and after 3 days of stabling. Values in parentheses are *P* values.
NA = Not applicable.

Relationships of ΔPpl_{max}, mucus score, and cough frequency to BALF neutrophil percentage and to total neutrophil count in BALF (plotted on a logarithmic scale) were determined (**Fig 5**). We included data from RAO-affected horses in experiment 1 and the pretreatment data from horses in experiment 2. Accumulations of mucus were observed when there were > 10% neutrophils or > 100 neutrophils/µL of BALF. Coughing and an increase in ΔPpl_{max} were observed once neutrophils in BALF exceeded 20% or 100 to 200 cells/µL.

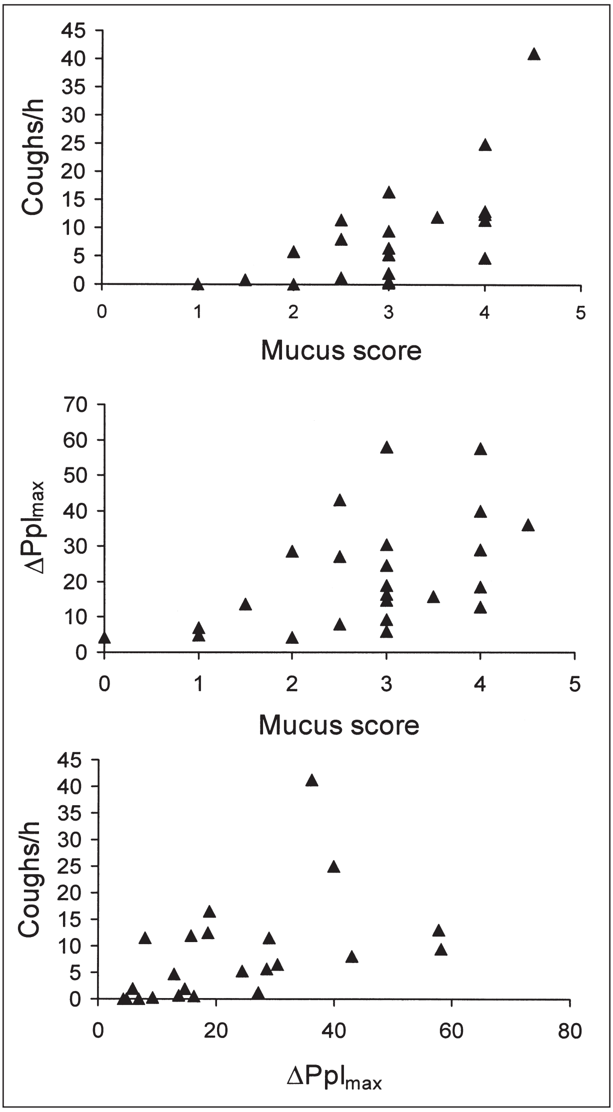

Figure 4—Relationships among mucus score, cough frequency, and maximal change in pleural pressure during tidal breathing (ΔPpl_{max}) for 6 RAO-affected horses at baseline and day 3 in experiment 1 and 12 RAO-affected horses after 3 days of stabling but before administration of dexamethasone in experiment 2.

Sensitivity and specificity of coughing and mucus accumulation as clinical signs—In RAO-affected horses, coughing was a highly specific and highly sensitive sign of a mucus score > 1.0, ΔPpl_{max} > 6 cm H_2O, > 100 neutrophils/µL of BALF, and > 20% neutrophils in BALF (**Table 3**). A mucus score of > 1.0 was a sensitive indicator of ΔPpl_{max} > 6 cm H_2O, > 100 neutrophils/µL of BALF, and > 20% neutrophils in BALF. It was also a specific indicator of > 100 neutrophils/µL of BALF. However, it was a less specific indicator of ΔPpl_{max} > 6 cm H_2O and > 20% neutrophils in BALF. Sensitivity and specificity were similar when RAO-affected horses were considered alone or in combination with control horses.

