Chronic obstructive pulmonary disease: Usefulness of clinical signs, bronchoalveolar lavage, and lung biopsy as diagnostic and prognostic aids

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Abstract

We examined 18 horses with clinical signs of chronic obstructive pulmonary disease (COPD) using physical examination, cytological and bacteriological evaluation of bronchoalveolar fluid, and percutaneous lung biopsy. In 16 cases, histological examination of lung tissue confirmed the diagnosis of COPD. Two horses were excluded: one had uncomplicated bacterial pneumonia and in the other a satisfactory lung biopsy could not be obtained. In horses with COPD, the most common historical complaint was coughing, which was reported in 88%. The most frequently detected abnormal finding on physical examination was abnormal lung sounds; these were detected in 69% of horses at rest and in 88% of horses breathing deeply into a bag. A novel finding was that 29% of horses had lung sounds that were quieter than would be expected for the degree of respiratory effort. Horses with COPD had increased percentages of neutrophils and decreased percentages of lymphocytes and macrophages in their bronchoalveolar lavage fluid. Bronchiolar neutrophil infiltration and peribronchiolar mast cell accumulation in lung biopsy tissue had the highest correlation with clinical condition. The severity of pathological changes in biopsies of lung did not predict whether the horse would die in the two to four year follow-up period. Horses that died in the follow-up period were more severely affected clinically at initial presentation than horses that were alive at the end of the follow-up period.

Résumé

Maladie pulmonaire obstructive chronique: la valeur des signes cliniques, du lavage broncho-alvéolaire et de la biopsie pulmonaire comme moyens de diagnostic et de pronostic

Les auteurs ont compilé les données observées chez 18 chevaux présentant des signes cliniques de maladie pulmonaire obstructive chronique (M.P.O.C.). Leur évaluation comprend l'examen physique de l'animal, une analyse cytologique et bactériologique du lavage broncho-alvéolaire et une biopsie pulmonaire transcutanée. Dans 16 cas, l'étude histologique des coupes pulmonaires a confirmé le diagnostic de maladie pulmonaire obstructive chronique. Deux cas ont été exclus, l'un présentait une pneumonie bactérienne non compliquée et, dans l'autre cas, les auteurs n'ont pu obtenir une biopsie pulmonaire adéquate pour analyse. Chez les chevaux atteints de M.P.O.C., la toux était dans 88 % des cas l'élément de l'anamnèse le plus souvent mentionné. À l'examen physique, des bruits respiratoires anormaux ont été décelés dans 69 % des chevaux au repos et ont été audibles dans 88 % des cas lors de respirations profondes effectuées dans un sac. Un fait nouveau était que, considérant l'effort respiratoire fourni, 29 % des chevaux présentaient des bruits respiratoires plus silencieux que prévus. Les résultats de la cytologie effectuée à partir du liquide du lavage broncho-alvéolaire ont révélé une augmentation du pourcentage de neutrophiles et une diminution du pourcentage de lymphocytes et de macrophages. Les deux observations issues de l'histologie pulmonaire présentant la meilleure corrélation avec les signes cliniques étaient l'infiltration bronchiolaire de neutrophiles et l'accumulation péribronchiolaire de mastocytes. La sévérité des changements pathologiques observés dans les coupes histologiques du poumon n'a pas permis de prédire la survie de l'animal durant les deux à quatre années d'observation. Les chevaux morts durant la période de suivi étaient ceux qui avaient démontré les signes cliniques les plus sévères lors de la première visite.

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Introduction

Chronic obstructive pulmonary disease (COPD) is a well recognized disease of horses. Affected animals are usually bright and alert, and suffer from varying degrees of dyspnea (1,2). Recently, bronchoalveolar lavage and lung biopsy have been advocated as potentially useful diagnostic and prognostic tests in COPD (3,4).

Bronchoalveolar lavage entails the sampling of fluid from the bronchi, terminal airways, and alveolar spaces. Lavage fluid contains fewer epithelial cells and more macrophages and lymphocytes than fluid collected from higher levels of the respiratory tract (5) and is thought to give a better representation of the inflammatory process in the lungs than does a transtracheal wash (3). Bronchoalveolar lavage is usually

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carried out by positioning a 180 cm endoscope in one of the main bronchi and advancing a thin tube through the biopsy port into a terminal airway. The airway is flushed with fluid and a sample is withdrawn through the tube (3,4).

Lung biopsy has been advocated as a safe method of obtaining a sample of tissue for diagnostic and prognostic purposes (3,6).

The objectives of the study reported herein were to investigate the usefulness of bronchoalveolar lavage and lung biopsy as diagnostic and prognostic tests in horses with COPD. We anticipated that bronchoalveolar lavage might reflect the severity of ongoing inflammatory change while lung biopsy would reflect both acute inflammation and chronic destruction.

