Received: 5 January 2024 | Accepted: 5 April 2024

DOI: 10.1111/jvim.17084

STANDARD ARTICLE

Journal of Veterinary Internal Medicine Open Access

ACVIM American College of Veterinary Internal Medicine

Clinical findings and outcome predictors for multinodular pulmonary fibrosis in horses: 46 cases (2009-2019)

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Abstract

Background: Prognostic indicators for equine multinodular pulmonary fibrosis (EMPF), an interstitial fibrosing lung disease, are poorly described.

Hypothesis/Objectives: Describe diagnostic findings and outcome predictors for EMPF.

Animals: Forty-six adult horses with EMPF.

Methods: Retrospective multicenter case series from 2009 to 2019. Radiographic (n = 27) and ultrasonographic studies (n = 19) from EMPF horses and bronchoalveolar lavage fluid (BALF) cytology from 6 EMPF and 13 asthma cases were independently reviewed and blinded to diagnosis and outcome. Associations between predictor variables and survival were assessed by predictor screening followed by Fisher's exact and Wilcoxon rank sum tests.

Results: Primary clinical findings were weight loss (36/46, 78%), increased respiratory effort (33/46, 72%), tachypnea (32/46, 70%), and fever (18/46, 39%). Macrophage atypia was seen in more EMPF than asthmatic horse BALF (67% vs. 8%; $P = .02$). Equine herpesvirus 5 (EHV-5) was detected in 24 of 30 (80%) and hyperfibrinogenemia in 25 of 28 (89%) cases. Twenty-seven of 46 horses (59%) and 11 of 45 (24%) survived to discharge and to 3 months, respectively. Three-month survival was associated with lower median (range) respiratory rates (30 [24-36] vs. 41 [30-60] breaths per minute; $P = .04$), and higher BALF lymphocyte:neutrophil ratios (4.7 [1.4-22] vs. 0.47 [0.11-1.9]; $P = .01$) and blood lymphocyte counts (1.25 [0.93-2.55] vs. 0.90 [0.70-1.24] $\times 10^9$/L; $P = .03$). Imaging findings, EHV-5 detection, and corticosteroid treatment were not associated with survival.

Conclusions and Clinical Importance: Fever is not a sensitive clinical sign of EMPF. Diagnostic testing should be pursued for horses with increased respiratory rate and

Abbreviations: ACVIM, American College of Veterinary Internal Medicine; AHV, asinine herpesvirus; BALF, bronchoalveolar lavage fluid; brpm, breaths per minute; EHV, equine herpesvirus; EMPF, equine multinodular pulmonary fibrosis; ICS, intercostal space; TNCC, total nucleated cell count; TW, tracheal wash.

effort and weight loss. The prognosis for EMPF horses is poor. Corticosteroid treatment does not improve 3-month survival.

KEYWORDS
asthma, equine herpesvirus-5 (EHV-5), bronchoalveolar lavage, tracheal wash

1 | INTRODUCTION

Equine multinodular pulmonary fibrosis (EMPF) is a clinical syndrome characterized by interstitial fibrosis causing a diffuse miliary or nodular pulmonary pattern on radiographic imaging.[1,2] An etiologic association has been made with the gammaherpesvirus equine herpesvirus-5 (EHV-5), based on virus isolation or detection of viral DNA in naturally infected cases,[2-6] experimental infections with EHV-5 resulting in pulmonary fibrosis in horses,[6] and evidence that gammaherpesviruses also contribute to pulmonary fibrosis in humans and mice.[7-9] However, some cases that have imaging and histologic features resembling EMPF but test negative for EHV-5.[10,11] Asinine herpesvirus-5 and EHV-2 have been detected in lung tissue of horses with a diagnosis of EMPF, suggesting additional possible etiologic agents.[4,12] Although many practitioners use the term EMPF specifically to indicate EHV-5-associated pulmonary fibrosis, others use it as an umbrella diagnosis, including any cause of nodular pulmonary fibrosis. Definitive antemortem diagnosis of EMPF is challenging, requiring a biopsy of pleural nodules and histopathologic assessment to confirm fibrosis. Detection of EHV-5 DNA by PCR in lung nodules or bronchoalveolar lavage fluid (BALF) or in situ hybridization of lung tissue is considered supportive of the diagnosis. However, lung nodules are not always present at the pleural surface for antemortem biopsy, especially early in the disease. In the absence of biopsy, a presumptive diagnosis of EMPF typically is made on finding a miliary or nodular pattern on thoracic imaging, BALF neutrophilia, and positive BALF EHV-5 PCR results. However, when EHV-5 testing is negative and biopsy is not performed, it can be difficult to distinguish between EMPF and other pulmonary conditions.

Obtaining an accurate diagnosis of EMPF is important because its treatment and prognosis can be substantially different from other pulmonary conditions with similar clinical presentations, such as severe asthma, bacterial or fungal pneumonia, or neoplasia. The case fatality rate of EMPF was high in 1 study, with reported short- and long-term survival of 57% and 14%, respectively.[11] The study found that short-term survival was associated with corticosteroid treatment but not EHV-5 viral load or severity of pulmonary lesions on thoracic radiographs. However, the study was hampered by low case numbers (14) and a requirement for histopathologic diagnosis for inclusion, perhaps biasing cases toward more severe disease and limiting the available prognostic information for EMPF.

In our study, clinical and diagnostic findings were retrospectively evaluated to determine prognostic indicators for EMPF. The description of clinical signs, hematologic and BALF cytologic results, and sonographic and radiographic findings were expanded beyond prior reports,[1,2,11,13] and short- and long-term survival were quantified in a larger cohort of horses than previously reported.[11] Because BALF neutrophilia is common to both EMPF[14] and equine asthma[15] and these diseases can have similar clinical presentations,[11,15] a detailed BALF cytologic analysis was done in horses with EMPF and asthma in an effort to identify features that could help distinguish these disorders or prompt testing for EMPF. Overall, the severity of clinical signs, imaging findings, increases in hematologic markers of inflammation, and BALF neutrophilia were hypothesized to be prognostic indicators.

2 | MATERIALS AND METHODS

2.1 | Animals

Cases were identified by contacting diplomates of the American College of Veterinary Internal Medicine (ACVIM) and the European College of Equine Internal Medicine who specialize in large animal medicine using the ACVIM listserv. Inclusion criteria were horses >2 years old, that were presented between 2009 and 2019 with increased respiratory rate, effort, or both, and that had a diagnosis of EMPF made by the attending clinician. Diagnoses were defined by clinicians in the field, therefore, biopsy with histologic confirmation of fibrosis was not required for inclusion. Six institutions performed a medical record review, using the search terms EMPF, pulmonary fibrosis, multinodular, and EHV-5 to identify cases (n = 41 cases). Three additional hospitals contributed a total of 5 cases without a full medical record search.