Effect of dexamethasone—Dexamethasone treatment significantly decreased ΔPpl_{max}, mucus score, cough frequency, clinical score, and percentage of neutrophils in BALF (**Fig 6**; Table 3). There was a non-

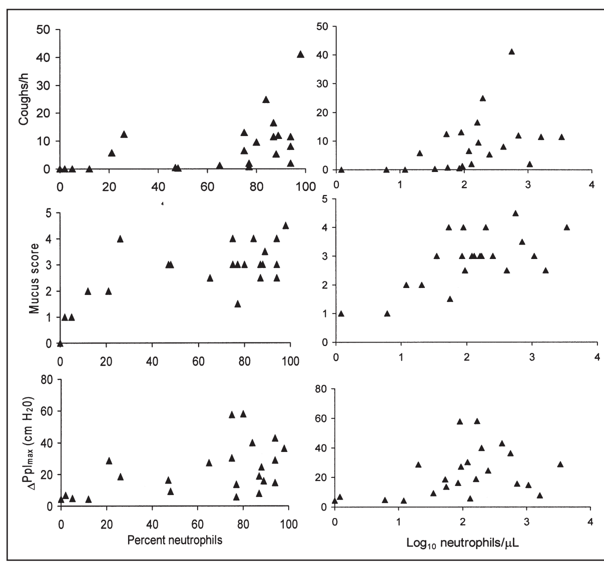

Figure 5—Mucus score, cough frequency, and ΔPpl_{max} in relation to neutrophil percentage (left column) and total neutrophil count (right column) in bronchoalveolar lavage fluid. Data are for 6 RAO-affected horses at baseline and day 3 in experiment 1 and 12 RAO-affected horses after 3 days of stabling but before administration of dexamethasone in experiment 2.

Table 3—Sensitivity and specificity of coughing and mucus score as clinical signs

		Mucus score > 1.0		ΔPpl_{max} > 6 cm H_2O		> 100 neutrophils/µL of BALF		> 20% neutrophils in BALF	
Clinical sign	Variable	RAO-affected horses	All horses	RAO-affected horses	All horses	RAO-affected horses	All horses	RAO-affected horses	All horses
Coughing (yes/no)	Sensitivity	0.95	0.88	0.95	0.92	0.95	0.88	1.00	0.91
	Specificity	1.00	0.92	1.00	0.92	1.00	0.90	1.00	0.91
Mucus score >1.0	Sensitivity	NA	NA	0.95	0.88	1.00	0.92	1.00	0.91
	Specificity	NA	NA	0.67	0.67	1.00	0.80	0.75	0.79

NA = Not applicable.

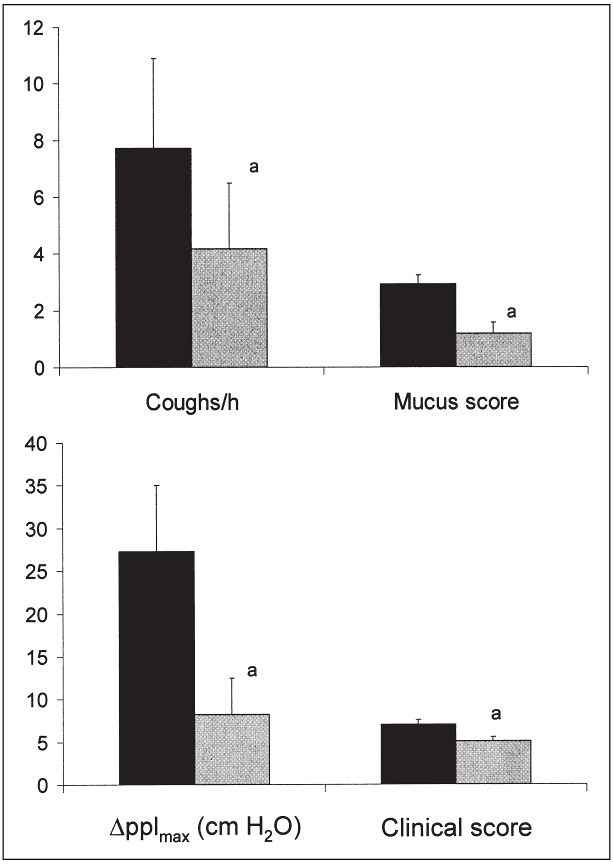

Figure 6—Mean ± SEM mucus score, cough frequency, clinical score, and ΔPpl_{max} before (black bars) and after (light gray bars) dexamethasone treatment in 12 RAO-affected horses. [a]Mean value differs significantly ($P < 0.05$) from mean value before treatment.