We postulated that, together, these two tests could be used to evaluate the proportion of clinical signs due to bronchospasm and inflammatory exudate (reversible), and that due to fibrosis and destruction of alveoli (irreversible). A secondary objective was to describe the clinical signs of horses with COPD in our practice area.

Table 1. Clinical scoring system for severity of chronic obstructive pulmonary disease

	Score[a]
Respiratory rate, (breaths/min)	
20	0
21-30	1
31+	2
Respiratory effort	
Normal	0
End expiratory abdominal lift	1
Flared nostrils/anal movement	2
Lung auscultation	
Normal	0
Localized wheezes or crackles	1
Generalized wheezes or crackles	2
Decreased air movement for respiratory effort:	
No	0
Yes	2

[a]Scores vary from 0 in normal horses to 8 in maximally affected horses

Materials and methods

Animals

Nine clinically normal adult horses, 9.9 ± 4.2 (mean ± 1 SD) years old with a range of 5–17 years, were used to generate bronchoalveolar lavage reference values. The breeds represented were Thoroughbred (n = 3), Arab-crossbred (n = 1), Quarter Horse (n = 2), and mixed (n = 3). They were housed outdoors at the Western College of Veterinary Medicine and showed no signs of anorexia, cough, or nasal discharge in the one month period prior to sampling. At the time of sampling, the horses had normal respiratory rates and were bright and alert.

A prospective study was performed on horses admitted to the Western College of Veterinary Medicine between January 1987 and April 1989, inclusive. Horses were initially included in the study if they had a tentative diagnosis of COPD based on a history of chronic (>1 month) respiratory disease, and signs of abnormal respiratory tract function including coughing, tachypnea (>16 respirations/minute), or the presence of abnormal lung sounds. Entry requirements included observations that the horses had a normal rectal temperature <38.4°C, were bright and alert, and were eating well. The demeanor requirement was waived for one horse which was depressed and anorexic, because it was thought that these changes were due to anoxia rather than infection. A diagnosis of COPD was made if horses that met the clinical inclusion criteria had histological features of bronchiolitis (7–9) present in a biopsy of the dorsal lung.

Severity of disease was assessed using the clinical scoring system shown in Table 1. Lung sounds were classified based on the terminology of Roudebush (10). Wheezes were high pitched, continuous sounds, and crackles were low pitched, discontinuous sounds. The term "squeaks" was used to describe high pitched sounds of short duration and probably corresponds to the term fine crackle as used by Roudebush. Auscultation was performed with the horses at rest and during use of a rebreathing bag to increase the depth of respiration. This was perfomed by holding a large (about 40 L) plastic bag over both nostrils for 1–5 min until there was a marked increase in the depth of respiration or the horse became uncomfortable. Once the horse was breathing deeply the amount of room available around the nose for entry of air into the bag was increased to allow auscultation while the horse breathed deeply.

Horses diagosed as suffering from COPD were given standardized treatments. If pathogenic bacteria were cultured from the bronchoalveolar wash, the horse was treated for seven to ten days with an appropriate antibiotic based on the *in vitro* Kirby-Bauer sensitivity of the microbe. All horses were treated with oral prednisone (Apo-prednisone, Apotex Inc., Toronto, Ontario) at an initial dose of 1–2 mg/kg for five to seven days, reducing the dose by half every five to seven days until a course of treatment at 0.5 mg/kg was completed. Then the horses were treated with prednisone at 0.5 mg/kg every other day for five treatments before the therapy was discontinued. Management advice was aimed at reducing exposure to dust and molds in feed and bedding (11,12) by keeping horses out of doors and feeding wet hay on the ground. The hay was thoroughly soaked by immersion in a tub for at least five minutes prior to feeding or, alternatively, alfalfa cubes were fed. A three-sided shed and horse blankets provided adequate protection during cold weather. Owners who insisted on keeping their horses indoors were advised to bed the stall with wood shavings, to feed alfalfa cubes rather than hay, and to provide good ventilation for the affected horse and those in adjacent stalls. The owners of horses that experienced COPD when kept outside were advised to feed alfalfa cubes and to avoid dusty locations.

Attempts were made to contact the owners of all horses in the study in July of 1989, January of 1991, and April of 1991. At each time the owners were asked if they still owned the horse, its present condition, use,

management, medication, and whether or not there was any exercise intolerance.