2.2 | Data collection

Clinical and diagnostic information was collected from the medical records (Table S1). Available radiographic images and BALF cytologic slides were independently reviewed.

2.3 | Image analysis: Independent review

Standing lateral radiographs and thoracic ultrasound images (static images and cine loops), obtained by the attending hospital at the time of initial examination and periodic follow-up examinations, were provided to the study investigators in digital imaging and communications in medicine or JPEG format and uploaded into picture archiving and communication system (PACS) software utilized by the Cornell

University Hospital for Animals (Carestream VuePACS; Rochester, NY). A second-year imaging resident with >10 years of clinical practice experience (ATD), blinded to ancillary diagnostic test results and clinical outcomes, evaluated the images on medical diagnostic-quality monitors (Dell U3219Q; Dell Technologies, Round Rock, TX). A qualitative evaluation of image quality was performed, as previously described[16] with input from the evaluator's clinical experience; poor-quality images were excluded.

The thorax was divided into 4 quadrants at the level of the carina.[16] Each quadrant was scored based on an ordinal system adapted from previous studies[11,16] to include the presence and severity of pulmonary nodules and the presence or absence of tracheobronchial lymphadenopathy. Each quadrant was scored for the presence and severity of alveolar, interstitial, and bronchial patterns and summed as a quadrant score. A total score was created by summation of each quadrant score plus a score for tracheobronchial lymphadenopathy (Table S2). The total score was corrected by dividing by the number of evaluated quadrants, and the corrected total score was used for analysis. For horses with multiple studies, all were assessed but only the first study was used in statistical analysis.

For clinical evaluation of ultrasonographic findings, all submitted images were evaluated for quality; those of poor quality or studies lacking images from at least 2 intercostal spaces (ICS) were not included. To our knowledge, a formal ultrasonographic scoring system has not been previously used for the evaluation of thoracic ultrasonographic images in horses, thus an ordinal scoring system was developed. Variables scored were: B-lines, nodules (<2 cm), consolidation or masses (>2 cm), and pleural margin thickness (Table S3).[17-24] The scores for pleural thickness were extrapolated from an abstract investigating normal pleural thickness in horses.[25] Images of normal ICS were not consistently recorded. Corrected scores (total score divided by the number of ICS evaluated) were tallied for each category and combined as a sum for analysis. For horses with multiple studies, all were assessed but only the first study was used in statistical analysis.

2.4 | Bronchoalveolar lavage and tracheal wash analysis in EMPF and asthma horses: Independent review

Available archived BALF cytospin (n = 6) and tracheal wash (TW) slides (n = 4) of EMPF cases from 1 institution (Cornell University) were reviewed by a board-certified clinical pathologist (T.S.) and compared to a control group of horses with asthma. The clinical pathologist was blinded to diagnosis and outcome. A random number generator was used to select 13 asthma cases for a 2:1 ratio of enrollment for BALF. The diagnosis of asthma was obtained from the medical record and based on a combination of clinical signs and the presence of inflammation in BALF (per consensus criteria[15]) or TW slides (>20% neutrophils[26]). Recorded results for BALF were total nucleated cell count (TNCC) and red blood cell counts, differential leukocyte percentages (400 cells) and morphologic features of macrophages, lymphocytes, and epithelial cells, when present, as medians or proportion of affected horses (Figure S1; Table S4). Recorded results for TW slides were differential leukocyte percentages (100 cells), the proportion of horses with mucus spirals, and specific macrophages (erythrophages, hemosiderophages, multinucleation, macro- or microvacuolation, phagocytic activity), or epithelial (atypia, necrosis) features. Epithelial atypia was defined as cells with cuboidal shapes or darker blue (more basophilic) cytoplasm than normal, or cells demonstrating anisokaryosis, blunted cilia, or both.

2.5 | Statistical analysis

Data were summarized using descriptive statistics and reported as median and range. Median results for BALF and TW findings in EMPF and asthma cases were compared using a Mann-Whitney *U* test. A Fisher's exact test was used to compare the proportions of horses in EMPF and asthma groups with BALF or TW results coded as present or absent.

Factors affecting survival were investigated using predictor screening in JMP (Version JMP Pro 14.0. SAS Institute Inc., Cary, NC, 1989-2021), which uses bootstrap forest partitioning to evaluate the contribution of predictors to the response. Predictor screening can identify predictors that might be weak alone but strong when used in combination with other predictors. Results can vary slightly on repeat iterations, therefore, the analysis was performed 4 independent times and the top 10 consistent predictors in 3 to 4 analyses are reported. Results were compared to bivariate analyses by Fisher exact or Wilcoxon rank sum tests followed by Benjamini-Hochberg correction for multiple comparisons with a false discovery rate of 0.05. Outcomes of short- and long-term survival were analyzed separately. Categorical predictors were: breed, sex, institution, BALF EHV-5 PCR, lung tissue EHV-5 PCR, historical fever, fever at admission, weight loss, nasal discharge, respiratory effort or distress or both, nodular, interstitial, miliary, or bronchointerstitial pulmonary radiographic patterns, nodular ultrasonographic lung patterns, antiviral treatment, and corticosteroid treatment. Continuous predictors were: age, duration of clinical signs, maximal rectal temperature, respiratory rate, peripheral blood total and differential leukocyte counts, plasma fibrinogen concentration as a percentage of the upper reference limit, BALF neutrophil, lymphocyte, macrophage, and mast cell percentages, BALF lymphocyte:neutrophil ratio, and corrected radiography and ultrasonography scores. Serum amyloid A concentration was excluded from predictor analysis because of low case numbers with this data. Where serial data was available, results from the first diagnostic test were used for statistical analysis (eg, for radiography scores). Significance was set at $P < .05$.

3 | RESULTS

3.1 | Signalments

Records of 46 horses from 9 clinics were obtained for analysis. Cases originated from the United States, Canada, Spain, Switzerland, and the

Netherlands. One of these cases was included in a previous publication.[10] There were 24 of 46 (52%) mares, 1 of 46 (2%) stallions, and 21 of 46 (46%) geldings. The median age was 15 years (range, 5-26 years). The breeds consisted of 13 of 46 (28%) Thoroughbreds, 19 of 46 (41%) Warmbloods, 4 of 46 (8%) Quarter Horses, and 10 of 46 (22%) other breeds.