Table 4—Mean ± SEM values for cytologic examination of BALF obtained from 12 RAO-affected horses after 3 days of stabling before treatment (baseline) and after 7 days of dexamethasone administration (0.1 mg/kg, IV, q 24 h). [a,b]Mean values with different superscript letters differ significantly ($P < 0.05$).

Variable	Baseline	Dexamethasone
Total number of cells ($\log_{10}$ number of cells/µL)	2.38 ± 0.21	2.15 ± 0.26
Percentage of neutrophils (%)	73.6 ± 9.3[a]	50.6 ± 9.4[b]
Total number of neutrophils ($\log_{10}$ number of cells/µL)	2.22 ± 0.26	1.81 ± 0.28

signficant ($P = 0.058$) decrease in number of neutrophils in BALF, and total cells in BALF did not decrease significantly after dexamethasone treatment (**Table 4**). Responses of specific horses to dexamethasone administration were determined (**Fig 7**). The ΔPpl_{max} decreased in all but 1 horse. In that horse, mucus score increased, and clinical score remained at the maximum value, but cough frequency decreased slightly. Cough frequency did not always decrease in parallel with mucus score or ΔPpl_{max}. For example, in the 3 horses with the highest cough frequency (16, 10.5, and 9.3 coughs/h) after dexamethasone treatment, mucus score was 1.5, 0.0, and 2.0, respectively, and ΔPpl_{max} was 3.1, 7.5, and 5.6 cm H_2O, respectively. However, in the 3 horses that stopped coughing, mucus score was ≤ 1.0, and ΔPpl_{max} was ≤ 6 cm H_2O.

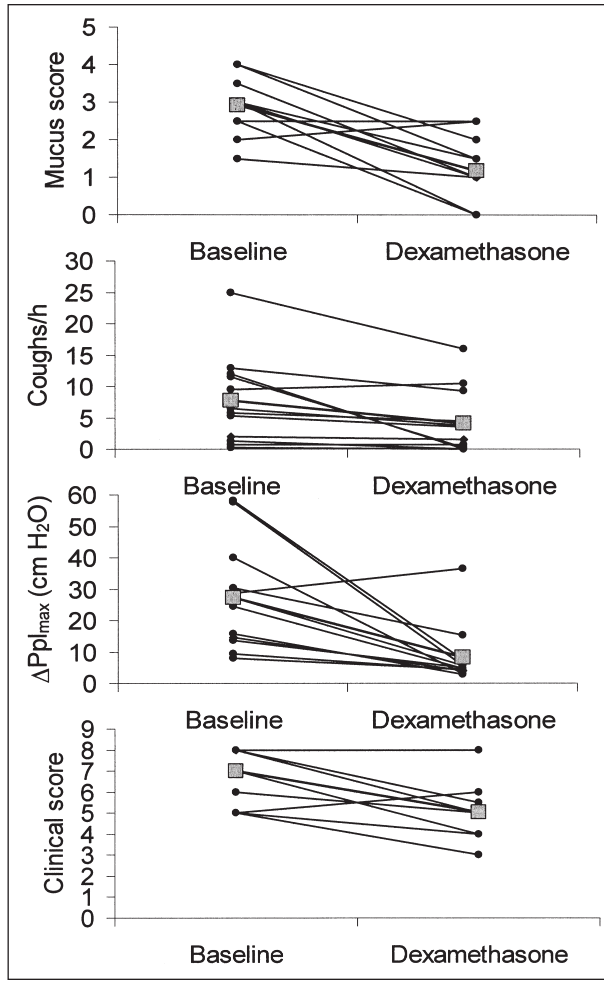

Figure 7—Mucus score, cough frequency, clinical score, and ΔPpl_{max} in individual RAO-affected horses after stabling for 3 days but before (baseline) and after administration of dexamethasone (0.1 mg/kg, IV, q 24 h for 7 days). Gray squares indicate mean values for each group of horses.