Bronchoalveolar lavage

We developed a collection technique, based on the passage of a guarded tube through the nose, which can be performed without an endoscope. The horse was sedated with intravenous xylazine (Rompun, Bayvet Division, Etobicoke, Ontario) at 0.6 mg/kg body weight. A 75 cm length of 8 mm internal diameter tube (Argyle, Sherwood Medical, St. Louis, Missouri, USA) was lubricated with viscous 2% lidocaine hydrochloride (xylocaine, Astra, Mississauga, Ontario). The horse was restrained with its neck extended as the tube was passed through the nasal cavity into the trachea. A plastic collar was placed around the tube at the level of the nostril to prevent further penetration of the tube. A sterile, 2.5 m long polypropylene tube, with an outside diameter of 6 mm and an internal diameter of 3 mm (Bev-A-Line V, Cole-Parmer, Chicago, Illinois, USA) was lubricated with viscous lidocaine and passed through the large tube. The small tube was advanced until its tip lodged in a bronchus after about 1.5 m had been passed. The horse usually coughed when the tube lodged. One hundred milliliters of lavage fluid were injected rapidly, followed by a 20 mL air bolus. The fluid was then aspirated. Lavage fluid consisted of 0.145 M sodium chloride, 0.0003 M ethylene di-sodium tetra-acetate, and 0.024 M N-2-hydroxyethylpiperazine-N-ethane sulfonic acid in distilled water. The solution was autoclaved prior to use. After autoclaving, 10 mL of 10% glucose, previously filtered through a 0.45 micron filter, was added to 990 mL of fluid.

Bronchoalveolar lavage — cytology

Three drops of the final cell suspension were applied to the funnel of a cytospin (Shandon Southern Instruments Corp., Pittsburg, Pennsylvania, USA) and centrifuged at 1000 rpm on low acceleration for five minutes at room temperature. The slides were stained on an automatic Wright-Giemsa stainer (Ames Hema-Tek slide stainer, Miles Scientific, Naperville, Illinois, USA). Differential cell counts were based on observation of 300 or 400 cells. Total cell counts were determined using a hemacytometer (Bright-Line Hemacytometer, Reichert Scientific Instruments, Buffalo, New York, USA).

Bronchoalveolar lavage — culture

Lavage fluid was cultured on blood agar plates incubated at 37°C aerobically, under carbon dioxide, and anaerobically. MacConkey's agar was also inoculated and incubated aerobically. Bacterial growths were identified. The number of bacteria present was scored as either a few scattered colonies on the plate or 1+ to 4+ in the case of heavier growths. A 1+ growth only grew on the first streakings from the inoculation site, a 4+ growth grew on the first, second, third, and fourth set of streakings.

Lung biopsy

A 5 cm square of skin was clipped between the seventh and ninth intercostal space, approximately 8 cm above the humeroradial joint (6). The horse was sedated with intravenous xylazine at 0.6 mg/kg body weight. The subcutaneous tissue and intercostal muscle were infiltrated with 2% lidocaine hydrochloride (M.T.C. Pharmaceuticals, Cambridge, Ontario), and the skin was given a sterile scrub with povidone-iodine (Betadine, Purdue Frederick Inc., Toronto, Ontario) and alcohol. A Tru Cut biopsy needle 15.2 cm long with a 2 cm specimen notch (Travenol Laboratories Inc., Deerfield, Illinois, USA) was carefully inserted through a small skin incision just cranial to the rib. The operator guarded the needle by placing his fingers about 9 cm from the tip to prevent the needle from advancing too deeply into the lung and to reduce the chance of penetrating a major blood vessel. The needle was inserted rapidly into the lung, the inner biopsy core advanced, the outer cannula slid over the biopsy, and the needle removed. Speed was thought to be important in minimizing trauma, so the needle was inserted into the lung for a maximum of one second. Lung biopsies were immediately placed in formalin. Repeat biopsies were taken if the sample was small (<1 cm long) or did not float. Biopsies from both sides of the thorax were submitted from each horse suspected of suffering from COPD. Following biopsy, the owner was instructed to rest the horse for two days. Horses that bled profusely from the nostrils following biopsy were given a three day course of penicillin (Ethacilin, rogar/STB Inc., London, Ontario) and were rested for at least three days. Biopsies were not taken from control horses.

Biopsies were stained with hematoxylin and eosin, periodic acid Schiff-alcian blue, Giemsa, Masson's trichrome, and neutral red. The severity of lesions was scored using the criteria in Table 2. This table was devised by ranking the known histological features of COPD (7–9) on a 3 point scale.

Statistics

The simple correlations between clinical, biopsy, and bronchoalveolar lavage scores were computed using Pearson correlation coefficients (13).

The value of lung biopsy and bronchoalveolar lavage findings for predicting clinical signs was investigated using step-wise linear regression with alpha-to-enter and alpha-to-remove set at 0.15. All variables for the bronchoalveolar lavage leukocyte percentages and for the scores for the individual and total pathology findings were initially entered into the model. The model was repeatedly reestimated and non-significant variables discarded until only significant variables ($p < 0.05$) were left (13).

Differences in findings at presentation between horses that did or did not survive for two years, and between bronchoalveolar lavage findings in normal and COPD affected horses, were compared using the *t*-test and Bartlett test. All calculations were performed using SYSTAT (13).

