Clinical signs, evaluation of bronchoalveolar lavage fluid, and assessment of pulmonary function in horses with inflammatory respiratory disease

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Objective—To evaluate the association among clinical signs, results of cytologic evaluation of bronchoalveolar lavage (BAL) fluid, and measures of pulmonary function in horses with inflammatory respiratory disease.

Animals—9 healthy horses, 5 horses with inflammatory airway disease (IAD), and 9 horses with chronic obstructive pulmonary disease (COPD).

Procedures—Clinical examination, lung function tests, and BAL were performed on each horse.

Results—Standard lung mechanics of horses with exacerbated COPD differed significantly from those of healthy horses; however, there were few differences among horses with IAD, horses with COPD during remission, and healthy horses. Most variables for forced expiration (FE) in horses with COPD or IAD differed significantly from those for healthy horses. Results of clinical examination had low to moderate sensitivity and predictive values for a diagnosis of COPD (range, 67 to 80%). Results of FE tests had high sensitivity, specificity, and predictive values for a diagnosis of COPD (79 to 100%), and results of standard lung mechanics tests had low sensitivity and predictive values (22 to 69%). Percentage of neutrophils in BAL fluid was highly sensitive (100%) but moderately specific (64%) for a diagnosis of COPD.

Conclusions and Clinical Relevance—Clinical examination is moderately accurate for establishing a diagnosis of COPD. Forced expiration tests can specifically detect early signs of airway obstruction in horses with COPD and IAD that may otherwise be inapparent. Cytologic evaluation of BAL fluid allows early detection of inflammatory respiratory disease, but it is not specific for COPD. (*Am J Vet Res* 2001;62: 538–546)

Chronic obstructive pulmonary disease (COPD), commonly called heaves, is considered the major cause of chronic respiratory tract disease in horses.[1,2] Chronic coughing, purulent nasal discharge, increased respiratory efforts, and exercise intolerance characterize the syndrome.[3] It is believed that COPD in horses is an allergic response to environmental dusts and molds that are particularly abundant in hay and straw,[4] and COPD has many similarities to asthma in humans.[5] Clinical signs of COPD are exacerbated (crisis) when susceptible horses are exposed to organic dusts for a period of a few days, which is often the case when horses are housed in barns. Clinical signs and lung function usually return to baseline values within 1 week after being housed in a pasture (remission).[6,7]

Inflammatory airway disease (IAD) is a mild form of respiratory tract disease commonly encountered in young performance horses.[8,9] Clinical signs of IAD include coughing, increased respiratory secretions, and decreased performance. The pathogenesis of IAD has not been established; however, bacteria commonly are isolated from tracheal exudate.[10,11] Viral infections and exposure to environmental allergens also have been suggested.[8,11,12]

A presumptive diagnosis of COPD or IAD may be made on the basis of medical history and clinical signs. Several tests can help confirm a diagnosis, including cytologic evaluation of **bronchoalveolar lavage (BAL)** fluid and lung function tests. The BAL fluid of healthy horses and horses with COPD fed moldy hay is characterized by a severe increase in the percentage of neutrophils.[4,13,14] However, only horses with COPD have changes in lung function consistent with airway obstruction. Values for BAL fluid of horses with COPD during remission usually are within acceptable limits.[15] The BAL fluid of horses with IAD may contain increased percentages of neutrophils, eosinophils, or mast cells[8,16]; however, standard lung function tests do not detect airflow obstruction.[17] Therefore, cytologic evaluation of BAL fluid alone does not enable clinicians to differentiate healthy horses exposed to moldy hay from horses with IAD or COPD during crisis. In addition, lung function testing does not discriminate among healthy horses, horses with IAD, and horses with COPD during remission.

During COPD crisis, bronchoconstriction, edema of airway wall, and accumulation of secretions in the airways result in airflow obstruction and stiffening of the lungs. These structural changes translate into functional changes such as increased **pulmonary resistance (R_L)** and decreased **dynamic compliance (C_{dyn})**.[6,18] However, measurements of R_L and C_{dyn} are not sufficiently sensitive to detect COPD during its early stages,

Received Feb 9, 2000.
Accepted May 19, 2000.
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Supported by the state of Indiana and the Purdue University School of Veterinary Medicine Research account. Funded by the total wager tax.
Presented in part at the 17th Annual Veterinary Medical Forum of the American College of Veterinary Internal Medicine, Chicago, June 10, 1999.
The authors thank Donna Griffey and Bianca Zenor for technical assistance.

COPD in horses in clinical remission, or IAD in horses.[3,7,17] In humans, **forced expiration (FE)** constitutes 1 of the most useful and commonly used pulmonary function tests for the early detection of airway disease.[19] The FE technique requires patients to inhale until they have achieved total lung capacity and to then exhale as hard and completely as possible while expiratory flow and volume are recorded. Such maneuvers have been performed in domestic animals, including horses, but they demand the use of general anesthesia to avoid interference of conscious respiratory movements with emptying of the lungs.[20] In another study,[21] we developed a minimally invasive FE method for use in horses that were sedated but not anesthetized. We documented that peripheral airflow obstruction was detectable through the analysis of the **forced expiratory flow-volume (FEFV)** curves. The purpose of the study reported here was to assess the association among clinical signs, results of cytologic evaluation of BAL fluid, and results of lung function tests in horses with inflammatory respiratory disease.