Discussion

Clinical signs of RAO include respiratory distress that is a consequence of airway obstruction, coughing, and mucus accumulation within the airways that is visible endoscopically. Although it is clear from the study reported here and others[12-14]that airway obstruction (indicated here by an increase in ΔPpl_{max}) is associated with inflammation in RAO-affected horses, to our knowledge, the natural history of coughing and its association with inflammation, mucus, and obstruction has not been described. Results of the study reported here clearly indicate that coughing is strongly associated with airway inflammation. After 3 days of stabling, RAO-affected horses coughed and had substantial accumulations of inflammatory cells in the airways, whereas control horses had neither of these (Table 1). Furthermore, cough frequency was significantly correlated with total cell count, percentage of neutrophils, and neutrophil count in BALF (Table 2), and coughing was a sensitive and specific indicator of > 100 neutrophils/µL of BALF or > 20% neutrophils in BALF (Table 3). An inflammation-induced increase in the sensitivity of cough receptors is a likely reason for the association between coughing and inflammation.[3,15] There also was a strong correlation between cough frequency and mucus score, suggesting that accumulation of mucus may have provided a physical stimulus to trigger coughing. Mucus in RAO-affected horses that are stabled has increased viscoelasticity and becomes more difficult to clear by mucociliary action and coughing.[16] Failure of coughing to clear viscid mucus may have necessitated an increase in cough frequency.

To investigate coughing, it was necessary to define the amount of time spent counting the number of coughs and conditions necessary for accurate determination of cough frequency. Because RAO is a response to inhalation of organic dust,[7] standardization of environmental conditions under which coughing was counted was essential. This was accomplished by counting coughs when the horses were undisturbed after cleaning of stalls and provision of feed had been completed. Accurate assessment of cough frequency was also made difficult by the paroxysmal nature of coughing (Fig 1). Analysis of the coefficient of variation of cough frequency in RAO-affected horses revealed that there was significantly more variability in cough frequency when it was based on 15-minute periods than when it was based on 1-hour periods (Fig 2). However, there was not a significant difference in variability between counts conducted during 1- and 4-hour periods. In addition, cough frequency based on 1-, 2-, 3-, or 4-hour counting periods did not differ significantly. Therefore, the number of coughs should be counted for at least 1 hour to quantify cough frequency in RAO-affected horses.

The study reported here also revealed that for clinical conditions, coughing could be used as a surrogate marker of increased ΔPpl_{max} in RAO-affected horses. There was a significant correlation between cough frequency and ΔPpl_{max}, and coughing was a highly specific and highly sensitive indicator of $\Delta Ppl_{max} > 6$ cm H_2O. It has been documented that although ΔPpl_{max} is not a direct measure of airway obstruction, it is an excellent overall indicator of the severity of lung dysfunction in RAO-affected horses,[17] and the major dysfunction in horses with RAO is airway obstruction.[7]

In another study[13] conducted by our laboratory group, we reported increased amounts of mucin glycoproteins in BALF collected from RAO-affected horses that were stabled. When these horses were returned to pasture, mucin glycoprotein concentrations decreased but not to amounts seen in control horses maintained continously at pasture. Results of the study reported here confirmed persistent accumulation of mucus in RAO-affected horses even when they are at pasture. Mucus scores were significantly greater in RAO-affected horses than in control horses, but there was not a significant effect attributable to stabling. Accumulation of mucoid secretions in horses with RAO is a result of increased expression of the principal mucin gene in equine airways (ie, eqMUC5AC)[18] and characteristics of mucus that decrease the ability to clear it from the airways.[16]

Correlation analysis indicated that mucus accumulation was significantly associated with inflammation. Total cell count, total number of neutrophils, and percentage of neutrophils in BALF were all significantly correlated with mucus score in RAO-affected horses. Furthermore, more than a few blobs of mucus (ie, mucus score > 1.0) was a highly sensitive and specific indicator of > 100 neutrophils/µL of BALF. The situation with regard to neutrophil percentage differed slightly. Whereas a mucus score > 1.0 was a sensitive indicator of > 20% neutrophils in BALF, it was somewhat less specific. This is because horses can have > 20% neutrophils and a low total cell count in BALF and yet apparently not have a sufficiently high total number of neutrophils to initiate increased production or secretion of mucus.