Results

Eighteen horses with clinical signs consistent with COPD were biopsied; in 16, histological features of COPD were seen in pulmonary tissue. One horse had

Table 2. Descriptors used to describe lung biopsies

	1+	2+
Bronchiolar epithelial hyperplasia[a]	One or more airways with mild, often eccentric or focal hyperplasia = >1 cell thick	One or more airways with concentric and severe hyperplasia
Goblet cell metaplasia	One or more airways contain one to a few epithelial goblet cells (PAS/Alcian blue) ± luminal mucin	One or more airways wherein most epithelial cells are "goblet" type plus abundant luminal mucin
Bronchiolar luminal exudate[b]	Mixtures of mucin, neutrophils, macrophages and cell debris in small amounts in one or more airways	Abundant similar material in one or more airways but with neutrophils predominating
Peribronchiolar fibrosis[c]	One or more airways surrounded by excess, concentric collagen fibers (Masson's trichrome stain)	
Peribronchiolar lymphoplasmacytic infiltrations	One or more airways surrounded by a few lymphocytes and lesser numbers of plasma cells	Densely cellular mantles of lymphocytes and/or plasma cells surrounding most airways ± follicle formations
Alveolar fibrosis and/or emphysema[c]	Excess collagen deposition in interalveolar septa, coassociated with dilatation and/or distortion of alveolar spaces	
Pneumocyte II hyperplasia[c]	Proliferated, large granular pneumocytes lining one or more alveoli	
Peribronchiolar mast cells	One to ten mast cells (Giemsa and/or neutral red stains) in lamina propria of, or surrounding one or more bronchioles	More than ten mast cells visible in same locations
Bronchiolar eosinophils	One to five eosinophils within the epithelial layer or surrounding one or more bronchioles	More than five eosinophils present
Bronchiolar neutrophils	A few neutrophils in the epithelial layer, or around airways and in adjacent alveoli	Numerous cells present in these locations

[a]Constricted airways with mucosal folds may artifactually appear to have hyperplastic epithelium
[b]The artifactual, biopsy-induced "prolapse" of airway epithelium into the lumen may be misinterpreted as exudates
[c]Criteria of equine COPD not visible in this series of cases

a normal biopsy; its bronchoalveolar lavage fluid sample contained 26% neutrophils, and *P. hemolytica* was cultured. This horse responded to antibiotic therapy. A diagnosis of low grade bacterial bronchitis or pneumonia was made. In the other horse, biopsies were taken on two separate occasions but insufficient tissue to make a histological diagnosis was obtained. This horse showed classical clinical signs of COPD (1,2), namely forced expiratory movements, flaring of the nostrils, and anal movements synchronous with respiration. On auscultation of the lungs, expiratory wheezes were heard. These latter two horses were excluded from further study.

The 16 horses with clinical and histological features of COPD were 12 ± 6.7 years old at presentation, with an age range of 3–27 years. There were eight castrated males, two entire males, and six females. The breed distribution was six Quarter Horses, four crossbreds, three Thoroughbreds, two Arabians, and one Standardbred. At the time of presentation, eight horses were permanently stabled, three were stabled at night, and five were kept outdoors day and night.

The majority of horses had classical signs of COPD: a bright demeanor, history of coughing, and exercise intolerance. The most common historical complaint was coughing, which was reported for 14 (88%) horses. Exercise intolerance was reported by nine (56%) owners. The most frequently detected abnormality on physical examination was abnormal lung sounds after the horse had been forced to breathe deeply by placing a bag over its nose; this was more sensitive than listening to the lungs at rest (Table 3). Five horses had decreased breath sounds, i.e. the intensity of normal bronchovesicular tones was decreased relative to that of a normal horse breathing with the same degree of effort. Of these, four horses also had adventitial lung sounds. In one horse the only abnormality detected on physical examination was an increase in respiratory rate.

Bronchoalveolar lavage was performed on 16 horses, but the slides from one horse were inadvertently discarded after processing. Elevated neutrophil percentages were most useful in differentiating between COPD and control horses because there was no overlap between the range of neutrophil percentages (Table 4). Heavy growths of pathogenic bacteria were isolated from the lavage fluid of two horses; one had *Streptococcus equi* (1+ growth), and the other had

Table 3. Clinical findings in horses with chronic obstructive pulmonary disease

Clinical finding	Horses affected	
	Number	Percent
Attitude		
Excited	1	6
Bright	12	75
Quiet	2	13
Depressed	1	6
Nasal discharge (serous)	2	13
Cough	7	44
Forced expiration	11	69
Flaring of nostrils	5	31
Anal movement with respiration	5	31
Barrel chested appearance	2	13
Abnormal tracheal sounds	7	44
Nature of abnormal tracheal sounds:		
Fluid sounds (Low pitched, discontinuous)	3	19
Wheezes	3	19
Crackles	1	6
Abnormal lung sounds:		
At rest	11	69
After bagging off[a]	14	88
Nature of abnormal lung sounds:		
Increased lung sounds	6	38
Inspiratory	4	25
Expiratory	2	13
Decreased lung sounds	5	29
Wheezes	14	88
Inspiratory	7	44
Expiratory	11	69
Crackles	8	47
Inspiratory	2	13
Expiratory	6	35
End inspiratory squeaks	2	13