3.2 | Diagnosis of EMPF

A definitive diagnosis of pulmonary fibrosis was established by histopathologic examination of biopsied (16/46, 35%) or necropsy-obtained (26/46, 57%) lung tissue or both in 34 of 46 (74%) cases. The 12 presumptive cases were diagnosed based on clinical findings of respiratory disease, pulmonary nodules on thoracic imaging, and the exclusion of bacterial pneumonia. Equine herpesvirus 5 DNA was detected in 24 of 30 (80%) tested cases in BALF (14/20, 70%), TW (4/7, 57%), lung tissue (7/11, 64%), or some combination of these. Of the 30 horses tested for EHV-5 DNA, 18 of 23 (78%) with a histopathologic diagnosis of EMPF tested positive for EHV-5, whereas 6of 7 (86%) with a presumptive diagnosis tested positive for EHV-5. Paired blood and nasal swab PCR[14] tests were not performed on any horse.

3.3 | Clinical examination and clinicopathologic testing

Median duration of clinical signs before presentation was 28 days (range, 5-360 days), with 2 horses having an unknown duration of signs. Eleven of 46 (24%) horses had a presumptive diagnosis of asthma before referral. In most cases this diagnosis was based on clinical examination, but 1 diagnosis was based on a BALF cytologic finding of neutrophilia.

Clinical signs at presentation included weight loss (36/46, 78%), increased respiratory effort (33/46, 72%), tachypnea >24 breaths per minute (brpm; 32/46, 70%), fever >101.5°F (18/46, 39%), nasal discharge (15/46, 33%), and respiratory distress (6/46, 13%). The median highest recorded rectal temperature during hospitalization was 102.6°F (range, 98.7-106.2°F). Many horses had an inflammatory or stress leukogram (Table 1), characterized by neutrophilia (23/36, 64%) with or without a left shift or monocytosis. Lymphopenia also was identified in 23 of 36 (64%) tested horses (Table 1). Twenty-five of 28 (89%) tested horses had hyperfibrinogenemia at admission, with a median plasma fibrinogen concentration of 6 g/L (range, 3.0-10.8 g/L). Serum amyloid A concentration was increased in all 11 cases in which this test was performed (median, 1123 mg/L; range, 406-2500 mg/L).

3.4 | Diagnostic imaging results

Radiographic imaging was performed in 41 horses and was bilateral (8/41, 20%), left- (22/41, 54%), or right- (11/41, 27%) sided. Observed lesions were reported as nodular (29/41, 71%), miliary (6/41, 15%), interstitial (32/41, 78%), and bronchointerstitial (23/41, 56%) patterns with most horses (32/41, 78%) having a combination of at least 2 patterns.

Thoracic sonography was performed in 37 cases, and nodules were observed in 22 cases (22/37, 59%). Pulmonary hypertension has been reported as a complication of EMPF,[1,11] and pulmonary artery diameter was examined in 5 cases, 4 of which were normal (80%). One horse had an enlarged right atrium and dilated pulmonary artery, suggesting pulmonary hypertension.

3.5 | Diagnostic imaging results: Independent review

Twenty-four radiographic studies from 5 institutions were independently evaluated. Of these, 16 of 24 (67%) consisted of complete radiographic studies that included the entire thorax, whereas 8 of 24 (33%) were incomplete, with the most commonly missing regions being craniodorsal (8/24, 100%) and cranioventral (4/24, 50%). Image quality was mostly good (9/24, 38%) to fair (11/24, 46%), with a few poor studies (4/24, 17%). The latter were excluded from further evaluation. Assessment of the remaining 20 thoracic imaging studies showed the following radiographic patterns: interstitial (20/20, 100%; Figure 1A), bronchial (15/20, 75%; Figure 1B), alveolar (14/20, 70%;

TABLE 1 Total and differential peripheral blood leukocyte counts for 37 cases of equine multinodular pulmonary fibrosis.

Cell type	Reference interval ($\times 10^9$ cells/L)	Case cell count ($\times 10^9$ cells/L)	Number (%) above interval	Number (%) below interval	n
White blood cells	5.2-10.1	10.8 (3.0-35.8)	20 (54%)	5 (14%)	37
Segmented neutrophils	2.7-6.6	8.2 (2.2-30.8)	22 (61%)	2 (6%)	36
Band neutrophils	0-0.1	0 (0-1.3)	7 (19%)	-	36
Lymphocytes	1.2-4.9	1 (0.3-4.9)	0	23 (64%)	36
Monocytes	0-0.6	0.3 (0-1.4)	6 (17%)	-	36
Eosinophils	0-1.2	0.1 (0-0.8)	0	-	36
Basophils	0-1.0	0.0 (0-0.9)	0	-	36

Note: Results are expressed as median (range) and number (percentage, %) of cases with results above or below the upper or lower reference limits, respectively. A differential leukocyte count was not available in 1 case.