Materials and Methods

Animals—Twenty-three horses were included in the study. Horses were allocated to 4 groups: healthy (control), IAD, COPD during remission, and COPD during crisis. Seventeen horses belonged to the Purdue Veterinary Teaching Hospital (9 healthy, 4 IAD, 3 COPD during remission, and 1 COPD during crisis), and 6 were client-owned horses (1 IAD, 2 COPD during remission, and 3 COPD during crisis). Healthy horses comprised 6 mares and 3 geldings (body weight [mean ± SD], 480 ± 43 kg; age, 8.3 ± 4.3 years). None had a history of chronic or recurrent respiratory tract disease. At the time of the study, abnormalities were not detected during physical examination and endoscopic examination of the airways. The other groups comprised 9 horses with COPD (5 mares and 4 geldings; body weight, 430 ± 74 kg; age, 19.0 ± 3.5 years) and 5 horses with IAD (4 mares and 1 gelding; body weight, 465 ± 52 kg; age, 16.6 ± 9.0 years).

Diagnosis of COPD was made on the basis of a history of chronic (> 2 years) coughing, intermittent mucopurulent nasal discharge, and recurrent episodes of increased respiratory efforts when horses were fed hay. Each horse with COPD had been examined at our veterinary teaching hospital at least once during disease exacerbation and remission. Clinical examinations were performed before lung function testing by a single clinician (CDC) who was unaware of each horse's medical history and group assignment. Clinical examination included clinical scoring, lung function testing, and cytologic evaluation of BAL fluid. At the time of the study, 4 horses with COPD were in crisis, and 5 were in remission. Clinical scores were assigned by use of a scale (range, 0 to 25) described by Tesarowski et al.[7] A COPD crisis was defined as a clinical score ≥ 12, and COPD remission was a clinical score ≤ 6. Diagnosis of IAD was made on the basis of a history of long-term (> 6 months) coughing and endoscopic evidence of an increased amount of mucopurulent respiratory secretions observed at least twice during the preceding 6 months. None of the horses with IAD had a history of increased respiratory efforts when housed in barns and fed hay.

An institutional animal care and use committee approved all procedures. Owners signed an informed consent form after reviewing a document explaining the purpose and protocol of the study.

Lung mechanics during spontaneous breathing—An airtight mask was fitted around the nose of each horse. Esophageal pressure was measured by means of a balloon catheter (internal diameter, 4.8 mm; outside diameter, 6.4 mm; 240 cm in length) advanced to the midthorax and connected to a pressure transducer.[a] The other side of the pressure transducer was connected to the mask via a similar catheter. **Transpulmonary pressure (P_L)** was defined as the difference between mask pressure and esophageal pressure. The port of the mask held a pneumotachometer[b] coupled with a pressure transducer[c] that generated a signal proportional to airflow. Output signals from the pressure transducers were recorded simultaneously by computer software.[d] Ten representative breaths were selected, and the mean value was determined for data analysis. Variables measured included **maximum change in transpulmonary pressure (ΔP_{Lmax})**, **tidal volume (V_T)**, **inspiratory time (Ti)**, **expiratory time (Te)**, **peak inspiratory flow rate (PIF)**, **peak expiratory flow rate (PEF)**, **breathing frequency (f)**, and **minute ventilation (V)**. Values for R_L and C_{dyn} were computed in accordance with the method described by Amdur and Mead.[22] Signals from pressure and flow transducers were phase-matched. Calibration of flow and P_L was performed before and after each experiment by use of a 3-L calibrated syringe[e] and a water manometer,[f] respectively.

Assessment of forced expiration—The method and apparatus have been described in detail elsewhere.[21] In summary, the mask was removed after collection of data to assess standard lung function, and each horse was sedated with a combination of detomidine hydrochloride (0.03 mg/kg of body weight, IV) and butorphanol tartrate (0.02 mg/kg, IV). A nasotracheal tube (internal diameter, 20 mm) was then advanced into the proximal third of the trachea. The nasotracheal tube was connected to a 3-way valve[g] with ports leading to a ventilator[h] or to the FE valve[i] of the vacuum reservoir (1,433 L, –220 cm H_2O). The esophageal catheter and associated transducer were used to measure P_L, as described previously. Initially, the FE valve was in a closed position. The horse was mechanically ventilated (V_T, 6 L; $\dot{V}$, 48 to 54 L/min) to maintain end-tidal CO_2 < 40 mm Hg, using an oxygen-air mixture (fractional concentration of oxygen > 70%). Then, the lungs were manually inflated to total lung capacity, defined as P_L = 30 cm H_2O. The 3-way valve was turned in the direction of the pressure reservoir. Airways were suddenly exposed to the vacuum reservoir by opening the FE valve, inducing FE. During FE, the **instantaneous pressure change (dP_r)** was measured at a central position within the reservoir. Data were collected at 100 Hz and stored in a computer. There is a linear relationship between **expired lung volume (dV_L)** and dP_r that can be calculated by using the following equation:

$$dV_L = 845.3\ (dP_r/P_r)$$

where P_r = initial reservoir pressure. This equation was used to estimate the forced expiratory volume as a function of time. Instantaneous flow rate (dV_L/dt) was calculated as the volume of air leaving the lungs during 1 sample collection interval in the digital data acquisition system. Combining data for flow versus time and volume versus time, we generated FEFV curves. Analysis of the curves yielded **forced vital capacity (FCV)**; **forced expiratory volume at 1, 1.3, 1.5, 1.7, 2, 2.5, and 3 seconds (FEV_x)**; **forced expiratory flow at 75, 80, 85, 90 and 95% of exhaled vital capacity ($FEF_{x\%}$)**; and **mean end-expiratory flow ($FEF_{75–95\%}$)**. The maneuver was repeated until 3 acceptable and repeatable FEFV curves were obtained in each horse. The FE variables were measured from the curve that provided the largest sum for forced vital capacity plus forced expiratory volume at 1 second.