[a]A large plastic bag was held over the nose until the horse became hyperpneic and took deep breaths. The entire lung field was ausculted during this deep breathing

Actinobacillus equuli (3+). Two horses had growths (1+ to 2+) of nonpathogenic *Alcaligenes* spp. One of these horses also had a growth (2+) of *Pseudomonas* spp. Three horses had light growths (less than 10 colonies per culture plate) of a variety of microorganisms, including *Streptomyces* spp., *Bacillus* spp., *A. equuli* and *P. hemolytica*. Washes from five of the clinically normal horses were cultured: three were sterile, one grew a single colony of *Streptomyces* spp., and one had a growth (1+) of *Neisseria* spp.

Lung biopsies were performed without complication in 15 horses. One horse bled from the nose following biopsy of the right lung and, consequently, a left lung biopsy was not taken. One to 15 bronchioles were present in each biopsy. The most common changes were bronchiolar goblet cell metaplasia, bronchiolar luminal exudate accumulation, peribronchiolar lymphoplasmacytic cell infiltration, and accumulations of neutrophils. Alveolar fibrosis and emphysema were not seen (Table 5).

The correlation of bronchoalveolar lavage cytology and biopsy findings with clinical score is shown in Table 6. Stepwise regression indicated that clinical score at presentation was more closely correlated with biopsy findings than with differential cytology of bronchoalveolar lavage fluid. Once biopsy findings were known, bronchoalveolar lavage data gave no additional predictive value. The final regression model predicted 64% of the variation in clinical score from the severity of peribronchiolar mast cell infiltration and the severity of bronchiolar neutrophil infiltration (both variables significant at $p < 0.03$, overall $r = 0.80$, $p = 0.001$).

Many of the horses were assessed clinically and cytologically on several occasions. Seven horses were assessed once, six horses twice, one horse three times, and one horse five times. In this subset there were moderate correlations between clinical score and the percentage of lymphocytes ($r = -0.49$, $p < 0.05$) or neutrophils ($r = 0.44$, $p < 0.05$).

Most owners reported that their horses responded favorably to therapy in both the short and long-term. It was possible to contact the owners of 15 of the horses at the final follow-up, two to four years after the horses were initialy presented. Twelve of these horses were still alive, eight were not receiving any medication, and four were medicated with clenbuterol (Ventipulmin, Boehringer Ingelheim Ltd., Burlington, Ontario) on an as-needed basis. In 11 of the 12 horses there had been no decrease in the amount or type of work being performed.

In the two-to-four year follow-up period, a total of three horses were killed because their owners judged

Table 4. Bronchoalveolar lavage findings in normal horses and in horses with chronic obstructive pulmonary disease

Item	COPD (n = 15)			Normal (n = 9)		
	Mean	SD	Range	Mean	SD	Range
Neutrophils[a] (%)	58.8	19.8	17–82	4.4	3.3	0–10
Lymphocytes[a] (%)	13.2	7.1	3–30	38.8	12.6	20–60
Macrophages[a] (%)	22.5	13.9	3–51	48.2	10.8	30–66
Eosinophils (%)	1.0	2.2	0–6.5	1.3	4	0–12
Mast cells (%)	4.3	4.0	1–12.9	7.3	4.7	2–15
Total cells $\times 10^6$	10.3	8.6	1.2–27	6.6	8.5	1.5–29

[a]Significant differences ($p = 0.001$) exist between horses with COPD and normal horses

Differentials are based on leukocytes only. Tissue cells were also seen: there were 5.8 ± 3.6% and 1.2 ± 2.6% epithelial cells in COPD-affected and normal horses, respectively

Table 5. Biopsy findings in horses with chronic obstructive pulmonary disease

Item	Mean	SD	Range
Bronchiolar epithelial hyperplasia	0.8	0.7	0–2
Goblet cell metaplasia	1.0	0.7	0–2
Bronchiolar luminal exudate	0.9	0.7	0–2
Peribronchiolar fibrosis	0.1	0.3	0–1
Peribronchiolar lymphoplasmacytic infiltration	1.1	0.6	0–2
Alveolar fibrosis or emphysema	0	0	0
Pneumonocyte type II hyperplasia	0	0	0
Peribronchiolar mast cells	0.8	0.6	0–2
Bronchiolar eosinophils	0	0	0
Bronchiolar neutrophils	0.8	0.9	0–2
Total pathology scores[a]	5.3	2.6	2–11

[a]Sum of scores in all categories

the COPD to be persistent and unmanageable. Deaths occurred 1, 7 and 14 months after presentation. All of these horses had clinical scores >5. Values at presentation were compared between those horses that died and those that lived throughout the follow-up period. The only variables that differed significantly were initial clinical score [3.4 ± 2.1 in long-term surviving horses versus 6.0 ± 2.0 in horses that died ($p = 0.06$)], and initial bronchoalveolar lavage macrophage percentage [25.6 ± 13.3 versus 8.0 ± 4.7 ($p = 0.05$)], respectively. Differences between all other variables were nonsignificant (p values >0.15).