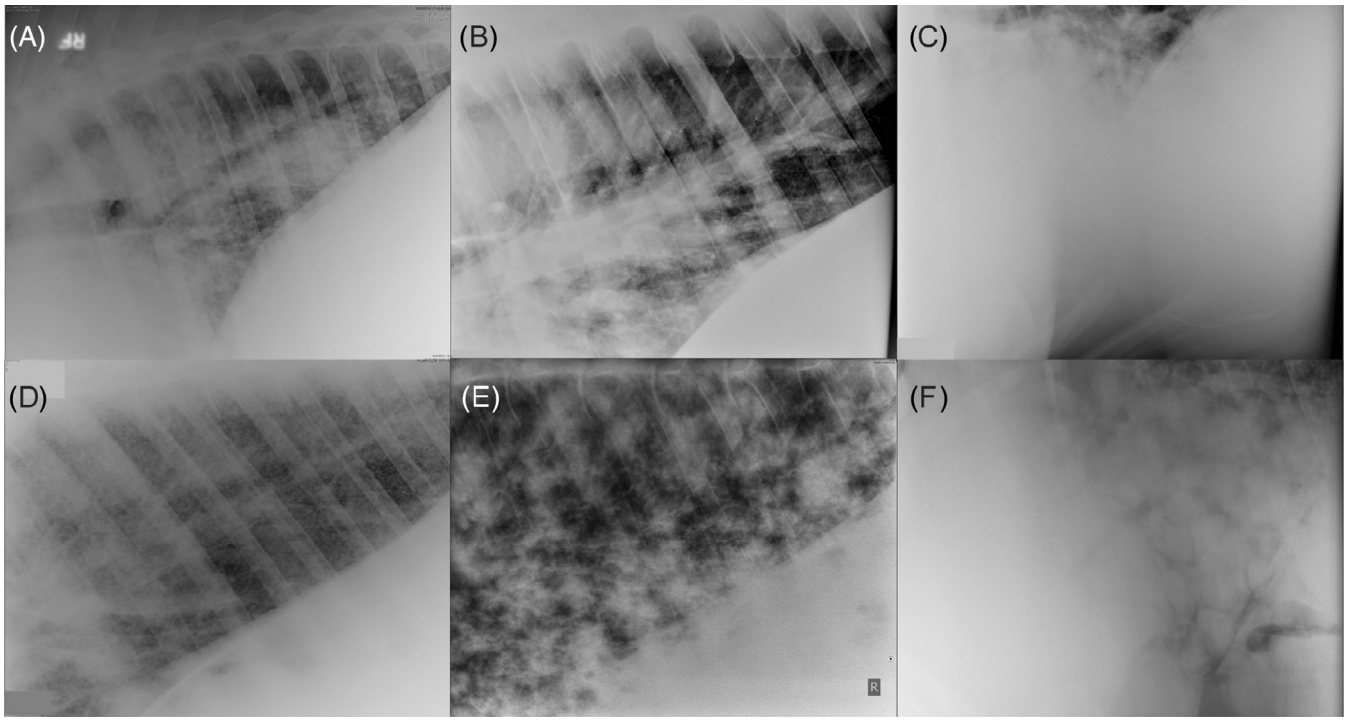

FIGURE 1 Examples of radiographic patterns observed in 20 horses with equine multinodular pulmonary fibrosis. A, Moderate interstitial pulmonary pattern in the caudodorsal lung field, with a mild bronchial component and a few, small, poorly discerned soft tissue opaque nodules. B, Moderate caudodorsal bronchial pulmonary pattern with a mild interstitial component. C, Severe caudoventral alveolar pulmonary pattern. D, Severe interstitial pattern with a micronodular (miliary) component. E, Innumerable, small to medium, poorly circumscribed, soft tissue opaque nodules with mild to moderate interstitial and mild bronchial patterns. F, Random innumerable, small to medium, well-circumscribed, soft tissue opaque nodules distributed diffusely through the lung field.

Figure 1C), and nodular (16/20, 78%; Figure 1D-F). Tracheobronchial lymphadenopathy was not assessable in 4 horses because of region effacement and was not detected in 11 of 16 horses (69%). The corrected scores were 4.9 (range, 0.5-8.5; Table 2). When present, alveolar patterns were predominantly noted in the caudoventral lung. Notably, 4 horses in the EMPF group did not have pulmonary nodules detected on radiography (4/20, 20%). Three of those 4 had nodules observed at the pleural surface sonographically and were confirmed to have fibrosing interstitial pneumonia by histopathology. The fourth horse without radiographic pulmonary nodules did not have histopathology performed but was EHV-5 positive in the BALF. Most studies exhibited mixed radiographic patterns consisting of ≥2 patterns (2 patterns, 3/20, 15%; 3 patterns, 7/20, 35%; 4 patterns, 9/20, 45%). Overall, the most prominent radiographic patterns, based on the highest total corrected score per pulmonary pattern, were nodular and interstitial (Table 2), with bronchiolar patterns being mild. Six horses had repeat thoracic radiographs 1 day to 8 months apart, which also were evaluated independently. Total corrected score improved in only 1 horse (1/6, 17%), from 5.3 to 2 after 3 months of treatment with prednisolone.

Twenty-two thoracic ultrasonographic studies from 4 institutions were independently evaluated. Five studies (5/22, 23%) were excluded because of insufficient (<2) ICS. Of the remaining 17 studies, the majority consisted of a combination of static images and cine loops (11/17, 65%), whereas fewer contained only static images (3/17, 18%) or only cine loops (3/17, 18%). Most studies provided images from both the left and right thorax (14/17, 82%), whereas fewer provided unilateral images (3/17, 18%). The quality of images was assessed as good (13/17, 76%) to fair (4/17, 24%), with none being of poor quality. All assessed horses had B-lines (Figure 2A) and nodules <2 cm (Figure 2B), whereas 12/17 (71%) horses had masses or regions of consolidation >2 cm (Figure 2C), and 9/17 (53%) had abnormal pleural thickness (Figure 2D). The median total corrected score was 3.6 (range, 2.4-5.7; Table 3). The horses with abnormal pleural margin thickness were

TABLE 2 Independent interpretation of thoracic radiographic imaging studies in 20 horses with equine multinodular pulmonary fibrosis, showing the proportion (%) of animals with specific radiographic patterns or lymphadenopathy and median (range) corrected individual and total scores.

	Proportion	Corrected score median (range)
Interstitial	20/20 (100%)	1.5 (0.5-2.5)
Bronchial	15/20 (75%)	0.25 (0-2)
Alveolar	14/20 (70%)	0.63 (0-3)
Nodular	16/20 (78%)	2 (0-4)
Tracheobronchial lymphadenopathy	4/20 (20%)	
Total		4.9 (0.5-8.5)