Bronchoalveolar lavage—The procedure was performed after completion of the pulmonary function tests while the

horses remained in stocks and were still sedated. A flexible videoendoscope (200 cm in length, 9 mm in diameter) was passed through the nasotracheal tube and advanced until wedged into a caudodorsal airway. Coughing was prevented by spraying airways with 0.2% lidocaine solution as the endoscope was advanced into the respiratory tract. A 250-ml bolus of sterile saline (0.9% NaCl) solution was infused under pressure through the endoscope biopsy channel. The BAL fluid was aspirated immediately into a container, using a suction pump, and the container was placed on ice. Fluid samples were processed within 20 minutes after collection and evaluated by a clinical pathologist (DBD) who was unaware of each horse's history and group assignment. Total nucleated cell counts were determined manually, using a hemocytometer. Cytologic specimens were prepared by centrifugation and processed with Wright's stain. Differential cell counts were determined by examination of 500 leukocytes/slide.

Correlation among variables—Associations among clinical signs, results of lung function tests, and results of cytologic examination of BAL fluid were assessed by use of Pearson correlation coefficients.

Evaluation of diagnostic tests—The usefulness of clinical examination, assessment of lung function, and cytologic evaluation of BAL fluid for establishing a diagnosis of COPD was evaluated by calculating sensitivity, specificity, and predictive values for clinical score, ΔP_{Lmax}, R_L, C_{dyn}, $FEF_{95\%}$, $FEF_{75-95\%}$, and $FEV_{1.5}$:FVC as well as neutrophil count and percentage of neutrophils in BAL fluid. Sensitivity was defined as the number of horses with COPD that had a positive test result divided by the total number of horses with COPD.[23] Specificity was defined as the number of horses that did not have COPD that had a negative test result divided by the total number of horses that did not have COPD. Positive-predictive value was defined as the number of horses with COPD that had a positive test result divided by the total number of horses that had a positive test result. Negative-predictive value was defined as the number of horses that did not have COPD that had a negative test result divided by the total number of horses that had a negative test result. The criterion-referenced standard for diagnosis of COPD was assessment of historical and clinical criteria, as described previously. A diagnostic test result was considered positive when the value was outside the 95% confidence interval, as determined on the basis of data generated from the healthy horses.

Statistical analysis—Differences among groups for variables of cytologic and lung function tests were evaluated by use of an ANOVA. Kruskal-Wallis tests were used to compare groups when variables were not normally distributed (FEV_1, $FEF_{75\%}$, $FEF_{80\%}$, $FEF_{85\%}$, $FEF_{90\%}$). Appropriate post-hoc tests were used for pairwise comparison of means when indicated. Results were expressed as mean ± SD. Significance was defined as $P < 0.05$.

Results

Clinical score—Horses with COPD during crisis had significantly higher mean ± SD clinical scores (15.0 ± 2.2; range, 13 to 18) than healthy horses (1.3 ± 1.6; range, 0 to 6) and horses with IAD (3.6 ± 1.9; range, 1 to 5). Clinical scores were significantly higher in horses with IAD and horses with COPD during remission (3.8 ± 1.6), compared with scores for healthy horses; however, there was overlap among data ranges.

Lung mechanics during tidal breathing—Data were summarized for test results (**Table 1**). Horses with COPD during crisis had significantly higher ΔP_{Lmax} than other horses and also had significantly greater R_L than healthy horses and horses during remission. In addition, R_L in horses with IAD was significantly higher than in healthy horses. Values for C_{dyn} were significantly lower in horses with COPD during crisis than in healthy horses. The V_T was significantly less in horses with COPD during crisis than in healthy horses. Horses with COPD during crisis had a significantly greater f than healthy horses and horses with IAD. The Ti was significantly shorter in horses with COPD during crisis than in healthy horses and horses with IAD. The PEF was significantly higher in horses with COPD during remission than in healthy horses and horses with IAD.

Forced expiration—The FEFV curves from 1 representative horse in each group were plotted (**Fig 1**). Data for FE were summarized (**Table 2**). Values for FEV_1 were significantly lower in horses with COPD during crisis than in healthy horses, but FVC and FEV_x at 1.3, 1.5, 1.7, 2, 2.5, and 3 seconds were not signifi-

Table 1—Value (mean ± SD) for variables of lung mechanics during tidal breathing for healthy horses, horses with inflammatory airway disease (IAD), horses with chronic obstructive pulmonary disease (COPD) during remission, and horses with COPD during crisis