Discussion

The most common physical findings in horses with COPD were tachypnea and abnormal lung sounds, which have been well described in standard texts (1,2). In one horse, the only physical abnormality was tachypnea. Veterinarians should be aware that tachypnea can be the only physical abnormality in a small percentage of horses with COPD. About a third of our cases were severely affected; these horses had quieter breath sounds than was anticipated from the amount of respiratory effort. A distended thorax, with prominent ribs giving a barrel-chested appearance, was seen in two horses. The quiet breath sounds and barrel-chested thorax are not mentioned in research articles or several current texts (1,2). These findings are presumably caused by collapse of the bronchioles under the increased intrapleural pressure that occurs on forced expiration, leading to reduced expiratory air flow, reduced tidal volume, and an increased end expiratory lung volume. All except one of the horses with decreased breath sounds also had adventitial lung sounds. Horses with COPD usually have a normal demeanor. However, one severely dyspneic horse was depressed, probably because of anoxia.

Several authors have stressed that exercise or use of a rebreathing bag to increase the depth of respiration improves the sensitivity of lung auscultation (1–3). This was borne out in our study, where a rebreathing bag increased the sensitivity of lung auscultation in detecting abnormal sounds from 69–88%. Wheezes and crackles were most commonly heard during the expiratory phase; this may be because narrowing of the airways increases the likelihood of air turbulence or of strands of mucus interfering with air flow, respectively. In a few horses with COPD, adventitial lung sounds were only heard on inspiration, a finding that is more characteristic of pneumonia. Adventitial lung sounds were present throughout the lungs in most horses with COPD, although some affected horses only had anteroventral abnormalities.

The technique we used for bronchoalveolar lavage enabled us to obtain samples in standing horses with minimal equipment, and is very similar to that recently described by Fogarty (14) for field use. Fogarty's studies suggest that the tip of the lavage tube is likely to pass into the dorsocaudal lung field (14). The use of a large tube to guard the lavage tubing in the nasopharynx appears to have been successful based on the low numbers of commensal organisms isolated from bronchoalveolar lavage samples. In comparison, transtracheal wash samples can yield very high levels of bacterial growth, even in normal horses (15). It is important that lavage fluid does not contain calcium and magnesium. These ions reduce cell yield, probably because they stimulate adhesion of cells to the alveolar walls (16). The exclusion of calcium and the inclusion of chelating agents in the lavage fluid used in our study were designed to maximize cell yield. The glucose provided a nutrient source to help maintain

Table 6. Correlation between clinical score, and initial bronchoalveolar lavage and biopsy findings in 15 horses with COPD

Variable	Correlation coefficient[a]
Bronchiolar neutrophils (biopsy score)	0.70
Total biopsy pathology (biopsy score)	0.58
Peribronchiolar mast cell infiltrate (biopsy score)	0.57
Bronchiolar luminal exudate (biopsy score)	0.36
Bronchiolar epithelial hyperplasia (biopsy score)	0.30
Peribronchiolar lymphoid hyperplasia (biopsy score)	0.26
Peribronchiolar fibrosis (biopsy score)	−0.22
Goblet cell metaplasia (biopsy score)	0.08
Bronchoalveolar lavage macrophages (%)	−0.32
Bronchoalveolar lavage neutrophils (%)	0.21
Bronchoalveolar lavage lymphocytes (%)	−0.05
Bronchoalveolar lavage eosinophils (%)	−0.09
Bronchoalveolar lavage mast cells (%)	0.01

[a]Values are Pearson correlation coefficients; coefficients >0.5 are significant at $p < 0.05$

cell function. The yield of cells obtained by our technique is somewhat lower than that reported by others, but this probably reflects the lower volume of fluid infused into the lungs. We used 100 mL of fluid compared to 300–500 mL of fluid in previous studies (4,17). The differential leukocyte counts in normal horses obtained by our guarded nasobronchoalveolar lavage technique are almost identical to those reported by Viel (4), and close to the values reported by Derksen (17,18). In contrast, reports from the British Isles often describe more macrophages and fewer lymphocytes than North American studies (5,14). All workers report that bronchoalveolar lavage fluid from the lungs of normal horses should contain less than 12% neutrophils (4,5,14,17,18).