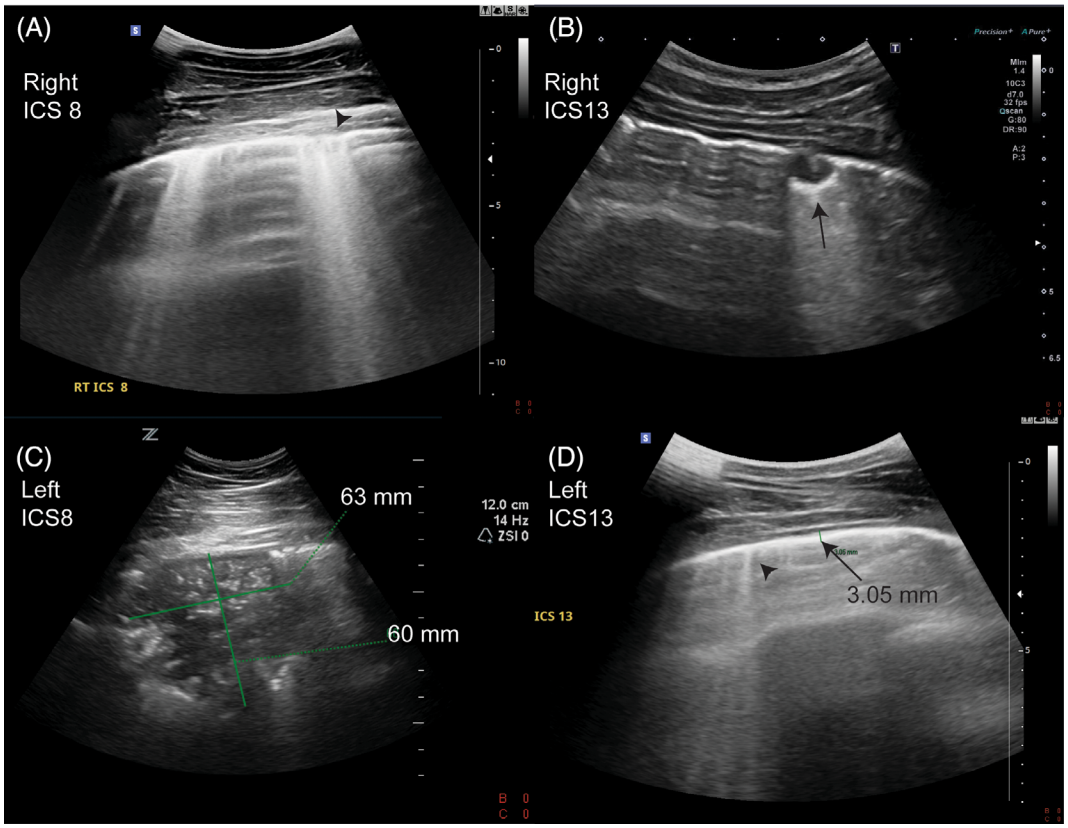

FIGURE 2 Examples of sonographic patterns observed in 17 horses with equine multinodular pulmonary fibrosis. A, Multiple B-lines with a mildly irregular pleural margin (arrowhead). B, Small, solitary, well-demarcated, pulmonary nodule (arrow). The adjacent pleural margin (toward left side of image) is mildly irregular. C, A large (>6 cm), irregularly margined, pulmonary consolidation or mass. D, Pleural thickness (combined parietal and visceral) is mildly increased (arrow, 3 mm) with a few B-lines (arrowhead).

TABLE 3 Independent interpretation of thoracic ultrasonographic imaging studies from 17 horses with equine multinodular pulmonary fibrosis, showing the proportion (%) of animals with specific results and median (range) corrected individual and total scores.

	Proportion	Corrected score median (range)
B-lines	17/17 (100%)	1.71 (0.6-2.6)
Nodules	17/17 (100%)	1.2 (0.67-2)
Consolidation	12/17 (71%)	0.25 (0-1.7)
Pleural thickness	9/17 (53%)	0.07 (0-1.3)
Total		3.6 (2.4-5.7)

marginally (defined as total corrected score 0-0.9; 5/9, 56%) or mildly thickened (defined as total corrected score 1-1.9; 4/9, 44%).

3.6 | Bronchoalveolar lavage fluid and TW results

Bronchoalveolar lavage fluid analysis was performed on 23 horses. Most horses had neutrophilic inflammation with low numbers of mast cells and eosinophils (Table 4). Cytologic descriptions were available for 15 cases. Neutrophils were described as non-degenerate in most cases (11/14, 79%, 1 did not describe neutrophil morphology), with a few cases having descriptions of granular lymphocytes (4/15, 27%). Microvacuolated macrophages were described in 6 of 15 cases (40%), all but 1 of which were from Cornell University.

Tracheal wash cytologic analysis and aerobic and anaerobic cultures were performed on 27 horses. Inflammation was seen in 17 of 27 (63%) horses and was neutrophilic (10/17, 59%) or mixed (neutrophils and macrophages; 7/17, 41%). Neutrophils were described as degenerate in 10 of 17 horses (59%). Twelve horses (12/27, 44%) had positive bacterial cultures. Aerobic cultures were positive in 11 samples with mixed flora (4/11, 36%), *Nicotella semolina* (2/11, 18%), and 1 case each with *Streptococcus equi* subsp *zooepidemicus*, alpha-hemolytic *Streptococcus* spp., *Rhodococcus equi*, *Staphylococcus delphini* B, and *Pantoea* spp. Anaerobic cultures were positive in 2 samples (2/12, 17%), with *Peptostreptococcus* spp. in 1 case and rare *Prevotella* and *Lactobacillus* spp. in another case.

3.7 | Bronchoalveolar lavage fluid and TW results in EMPF and asthma horses: Independent review

The BALF and TW slides were examined in archived specimens from a single institution by a single-blinded clinical pathologist. In BALF, 5 of

Result	Consensus cut-off	Case results	Number (%) above cut-off	n
TNCC ($\times 10^6$/L)	<530	472 (146-8278)	6 (46%)	13
Neutrophils (%)	>10	27 (1-87)	17 (74%)	23
Lymphocytes (%)	NA	27 (0-75)	-	23
Macrophages (%)	NA	34 (3-88)	-	23
Eosinophils (%)	>5	0 (0-1)	0	23
Mast cells (%)	>5	1 (0-3)	0	23

TABLE 4 Total nucleated (TNCC) and differential cell counts in bronchoalveolar fluid in horses with equine multinodular pulmonary fibrosis (n = 23).

Note: Results are expressed as median (range) and number (%) of cases with results above consensus[15] criteria cut-offs for asthma. A TNCC was not available for 10 horses.
Abbreviation: NA, not available.