			COPD	
Variable	Healthy (n = 9)	IAD (n = 5)	Remission (n = 5)	Crisis (n = 4)
ΔP_{Lmax} (cm H_2O)	5.5 ± 1.6	8.0 ± 5.0	5.7 ± 1.7	19.8 ± 9.8[a,b,c]
R_L (cm H_2O/L per s)	0.49 ± 0.30	1.06 ± 0.36[a]	0.61 ± 0.17	1.39 ± 0.89[a,c]
C_{dyn} (L/cm H_2O)	2.26 ± 0.60	2.01 ± 1.42	2.16 ± 1.00	0.96 ± 0.45[a]
Ti (s)	2.38 ± 0.29	2.20 ± 0.91	1.64 ± 0.38[a]	1.29 ± 0.46[a,b]
Te (s)	2.99 ± 0.88	2.55 ± 1.00	1.93 ± 0.38[a]	1.79 ± 0.59[a]
PIF (L/s)	4.40 ± 1.07	3.63 ± 0.97	5.19 ± 1.41	5.43 ± 1.86
PEF (L/s)	3.88 ± 1.03	3.35 ± 0.93	5.92 ± 1.69[a,b]	4.47 ± 1.56
V_T (L)	6.26 ± 1.51	5.41 ± 1.70	5.64 ± 0.94	4.48 ± 0.72[a]
f (breaths/min)	11.7 ± 2.6	14.2 ± 5.0	17.3 ± 2.8[a]	21.1 ± 6.2[a,b]
$\dot{V}_E$ (L/min)	71.2 ± 15.7	72.1 ± 19.9	98.3 ± 29.2	92.0 ± 24.7

[a]Value is significantly ($P < 0.05$) different from value for healthy horses. [b]Value is significantly ($P < 0.05$) different from IAD value. [c]Value is significantly ($P < 0.05$) different from COPD remission value.

ΔP_{Lmax} = Maximum change in transpulmonary pressure. R_L = Pulmonary resistance. C_{dyn} = Dynamic lung compliance. Ti = Inspiratory time. Te = Expiratory time. PIF = Peak inspiratory flow rate. PEF = Peak expiratory flow rate. V_T = Tidal volume. f = Breathing frequency. $\dot{V}_E$ = Minute ventilation.

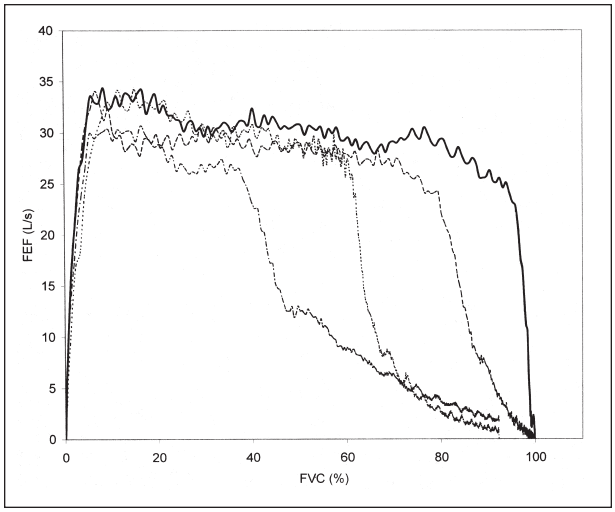

Figure 1—Forced expiratory flow-volume curves obtained for a healthy horse (—), a horse with inflammatory airway disease (- - -), a horse with chronic obstructive pulmonary disease (COPD) during remission (….), and a horse with COPD during crisis (– · –). FEF = Forced expiratory flow. FVC = Forced vital capacity.

cantly different among groups. However, significant differences were observed in FEV_x:FVC among groups. Among FEV_x:FVC values, $FEV_{1.5}$:FVC and $FEV_{1.7}$:FVC were significantly lower among horses with COPD, compared with values for horses with IAD, and healthy horses. The $FEF_{95\%}$ was significantly lower in horses with COPD, compared with values for healthy horses ($P < 0.001$) and horses with IAD ($P < 0.006$). The $FEF_{95\%}$ also was significantly ($P < 0.001$) lower in horses with IAD than in healthy horses. All mean values for variables of end-expiratory flow were significantly different among groups.

Bronchoalveolar lavage cytologic evaluation—Total nucleated cell counts were not significantly different among groups. Neutrophil counts of horses with COPD during crisis were significantly higher than for healthy horses (**Table 3**). We repeated the analysis after removing data for 1 horse with an unusually high total nucleated cell count (3,564 cells/µl, including 3,278 neutrophils/µl) and found that neutrophil counts of horses with COPD during crisis (90 ± 84 neu-

Table 2—Values (mean ± SD) for forced expiration variables for healthy horses, horses with IAD, horses with COPD during remission, and horses with COPD during crisis

Variable	Control	IAD	COPD	
			Remission	Crisis
FVC (L)	41.6 ± 5.8	39.6 ± 4.8	41.6 ± 9.3	35.1 ± 5.5
FEV_1 (L)	31.9 ± 0.8	30.4 ± 1.9	28.4 ± 7.1	26.3 ± 5.0[a]
FEV_1:FVC	0.777 ± 0.100	0.773 ± 0.063	0.679 ± 0.050[a]	0.748 ± 0.070
$FEV_{1.5}$:FVC	0.981 ± 0.019	0.972 ± 0.025	0.867 ± 0.093[a,b]	0.882 ± 0.046[a,b]
$FEV_{1.7}$:FVC	0.998 ± 0.002	0.984 ± 0.017	0.896 ± 0.089[a,b]	0.907 ± 0.040[a,b]
$FEV_{2.0}$:FVC	0.999 ± 0.001	0.992 ± 0.011	0.926 ± 0.079[a,b]	0.932 ± 0.032[a,b]
$FEF_{75\%}$ (L/s)	28.3 ± 0.8	27.9 ± 1.5	22.1 ± 10.7	17.5 ± 9.8[a,b]
$FEF_{80\%}$ (L/s)	27.8 ± 0.9	26.4 ± 1.0	18.0 ± 10.1[a,b]	15.1 ± 10.3[a,b]
$FEF_{85\%}$ (L/s)	27.4 ± 1.3	24.5 ± 3.8	14.8 ± 9.8[a]	9.6 ± 7.0[a,b]
$FEF_{90\%}$ (L/s)	26.4 ± 1.5	21.4 ± 8.0	9.8 ± 8.6[a]	4.9 ± 2.1[a,b]
$FEF_{95\%}$ (L/s)	23.4 ± 2.2	13.0 ± 8.7[a]	4.4 ± 2.7[a,b]	2.0 ± 0.5[a,b]
$FEF_{75–95\%}$ (L/s)	25.9 ± 1.2	20.5 ± 4.7[a]	13.3 ± 6.3[a,b]	9.8 ± 4.7[a,b]
$FEF_{90–95\%}$ (L/s)	24.9 ± 1.6	17.2 ± 7.8[a]	7.1 ± 5.7[a,b]	3.4 ± 1.0[a,b]