Ancillary tests increased diagnostic precision, but they made a major contribution in only two of 18 horses. One of these was initially thought to be suffering from COPD, based on the presence of a chronic cough, normal food intake, and alert attitude. The horse was finally classified as suffering from bacterial pneumonia, because the lung biopsy showed normal tissue and the bronchoalveolar lavage fluid contained an increased percentage of neutrophils and *P. hemolytica*. In the second horse, tachypnea was the only detectable clinical abnormality. Bronchoalveolar lavage fluid showed a neutrophilic infiltration, thus localizing the problem to the lungs, and lung biopsy revealed histological features of COPD.

A third horse remained a diagnostic challenge despite the use of ancillary tests. This horse had classical signs of COPD, but bronchoalveolar lavage fluid contained a normal percentage of neutrophils and, despite repeated lung biopsies, only small amounts of normal lung tissue were obtained.

The major change in bronchoalveolar lavage fluid cytology in COPD was a relative and absolute neutrophilia. There was also a relative and absolute decrease in the number of lymphocytes. Previous workers have noted a statistically nonsignificant decrease in the numbers of lymphocytes in lavage fluid from ponies with COPD (17). In our study, bronchoalveolar lavage fluid was always abnormal in horses with COPD in that the percentage of neutrophils was elevated in all affected horses. The sensitivity of bronchoalveolar lavage in detecting pneumonia is much lower, neutrophilia occurring in only about half the cases (19).

Bronchoalveolar lavage had a very poor correlation with the severity of clinical signs on initial examination (Table 6), although decreases in neutrophil percentages in a given horse did tend to parallel improvements in clinical condition. It had some prognostic value: there was a lower percentage of macrophages in the fluid from horses that were eventually euthanized.

Bronchoalveolar lavage is less invasive than lung biopsy, but its diagnostic value is limited to confirming the presence of a lung problem. An increased percentage of neutrophils in the fluid is characteristic of both COPD and pneumonia. The absence of bacteria on culture from a horse with a neutrophilic exudate suggests that COPD is the more likely diagnosis. Problems in interpretation arise when bacteria are cultured, as happened several times in our study. Their presence may be due to contamination or to transient colonization of the airways, as shown by finding bacteria in the fluid from normal horses. In horses with COPD, contamination of the airways with bacteria may be more likely because normal clearance mechanisms function less efficiently. Alternately, bacteria may be present as a result of a primary pneumonia or secondary infection of a lung afflicted by COPD. Furthermore, bronchoalveolar lavage is not as reliable in detecting bacterial infection as a transtracheal wash, which samples the lower trachea through which inflammatory exudate from the whole lung passes (19). In contrast, bronchoalveolar lavage samples only a small lung segment and may miss a localized infection (19,20). The use of endoscopy to guide the bronchoalveolar lavage tube should increase the chance that abnormal airways are sampled, but it is still unreliable in detecting bacterial pneumonia (19). Bronchoalveolar lavage does have advantages over transtracheal wash in that the cytology of the sample is more representative of the lower airways (5), and spurious contamination by inhaled microorganisms is less likely.

Biopsy of the dorsal lung lobes was more useful than bronchoalveolar lavage as a diagnostic aid. It was particularly useful in confirming the presence of COPD in two horses that had pathogenic bacteria cultured from their bronchoalveolar lavage. In our study, lung biopsy histology had a good correlation with clinical signs (Table 6). Used together, the degree of bronchiolar neutrophil infiltration and goblet cell metaplasia gave the best correlations. This suggests that these changes are particularly important in the pathogenesis of signs. Furthermore, as these two changes are potentially reversible, it suggests tht most horses were capable of significant improvement in condition. This is borne out by the high long-term survival rate and by the fact that many owners thought there was little long-term compromise resulting from COPD. There is a tendency by some veterinarians to believe that horses severely clinically affected by COPD have irreversible changes. However, our study suggests that it is important to give severely affected horses a course of appropriate treatment before establishing a prognosis, because many are capable of substantial improvement.

One might think that since COPD is a diffuse lung disease, a blind biopsy gives a representative sample. However, lung changes in COPD are not uniform so an isolated biopsy may not be completely representative of overall pathological status (7,8). Obtaining samples from both sides of the lung increases the reliability of biopsy. The disadvantages of biopsy are that it is an invasive technique, although in our study complications were minimal. Only one horse bled from the nose following lung biopsy, and there were no permanent complications. Complications with hemorrhage were less frequent than reported previously (6), which may be due to the fact that the biopsy was taken rapidly and only from the peripheral areas of the lung.

Some researchers have suggested that horses with COPD can be divided into two populations based on whether they have an eosinophilic or neutrophilic exudate in their bronchoalveolar lavage fluid (2).

In our study, all horses had a neutrophilic exudate. Eosinophils were not seen in the lung biopsies. These findings are consistent with other studies indicating that COPD can be caused by a type III mediated hypersensitivity to *Micropolyspora faeni* and possibly *Aspergillus fumigatus* (2,9,18,21). In type III hypersensitivity, neutrophils are attracted into the inflamed tissue as a result of activation of complement and other neutrophil chemoattractants (18).