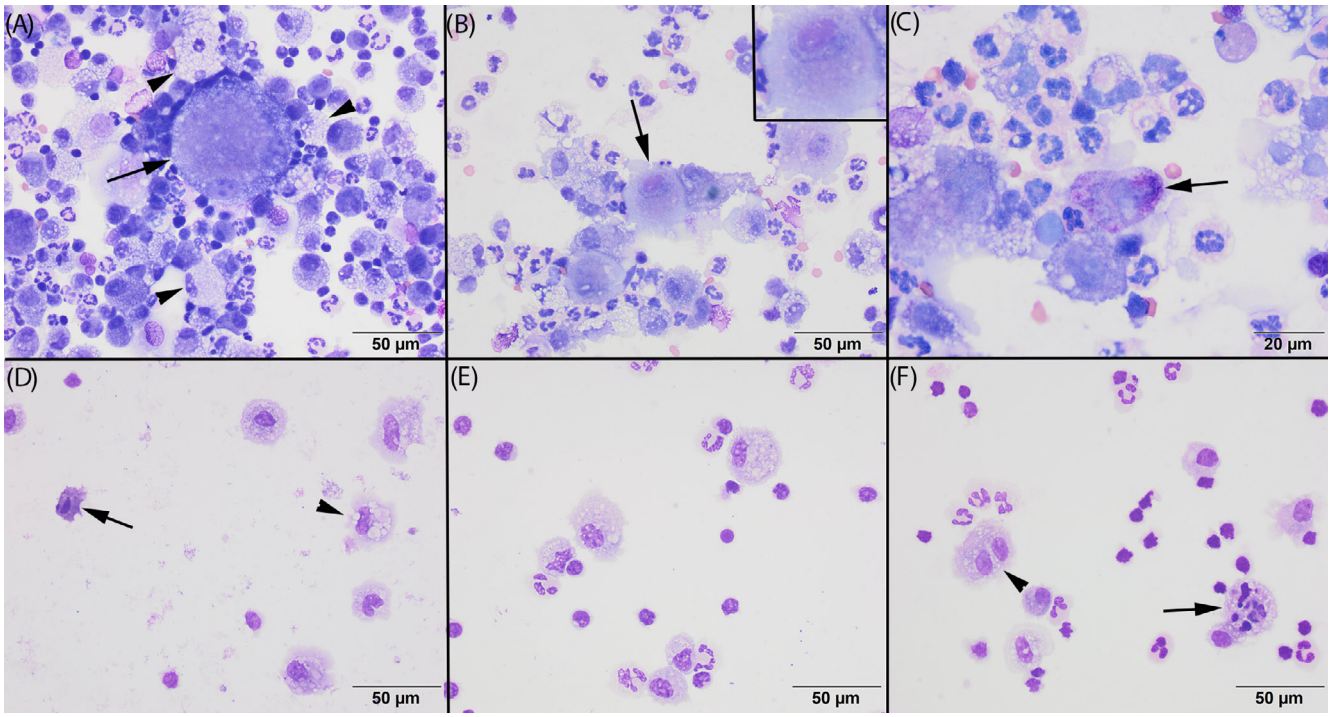

FIGURE 3 Examples of cytologic features of bronchoalveolar lavage fluid cells in horses with EMPF (n = 3) or asthma (n = 2). A, EMPF horse 1: A multinucleated macrophage with atypia (arrow; intracellular anisokaryosis, multiple prominent nucleoli within individual nuclei) and numerous microvacuolated macrophages (several labeled with arrowheads). B, EMPF horse 2: A large pink intranuclear inclusion is evident in the macrophage (arrow; inset: cropped image). Macrophages demonstrate atypia, including darker blue and more abundant cytoplasm than normal. C, EMPF horse 2: A macrophage with variably sized purple globular cytoplasmic material (arrow). This material was only seen in macrophages from EMPF horses. D, EMPF horse 3: A dying epithelial cell with dark purple cytoplasm and a pyknotic nucleus (arrow). Macrophages are lightly vacuolated with 1 macrovacuolated cell (arrowhead). E, Asthma horse 1: Lightly vacuolated macrophages, small non-reactive lymphocytes and increased neutrophils. F, Asthma horse 2: A leukophagocytic (arrow) and binucleated (arrowhead) macrophage. Macrophages are lightly vacuolated.

6 (83%) horses with EMPF had mixed inflammation (neutrophils and macrophages), with increased TNCC (>530 cells/µL[15]) in 3 of 6 (50%) horses. One horse had mild mastocytosis (3%; normal, ≤2%, below the cut-off for asthma in horses[15]) and epithelial necrosis as the only BALF abnormalities. In contrast, only 1 of the 13 (7.7%) horses with asthma had mildly increased TNCC, but 12 of 13 (92%) horses had neutrophilic (>10% neutrophils; 11/13, 85% of horses) or eosinophilic (>5% eosinophils; 1/13, 7.7% of horses) inflammation in the BALF.[15] The remaining horse had mild BALF neutrophilia (6.5% neutrophils) but had neutrophilic inflammation (94% neutrophils) in the TW and clinical signs compatible with asthma. With respect to macrophage features, there was no difference in the percentage of macrophages with macro- and micro-vacuolation, but phagocytic activity and macrophage atypia (larger or more prominent nuclei than normal, multiple nucleoli, anisokaryosis, intracellular anisokaryosis, ballooned cells, epithelioid cells, or some combination of these) were present in BALF smears of significantly more EMPF horses than asthmatic horses (Figure 3; Table 5). A single macrophage with a large pink intranuclear inclusion was identified in 1 horse with EMPF (Figure 3B). Macrophages with pink to purple globular material in their cytoplasm only were identified in EMPF cases (Figure 3C). Bacteria were not seen in any BALF smear.

TABLE 5 Cytologic results for bronchoalveolar fluid from horses with equine multinodular pulmonary fibrosis (EMPF; n = 6) or asthma (n = 13).

Test result	EMPF (n = 6)	Asthma (n = 13)	*P* value[a]
TNCC (× 10^6/L)	472 (146-8278)	275 (60-593)	.18
RBC count (× 10^6/L)	300 (3-4989)	10 (3-478)	.08
Differential cell count			
Neutrophils (%)	17 (2-56)	17 (4-70)	1.00
Lymphocytes (%)	25 (8-62)	32 (10-64)	.37
Macrophages (%)	42 (22-80)	37 (18-61)	.37
Mast cells (%)	0.2 (0-3)	1.3 (0-3.5)	.07
Eosinophils (%)	0 (0-0)	0 (0-6)	.26
Macrophage features (% of macrophages)			
No vacuolation	17 (4-33)	31 (4-84)	.29
Light vacuolation	50 (26-88)	61 (7-88)	.78
Macro-vacuolation	15 (4-33)	6 (1-21)	.11
Micro-vacuolation	4 (0-15)	1 (0-6)	.14
Macro- plus micro-vacuolation	19 (4-40)	3 (1-10)	.11
Phagocytic	**9 (2-29)**	**2 (0-5)**	**.001**[b]
Erythrophages	2/6 (33%)	1/13 (8%)	.22
Hemosiderophages	1/6 (17%)	1/13 (8%)	1.00
Binucleated	1 (0-4)	1 (0-8)	.92
Multinucleated	3 (1-10)	3 (1-9)	.95
Atypia	**4/6 (67%)**	**1/13 (8%)**	**.02**
Lymphocyte features (% of lymphocytes)			
Reactive	1 (0-19)	0 (0-2)	.35
Granular	13 (8-19)	16 (1-42)	.40
Epithelial cell death	2/6 (33%)	0/13 (0%)	.09

Note: Results are expressed as median (range) or number (%) of horses, with bivariate *P* values. Bolded results were significantly different between groups on single comparisons.
Abbreviations: RBC, red blood cell; TNCC, total nucleated cell count.
[a]Mann-Whitney *U* test for medians and Fisher's exact test for proportions.
[b]Remained significant with Benjamini-Hochberg test for multiple comparisons.