[a]Value is significantly ($P < 0.05$) different from value for healthy horses. [b]Value is significantly ($P < 0.05$) different from IAD value.
FVC = Forced vital capacity. FEV_x = Forced expiratory volume at x seconds (x = 1, 1.5, 1.7, and 2 seconds). $FEF_{y\%}$ = Forced expiratory flow rate at y% of expired FVC (y = 75, 80, 85, 90, and 95). $FEF_{y–95\%}$ = Mean forced expiratory flow rate (y = 75 and 90).

Table 3—Results (mean ± SD) of cytologic examination of bronchoalveolar lavage fluid obtained from healthy horses, horses with IAD, and horses with COPD during remission and during crisis

Variable	Healthy (n = 9)	IAD (n = 5)	COPD	
			Remission (n = 5)	Crisis (n = 4)
TNCC (cells/µl)	321 ± 100	535 ± 187	233 ± 110	1045 ± 1681
Mast cells (cells/µl)	4 ± 2	7 ± 5	2 ± 2[b]	3 ± 2[b]
Neutrophils (cells/µl)	22 ± 12	110 ± 76	143 ± 156	887 ± 1595[a]
Lymphocytes (cells/µl)	104 ± 43	212 ± 69[a]	46 ± 32[b]	101 ± 75[b]
Macrophages (cells/µl)	190 ± 78	247 ± 112	53 ± 32[a,b]	49 ± 75[a,b]
Eosinophils (cells/µl)	1 ± 1	2 ± 3	2 ± 3	4 ± 9
Mast cells (%)	1.5 ± 0.8	1.5 ± 1.3	1.5 ± 1.7	1.5 ± 1.0
Neutrophils (%)	6.8 ± 2.7	20.4 ± 9.3	48.8 ± 40.3[a]	53.8 ± 32.4[a,b]
Lymphocytes (%)	31.4 ± 13.0	36.3 ± 6.8	28.5 ± 18.6	27.0 ± 18.3
Macrophages (%)	57.1 ± 10.3	42.0 ± 17.6	30.3 ± 18.7[a]	15.0 ± 11.2[a,b]
Eosinophils (%)	0.3 ± 0.5	0.5 ± 1.0	1.3 ± 2.5	1.8 ± 3.5

[a]Value differs significantly ($P < 0.05$) from value for healthy horses. [b]Value differs significantly ($P < 0.05$) from value for horses with IAD.
TNCC = Total nucleated cell count.

Table 4—Correlations among clinical signs, variables of lung function, and results of analysis of bronchoalveolar lavage fluid

Variable	Clinical score	Neutrophils (cells/μl)	Lymphocytes (cells/ml)	Macrophages (cells/μl)	Neutrophils (%)	Macrophages (%)
Clinical score	NA	0.58	—	—	0.59	−0.71
FEV_1 (L)	—	—	—	0.53	−0.78	0.72
$FEV_{1.5}$:FVC	—	—	0.53	0.66	−0.68	0.66
$FEF_{75\%}$ (L/s)	—	—	0.51	0.59	−0.68	0.65
$FEF_{80\%}$ (L/s)	—	—	0.55	0.65	−0.61	0.65
$FEF_{85\%}$ (L/s)	−0.60	—	—	0.72	−0.65	0.76
$FEF_{90\%}$ (L/s)	−0.68	—	—	0.69	−0.68	0.79
$FEF_{95\%}$ (L/s)	−0.66	—	—	0.66	−0.67	0.80
$FEF_{75\text{-}95\%}$ (L/s)	−0.65	—	—	0.71	−0.76	0.83
ΔP_{Lmax} (cm H_2O)	0.74	—	—	—	—	−0.52
R_L (cm H_2O/L per s)	0.58	—	—	—	—	—
C_{dyn} (L/cm H_2O)	−0.53	—	—	—	−0.56	0.58
Ti (s)	−0.55	—	—	0.58	−0.54	0.68

NA = Not applicable. − = Weak correlation ($r \leq 0.5$) or not significantly ($P > 0.05$) correlated.
See Table 1 and 2 for key.