The majority of cases of COPD can be correctly diagnosed on physical examination. Bronchoalveolar lavage is useful in confirming the presence of pneumonopathy in doubtful cases. Transtracheal wash is more useful in detecting bacterial pneumonia (19). However, interpretation of the significance of bacterial cultures may be difficult and, in these horses, lung biopsy can be used to determine if COPD exists. It was difficult to prognosticate based on initial clinical and laboratory findings. In general, horses with the most severe clinical signs were most likely to be euthanized, but some horses with severe signs lived for more than four years following presentation. Many horses with COPD experienced few long-term problems following changes in management and an initial treatment with prednisone. It thus seems best to base the prognosis on the response to treatment rather than on the clinical or laboratory findings. In this study, management changes and treatment with prednisone or clenbuterol during high-risk periods helped many horses.

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References

1. Beech J. Chronic obstructive lung disease. In: Smith BP, ed. Large Animal Internal Medicine. St Louis: The CV Mosby Company, 1990: 533-536.
2. Derksen FJ. Chronic obstructive pulmonary disease. In: Beech J, ed. Equine Respiratory Disorders. Philadelphia: Lea & Febiger, 1991: 223-235.
3. Viel L. Diagnostic procedures, prognosis and therapeutic approaches of chronic respiratory diseases in horses. Can Vet J 1985; 26: 33-35.
4. Viel L, Valli VEO, Willoughby RA, McDonell WN. Bronchoalveolar lavage in evaluation of small airway disease (COPD) in light horses. Proc XXIII World Veterinary Congress, 1987: 11.8.5.
5. Mair TS, Stokes CR, Bourne FJ. Cellular content of secretions obtained by lavage from different levels of the equine respiratory tract. Equine Vet J 1987; 19: 458-462.
6. Raphael CF, Gunson DE. Percutaneous lung biopsy in the horse. Cornell Vet 1981; 71: 439-448.
7. Kaup FJ, Drommer W, Damsch S, Deegen E. Ultrastructural findings in horses with chronic obstructive pulmonary disease (COPD). II: Pathomorphological changes of the terminal airways and the alveolar region. Equine Vet J 1990; 22: 349-355.
8. Kaup FJ, Drommer W, Deegen E. Ultrastructural findings in horses with chronic obstructive pulmonary disease (COPD). I: Alterations of the larger conducting airways. Equine Vet J 1990; 22: 343-348.
9. Winder NC, von Fellenberg R. Chronic small airway disease in the horse: Immunohistochemical evaluation of lungs with mild, moderate and severe lesions. Vet Rec 1988; 122: 181-183.
10. Roudebush P. Lung sounds. J Am Vet Med Assoc 1982; 181: 122-126.
11. Clarke AF, Madelin T. Technique for assessing respiratory health hazards from hay and other source materials. Equine Vet J 1987; 19: 442-447.
12. Clarke AF. Air hygiene and equine respiratory disease. In Practice 1987; 9: 196-204.
13. Wilkinson L. SYSTAT: The system for statistics. Evanston, Illinois: SYSTAT Inc., 1988.
14. Fogarty U. Evaluation of a bronchoalveolar lavage technique. Equine Vet J 1990; 22: 174-176.
15. Darien BJ, Brown CM, Walker RD, Williams MA, Derksen FJ. A tracheoscopic technique for obtaining uncontaminated lower airway secretions for bacterial culture in the horse. Equine Vet J 1990; 22: 170-173.
16. Brain JD, Frank R. Alveolar macrophage adhesion: wash electrolyte composition and free cell yield. J Appl Physiol 1973; 34: 75-80.
17. Derksen FJ, Scott JS, Miller DC, Slocombe RF, Robinson NE. Bronchoalveolar lavage in ponies with recurrent airway obstruction (heaves). Am Rev Desp Dis 1985; 132: 1066-1070.
18. Derksen FJ, Robinson NE, Scott JS, Stick JA. Aerosolized *Micropolyspora faeni* antigen as a cause of pulmonary dysfunction in ponies with recurrent airway obstruction (heaves). Am J Vet Res 1988; 49: 933-938.
19. Rossier Y, Sweeney CR, Ziemer EL. Bronchoalveolar lavage fluid cytologic findings in horses with pneumonia or pleuropneumonia. J Am Vet Med Assoc 1991; 198: 1001-1004.
20. Mair TS, Sweeney CR. Advances in the diagnosis of equine lung disease: sampling from the lower airways. Equine Vet J 1990; 22: 147-148.
21. Lawson GHK, McPherson EA, Murphy JR, *et al.* The presence of precipitating antibodies in the sera of horses with chronic obstructive pulmonary disease (COPD). Equine Vet J 1979; 11: 172-176.