Tracheal wash smears from 4 EMPF and 7 asthmatic horses were reviewed by 1 blinded clinical pathologist. Slides from 1 horse with asthma were too poorly cellular for evaluation. Samples from all horses contained mucus, with or without mucus spirals, and featured neutrophilic inflammation, with low numbers of horses in each group having epithelial atypia (1 horse in each group) or necrosis (2/4, 50% EMPF horses, 1 asthmatic horse, Figure S2; Table S5). Rare bacteria were identified intracellularly in 1 horse with EMPF, which yielded *S. delphini* on aerobic culture. No significant differences were found when the results of the 2 groups were compared (Table S5), although macrophages with macro- or micro-vacuolation were only seen in TW smears from horses with EMPF.

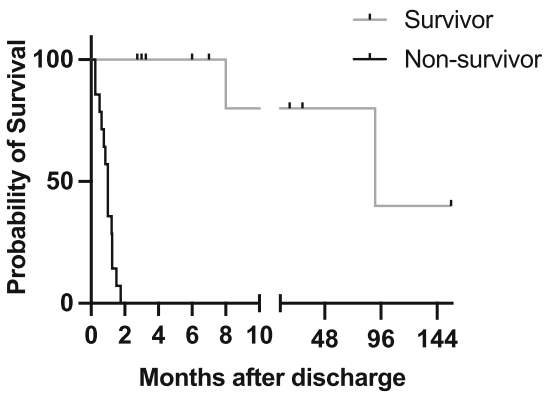

FIGURE 4 Survival times after discharge from hospital for horses with equine multinodular pulmonary fibrosis (n = 26). Horses are grouped by survival to 3 months after discharge. Tick marks indicate censoring at last follow-up.

3.8 | Histopathologic results from lung biopsies or necropsy examination

The histopathologic interpretation on lung biopsies from 18 horses was interstitial fibrosis with type II pneumocyte hyperplasia and alveolar inflammation, which was described as histiocytic (8/18, 44%), granulomatous (4/18, 22%), purulent (9/18, 50%), and lymphoplasmacytic (4/18, 22%) alone or in combination. Eosinophils and intranuclear inclusion bodies were only observed in 1 case each.

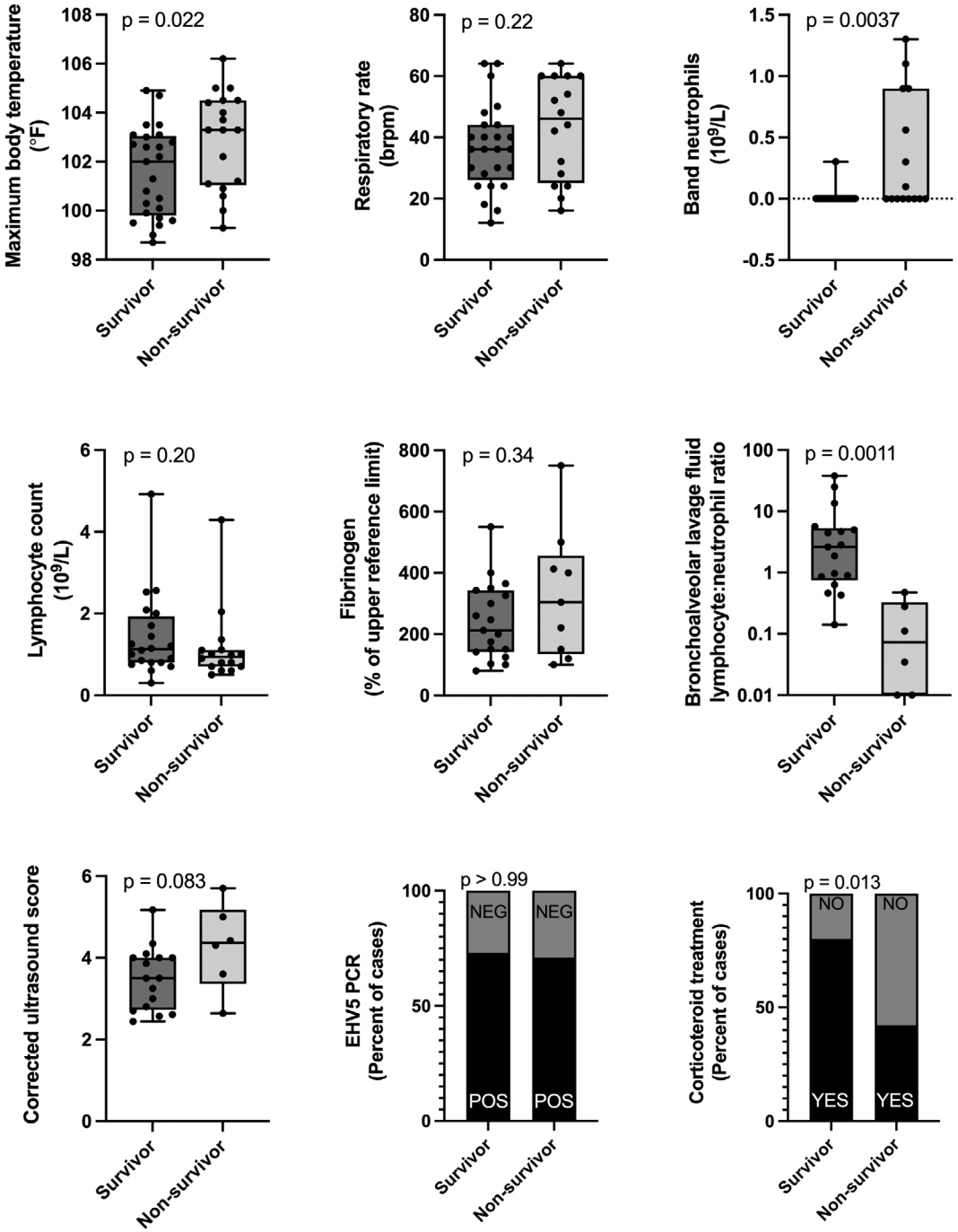

FIGURE 5 Selected test results as prognostic predictors for short-term survival in 46 horses with equine multinodular pulmonary fibrosis. Maximal body temperature, band neutrophil count, bronchoalveolar lavage fluid (BALF) lymphocyte:neutrophil ratio, and corticosteroid treatment remained significant with Benjamini-Hochberg test for multiple comparisons.