Table 5—Evaluation of clinical scoring, results of lung function tests, and cytologic examination of bronchoalveolar lavage fluid for establishing a diagnosis of COPD

Variable	Sensitivity	Specificity	PPV	NPV
Clinical score	66.7	85.7	75.0	80.0
ΔP_{Lmax} (cm H_2O)	44.4	84.6	66.6	68.8
R_L (cm H_2O/L per s)	22.2	69.2	33.3	56.3
C_{dyn} (L/cm H_2O)	33.3	76.9	50.0	62.5
$FEF_{95\%}$ (L/s)	100.0	78.6	75.0	100.0
$FEF_{75\text{-}95\%}$ (L/s)	100.0	78.6	75.0	100.0
$FEV_{1.5}$:FVC	88.9	100.0	100.0	93.3
Neutrophil count (cells/μl)	55.5	71.4	55.5	71.4
Neutrophils (%)	100.0	64.3	64.3	100.0

Values are expressed as percentages.
PPV = Positive-predictive value. NPV = Negative-predictive value.

trophils/μl) were not significantly ($P = 0.26$) different from neutrophil counts of healthy horses (22 ± 12 neutrophils/μl). Percentages of neutrophils in BAL fluid of horses with COPD during crisis and remission were significantly higher than for healthy horses. Percentage of neutrophils in BAL fluid of horses with COPD during crisis was significantly higher than in horses with IAD. Lymphocyte counts were significantly higher in horses with IAD than in healthy horses and horses with COPD. Macrophage counts and percentage of macrophages in horses with COPD during crisis and remission were significantly ($P = 0.01$) lower than those of healthy horses. Mast cell counts were significantly lower in horses with COPD than in horses with IAD; however, mast cell counts were not significantly different between horses with IAD and healthy horses. Eosinophil counts and percentage of eosinophils were not significantly different among groups.

Correlation among variables—Clinical score was negatively correlated with FEF variables, C_{dyn}, and Ti, and it was positively correlated with ΔP_{Lmax} and R_L (**Table 4**). Numbers of neutrophils and alveolar macrophages in BAL fluid were moderately correlated with variables assessed during lung function tests.

Evaluation of diagnostic tests—Sensitivity of FE variables for the diagnosis of COPD during crisis or remission was high and always better than the sensitivity of variables for standard lung function tests (**Table 5**). Specificity of FE variables also was high and as good as, or better than, the specificity of variables for standard lung function tests. Positive- and negative-predictive values of FE variables were high. Percentage of neutrophils in BAL fluid was highly sensitive but not specific for the diagnosis of COPD. Clinical scoring of horses was moderately specific but poorly sensitive for the diagnosis of COPD. These calculations were not performed for horses with IAD because of the small sample size and lack of a satisfactory criterion-referenced standard for the disease.

Discussion

During exacerbation of disease, horses with COPD had significant changes in clinical score, results of lung function tests, and results of cytologic examination of BAL fluid; similar changes have been characterized elsewhere.[7,15,18,24] Standard measurements of lung function (ΔP_{Lmax}, R_L, C_{dyn}) in horses with COPD during disease remission were within reference ranges established here and in prior studies.[7,25] However, FE revealed substantial airway obstruction in this group. Many differences were observed among variables measured in healthy and affected horses; however, the most consistent difference was evidence of airflow obstruction detected by FE in horses with airway disease. The magnitude of airway obstruction was highest in horses with COPD during crisis, lowest in horses with IAD, and intermediate in horses with COPD during remission.

Increased clinical scores for horses with COPD during crisis resulted from severe respiratory abnormalities classically described for the disease.[26] Horses with IAD and horses with COPD during remission had significantly higher clinical scores than healthy horses; however, this difference was not considered clinically relevant because of the large overlap in values among groups. Clinical signs often do not differ between horses with COPD during remission and healthy horses.[7] Therefore, results of clinical examination alone may not be sufficient to diagnose mild respiratory conditions such as IAD or COPD during remission.

During crisis, standard lung mechanics of COPD

horses are characterized by increased ΔP_{Lmax} and R_L and decreased C_{dyn}.[6,18] During disease remission, values for these measures of standard lung mechanics return to reference ranges. However, some variables such as Ti and respiratory flows may remain significantly different from values for healthy control horses.[27] Similar findings were obtained in the study reported here, because our horses with COPD during remission had significantly lower Ti and Te and significantly higher PEF than healthy horses. These subtle changes are attributed to the disappearance of the biphasic breathing pattern that is observed in most control horses.[27] Horses with IAD had significantly higher R_L than healthy horses, suggesting some degree of airway obstruction. Because of the overlap of values among healthy horses, horses with IAD, or horses with COPD during remission, measures of standard lung mechanics appear to be of limited value for early detection of obstruction of the distal airways.

Forced expiratory maneuvers rarely are performed in horses, because the methods used are invasive and cumbersome.[20,28] The method used in the study reported here was safe, minimally invasive, and tolerated well by the horses. Previously, we documented that histamine-induced and naturally occurring airway obstructions could be detected through analysis of FEFV curves.[29] In the study reported here, we observed that several variables derived from the FEFV curve were significantly different among healthy horses, horses with IAD, and horses with COPD during crisis and remission. However, clinical scores of horses with IAD and horses with COPD during remission were low and overlapped with clinical scores of healthy horses. These findings indicate that airflow obstruction is detectable in horses with mild respiratory disease even when clinical signs of the disease may not be apparent.