When examining the relationship between mucus score and cough frequency, it was obvious that horses that had a mucus score of ≤ 2 coughed infrequently. For scores > 2, the greater the frequency of coughing, the higher the mucus score. However, as mentioned previously, horses could have mucus scores up to 3.0 and cough little. Although the number of horses in our study are small, this observation supports those of larger field studies[5,6] of racehorses in which it was observed that horses could have mucus and not cough but always had mucus accumulations when they were coughing. It also is clear that as airway obstruction becomes worse (ΔPpl_{max} increases), mucus score increases. However, some horses had a high mucus score with a low ΔPpl_{max}. For this reason, a mucus score > 1.0 was not a specific indicator of increased ΔPpl_{max}.

It was somewhat surprising that when the number of coughs was counted for 4 hours, we discerned a transient increase in cough frequency in some control horses when they were first stabled. In these horses, coughing was so infrequent that it would have been missed during counting periods of < 4 hours. Although the reason for this transient increase in cough frequency is currently unknown, it is possible that it represents the onset of inflammation that is then turned off by an increased production of anti-inflammatory cytokines and a reduced production of proinflammatory cytokines. Such a response has been observed in mice challenge exposed with particulate antigen.[19]

Efficacy of dexamethasone for the relief of airway obstruction in horses with RAO is accepted,[9,20] and we confirmed it in the study reported here. Seven days of treatment with dexamethasone (0.1 mg/kg, IV, q 24 h) reduced ΔPpl_{max} in 11 of 12 RAO-affected horses. The nonresponding horse had severe RAO (clinical score, 8), and examination of a recent lung biopsy specimen obtained from this horse revealed extensive deposition of collagen around the small airways. The reduction in cough frequency that accompanied dexamethasone treatment can be explained by several mechanisms, including a decrease in the sensitivity of the cough reflex[15] and less accumulation of mucus, the latter being indicated by the significantly reduced mucus score. Similar beneficial effects of glucocorticoids on coughing have been observed in humans with asthma[15] or eosinophilic bronchitis[21] and in ozone-challenged rats.[22] In the latter study, fluticasone treatment alleviated airway inflammation and metaplasia of mucous cells. Synthesis and secretion of mucus are increased by mediators, especially neutrophil elastase, that are released in response to neutrophilic inflammation such as occurs in RAO. Glucocorticoids down regulate the expression of proinflammatory genes and production of inflammatory mediators; therefore, it is not surprising that there is a reduction in mucus score. However, it is also clear from our data that cough frequency is not solely determined by the accumulation of mucus, because 3 horses with the highest cough frequencies did not have particularly high mucus scores.

In the investigation of coughing in RAO-affected horses reported here, we documented that it is necessary to count the number of coughs for 1 hour to accurately assess cough frequency, increased cough frequency during stabling is associated with increased numbers of neutrophils in BALF, and coughing is a sensitive and specific indicator of airway obstruction and inflammation. Furthermore, although coughing horses have mucoid accumulations in their airways, such accumulations are not necessarily associated with coughing and airway obstruction. Finally, we determined that cough frequency decreases when RAO-affected horses are treated with dexamethasone.

[a]Trojan condom, Carter-Wallace Inc, NY.
[b]PE240, Becton, Dickinson & Co, Franklin Lakes, NJ.
[c]Model DP/45-35, Validyne, Northridge, Calif.
[d]Dash model II, Astro-Med, West Warwick, RI.
[e]*Dieckmann MP. Zur Wirksamkeit von Ambroxolhydrochlorid (Mukovent) bei lungenkranken Pferden - Klinische, Funktionelle und Zytologische Untersuchungen. PhD Thesis, Tieraerztliche Hochschule, Hannover, Germany, 1987.*
[f]SigmaStat, SPSS Science, Chicago, Ill.

Appendix

System used to score mucus observed during endoscopy in the airways of horses with recurrent airway obstruction

Score	Description
0	No visible mucus
1	Single small blobs of mucus
2	Multiple blobs of mucus partially confluent
3	Mucus confluent in a stream in the ventral aspect of the lumen or multiple large blobs around the circumference of the airway
4	Large pool of mucus in the ventral aspect of the airway
5	Profuse amounts of mucus occupying more than 25% of tracheal lumen

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