In humans, FEV_1 is most commonly used for the detection of airflow obstruction.[29] In particular, a substantial decrease in FEV_1 is observed in humans with asthma and COPD. In our study, FEV_1 was significantly lower in horses with COPD during crisis but not during remission, even though analysis of FEF variables suggested a major airflow obstruction in horses with COPD during remission. This lack of sensitivity of FEV_1 is attributable to the fact that, in control horses, FEF is limited by the nasotracheal tube diameter during the first 90% of exhaled FVC.[21] Only the late part of the FEFV curve is dependent on the distal airways of each horse. Therefore, FEV_1 is of limited value with our methods. Nevertheless, $FEV_{1.5}$:FVC, $FEV_{1.7}$:FVC, and $FEV_{2.0}$:FVC in horses with COPD during crisis or remission were significantly lower than in healthy horses and horses with IAD. Most healthy horses and horses with mild airway obstruction completed FVC expiration in < 1.5 seconds. As airway obstruction became more severe, expiratory flow was further reduced, particularly during late expiration. Values for FVC were not significantly different among groups, but the time needed to exhale FVC was prolonged. Consequently, FEV_x:FVC for all time points ≥ 1.5 seconds was lower in horses with moderate to severe airway obstruction than in healthy horses and horses with mild airway obstruction.

The most significant difference among groups was the severe decrease in FEF recorded in horses with COPD, compared with healthy horses, particularly during late expiration ($FEF_{95\%}$ and $FEF_{90–95\%}$). Similar findings were reported in 1 horse with COPD in which FEFV curves were obtained by use of plethysmography.[j] The FEFV curves calculated for healthy horses typically are composed of 3 phases.[21] The first phase is a quick increase in flow to a peak value as soon as airways are exposed to the subatmospheric pressure of the vacuum reservoir, the second phase is a relative plateau extending over most of FVC, and the third phase is characterized by a rapid decrease in flow. In horses with obstructions of the distal airways, a fourth phase is recognized in which flow decreases at a much slower rate, resulting in a concave tail of the FEFV curve.[21] Similar findings have been reported in dogs in which obstruction of the peripheral airways was induced by IV administration of histamine or inhalation of papain.[30,31] The FEF is determined by the choke point, the airway segment that allows the smaller maximal flow which varies in location depending on lung volume.[32] A decrease in flow seems to be related to relocation of the choke point to an area located more toward peripheral airways.[32] Reduction in FEF during the early part of FE (high, lung volume) without a change in FEFV shape is consistent with obstruction of large airways. Reduction in FEF during the later part (low lung volume) that affects FEFV shape is consistent with obstruction of smaller airways.[31] The latter changes were observed in horses with COPD and, to a lesser extent, horses with IAD. These observations suggest that horses with chronic IAD have detectable airflow obstruction. Severity of the obstruction gradually is worse in horses with IAD, horses with COPD during remission, and horses with COPD during crisis, respectively.

The FE maneuvers were performed while horses were sedated with a combination of detomidine and butorphanol. The nasopharyngeal and laryngeal areas were bypassed by use of a nasotracheal tube, which helped minimize the effects of sedation on lung mechanics. Nevertheless, sedation of horses with COPD during crisis by administration of xylazine hydrochloride, an α_2-agonist similar to detomidine, causes significant reduction in obstruction of the distal airways, presumably as a result of relaxation of airway smooth muscles.[33] However, relaxation of airway smooth muscles probably is limited, because histamine-induced bronchoconstriction is detectable by FE in horses sedated with detomidine.[21] Overall, the degree of airflow obstruction detected in the horses of our study may have been underestimated. Other pulmonary changes contributing to airflow obstruction include mucus plugging of airways and thickening of airway walls, which are unlikely to be affected by sedation.

Results of cytologic evaluation of BAL fluid were consistent with neutrophilic inflammation in horses with COPD, particularly during crisis. This inflammatory response has been described elsewhere.[13-15] A surprising finding was the high percentage (49%) of neutrophils in BAL fluid of horses with COPD during

remission. Two of these horses had extremely high values (90 and 95%), compared with values for the other 3 horses during remission (15, 15, and 29%). One of the horses with a high percentage of neutrophils (95%) in BAL fluid had cytologic evidence of minimum sepsis, which probably accounted for the severe neutrophilia. Bacterial infections commonly are detected in the airways of horses affected with COPD,[34,35] and similar to results of the study reported here, they may dramatically affect cellular components of BAL fluid. Results of cytologic evaluation of BAL fluid of horses with COPD during remission usually do not differ from results for clinically normal horses.[15] However, severe airway inflammation also may be observed in horses with COPD during remission.[36,37] This discrepancy between cytologic results for BAL fluid and clinical examination suggests that resolution of airway inflammation may lag behind resolution of clinical signs and lung dysfunction.

Horses with IAD had neutrophil counts and percentages that were higher than for healthy horses; however, the values did not differ significantly. Other investigators have reported that the BAL fluid of horses with IAD is characterized by an increase in total nucleated cell count with the predominant inflammatory cell type being neutrophilic, eosinophilic, or mastocytic.[8,16] In the study reported here, horses with IAD had significantly higher lymphocyte counts in BAL fluid, compared with counts for healthy horses and horses with COPD. This mixed inflammatory profile corroborates results of another study[16] and suggests a differing pathogenesis for IAD and COPD.

Inflammatory airway disease has been described in young racehorses.[11,16] Horses with IAD in our study ranged from 5 to 29 years old. They had been examined multiple times over an extended period, and results of clinical examination and analysis of BAL fluid were repeatable. All of these horses had been housed indoors several times and fed hay, and none had developed increased respiratory efforts typical of COPD. At the time of the study, 1 horse (5 years old) that was actively competing was housed indoors on straw and fed hay and pelleted feed. The other 4 horses with IAD (13 to 29 years old) were nonathletic and had been housed on pasture and fed supplemental hay for at least 2 months prior to examination. Analysis of results of this study suggests that some older horses have a condition that resembles IAD of racehorses but differs from COPD.

Associations among test variables were evaluated, using correlation coefficients. The clinical scoring system used in this study was moderately but significantly correlated with several variables of lung function and analysis of BAL fluid. The discrepancy between clinical signs and objective assessments of lung function was particularly evident in horses with COPD during remission when clinical scores were low, even though FE variables indicated substantial airflow obstruction. Poor correlation between clinical signs and variables of lung mechanics has been reported in horses.[38] Similar findings are described in humans with asthma or COPD.[39,40] Analysis of results obtained in our study suggests that objective measurement of airway obstruction by use of lung function tests, especially FE, is preferable to clinical signs when evaluating horses with chronic airway disease.

Usefulness of clinical examination, standard lung mechanics, FE, and cytologic evaluation of BAL fluid for diagnosing COPD was assessed by calculating sensitivity, specificity, and predictive values for test variables. Such calculations may be used when certain criteria are met.[23] First, assessment of diagnostic tests depends on an appropriate definition of the disease (criterion-referenced standard). Historical and clinical criteria that were used to diagnose COPD in the study reported here, particularly for recurrence of signs of respiratory tract disease when horses were fed hay, have been commonly adopted, because they are pathognomonic for COPD.[4,7] Second, a large spectrum of horses with chronic respiratory tract disease was tested, including horses with COPD during crisis and remission and horses with IAD. The purpose was to assess the validity of these tests in situations that were clinically challenging and relevant. Third, investigators assigning clinical scores and evaluating cellular components of BAL fluid were not aware of the group assignment of each horse.

The percentage of horses with COPD that had abnormal values for variables of lung mechanics during tidal breathing was low; hence, sensitivity of the test was poor. This was attributed mainly to the fact that all horses with COPD during remission had values within the reference range (negative result). The percentage of horses that did not have COPD that had normal values for variables of lung mechanics was moderately high. Therefore, specificity of the test was good. More importantly, given a horse with abnormal values for lung mechanics, the likelihood that it had COPD (positive-predictive value) was low. Similar results were obtained for the negative-predictive values. Therefore, values for standard lung mechanics constitute a poor clinical test for COPD. The FE variables were highly sensitive and specific for COPD. In addition, high positive- and negative-predictive values indicated that FE may be a useful screening test for COPD. Predictive values of a test are improved when disease prevalence is high.[23] Because prevalence of COPD is high,[41] predictive values of FE tests observed in the study reported here are likely to be accurate. Prospective studies involving a larger sample size would allow investigators to critically evaluate these data.

Between 20 and 80% of R_L in horses is determined by airways of the proximal respiratory tract.[42,43] In the lungs, larger airways (trachea, bronchi) account for most of R_L with little contribution from small airways.[44] In horses with COPD, mainly small airways (bronchioles) are obstructed, and only severe disease will increase R_L; hence, the low sensitivity of the test.[3] The FE method described here is mainly affected by changes in diameter of small airways; therefore, it is a more sensitive test of COPD than R_L.[k]

Clinical score was poorly sensitive for COPD, because 3 horses during remission had scores typical of clinically normal horses. Predictive values suggest that, based on clinical score, a correct diagnosis of COPD

may be made in, at most, 75 to 80% of cases. However, clinical score often does not differ between control horses and horses with COPD during remission.[7] Percentage of neutrophils in BAL fluid was highly sensitive but not specific for a diagnosis of COPD. The poor specificity and positive-predictive value of the test were attributable to the fact that 1 healthy horse and 4 horses with IAD had values higher than the reference range. The high negative-predictive value of the test allows clinicians a high degree of confidence in ruling out COPD as an entity in horses with a normal percentage of neutrophils. Results of the study also revealed that neutrophilic inflammation of the respiratory tract's smaller airways may be observed in horses that do not have COPD and that airway inflammation is not necessarily associated with lung dysfunction. These findings are in agreement with studies in which investigators observed an influx of neutrophils in airways of equids exposed to molds[4,37,45]; however, only equids with COPD had changes in lung function. We speculate that when the inflammatory process is sufficient in duration or severity, changes in morphologic characteristics of the airways will develop, leading to impairment of lung function, such as has been proposed in humans with asthma.[46] When inflammation does not induce airway remodeling, the only manifestation will be an abnormal cellular composition of BAL fluid and, possibly, coughing.

[a]DP/45–30, Valydine Engineering Corp, Northridge, Calif.
[b]No. 4 Fleisch, EMKA Technologies, Paris, France.
[c]DP/45–14, Valydine Engineering Corp, Northridge, Calif.
[d]Pulmonary mechanics analyzer, XA version, Buxco Electronics Inc, Sharon, Conn.
[e]Model 5530, Hans Rudolph Inc, Kansas City, Mo.
[f]Slack-tube manometer, Dwyer Instruments, Michigan City, Ind.
[g]22-mm internal diameter, model 2100B, Hans Rudolph Inc, Kansas City, Mo.
[h]NELAC, North American Dragger, Telford, Pa.
[i]Asco Red Hat, 3.8 cm ID, Automatic Switch Co, Florham Park, NJ.
[j]Leith DE, Gillespie JR. Respiratory mechanics of normal horses and one with chronic obstructive lung disease (abstr), *Fed Proc* 1971;30,556.
[k]Couetil L, Rosenthal F, Simpson C. Forced expiration in horses with small airway disease (abstr), in *Proceedings*. Am Coll Vet Intern Med 1999;242.

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