Necropsy was performed on 26 horses. Three horses only had brief gross examinations with findings of white firm nodules throughout the lungs, consistent with EMPF. Histopathologic descriptions were provided for 23 horses. The primary finding in all cases was nodular or multifocal to coalescing chronic interstitial fibrosis that was accompanied by type II pneumocyte hyperplasia in 10 of 23 (43%) horses. There was concurrent alveolitis, consisting of histiocytic (12/23, 52%), granulomatous (2/23, 9%), purulent (10/23, 43%; degenerate neutrophils in 2/23, 9%), and lymphocytic or lymphoplasmacytic (5/23, 22%) infiltrates alone or in combination. Alveolar macrophages were described as foamy in 3 cases (3/23, 13%; likely indicating micro-vacuolation). Rare intranuclear inclusions or epithelial syncytia were observed in 8 of 23 (35%) and 2 of 23 (9%) cases, respectively. Two horses had concurrent acute bacterial bronchopneumonia, including fibrinosuppurative pleuritis (1/23, 4%) and acute necrotizing bronchopneumonia (1/23, 4%). Necropsy cultures were not performed in either case.

TABLE 6 Prognostic predictors for short-term survival (to hospital discharge) in 46 horses with equine multinodular pulmonary fibrosis with bivariate analysis *P* values.

Test result	Non-survivor (n = 19)	Survivor (n = 27)	*P* value[a]
Clinical and demographic findings			
Institution	A—1/19, 5%	A—1/27, 4%	
	B—3/19, 16%	B—7/27, 26%	
	C—1/19, 5%	C—1/27, 4%	
	D—4/19, 21%	D—7/27, 26%	
	E—1/19, 5%	E—0/27, 0%	
	F—0/19, 0%	F—2/27, 7%	
	G—2/19, 11%	G—2/27, 7%	
	H—7/19, 37%	H—6/27, 22%	
	I—0/19, 0%	I—1/27, 4%	
Age (years)	15 (8-22)	15 (5-26)	
Breed	WB—9/19, 47%	WB—10/27, 37%	
	TB—5/19, 26%	TB—8/27, 30%	
	QH—2/19, 11%	QH—2/27, 7%	
	Other—3/19, 16%	Other—7/27, 26%	
Sex	Female—11/19, 58%	Female—13/27, 48%	
	Gelding—8/19, 42%	Gelding—13/27, 48%	
	Stallion—0/19, 0%	Stallion—1/27, 4%	
Duration of clinical signs (days)	29 (7-210)	21 (5-360)	
Historical fever	10/19, 53%	16/27, 59%	
Fever at admission	9/19, 47%	9/27, 33%	
Maximum rectal temperature	**103.3 (99.3-106.2)**	**102 (98.7-104.9)**	**.02**[b]
Weight loss	17/19, 89%	19/27, 70%	
Nasal discharge	7/19, 37%	9/27, 33%	
Increased respiratory effort	16/19, 84%	18/27, 67%	
Respiratory distress	5/19, 26%	1/27, 4%	
Respiratory rate (brpm)	46 (16-64)	36 (12-64)	.22
Hematology			
Total leukocytes ($\times 10^9$/L)	10.96 (3-35.79)	9.62 (4.46-20.5)	
Neutrophils ($\times 10^9$/L)	9.25 (2.2-30.78)	7.5 (3.3-19.2)	
Band neutrophils ($\times 10^9$/L)	**0 (0-1.3)**	**0 (0-0.3)**	**.004**[b]
Lymphocytes ($\times 10^9$/L)	0.94 (0.5-4.29)	1.13 (0.3-4.92)	
Monocytes ($\times 10^9$/L)	0.3 (0-0.92)	0.27 (0-1.4)	
Eosinophils ($\times 10^9$/L)	0.02 (0-0.4)	0.1 (0-0.8)	
Basophils ($\times 10^9$/L)	0.01 (0-0.9)	0.015 (0-0.4)	
Fibrinogen (% of max reference interval)	304 (100-750)	212 (80-550)	
Bronchoalveolar fluid (BALF) differential count			
Neutrophils (%)	57.5 (39-87)	18 (1-85)	
Lymphocytes (%)	4.5 (0-18.5)	38 (3-75)	
Macrophages (%)	33 (10-48.5)	34 (3-88)	
Mast cells (%)	0.5 (0-3)	1 (0-3)	
Lymphocyte:neutrophil ratio	**0.073 (0-0.47)**	**2.6 (0.14-38)**	**.001**[b]
Virology			
EHV-5 PCR+ BALF, TW, or biopsy	7/9, 78%	18/22, 82%	
EHV-5 PCR+ BALF	4/4, 100%	10/16, 63%	
EHV-5 PCR+ lung	1/4, 25%	7/10, 70%	

(Continues)

TABLE 6 (Continued)

Test result	Non-survivor (n = 19)	Survivor (n = 27)	*P* value[a]
Imaging			
Nodular pattern radiographs	14/17, 82%	15/24, 63%	
Miliary pattern radiographs	4/17, 24%	2/24, 8%	
Interstitial pattern radiographs	11/17, 65%	21/24, 78%	
Bronchointerstitial pattern radiographs	11/17, 65%	12/24, 50%	
Radiograph score with lymph nodes	17 (7-25)	16 (2-31)	
Radiograph score corrected	5.67 (2.5-8.5)	4.75 (0.5-8.5)	
Nodular pattern ultrasound	8/11, 73%	16/23, 70%	
Ultrasound score	43 (10-84)	26.5 (3-113)	
Ultrasound score corrected	4.37 (2.64-5.7)	3.5 (2.44-5.17)	.08
Treatment			
Antivirals	5/19, 26%	10/25, 40%	
Corticosteroids	**8/19, 42%**	**20/25, 80%**	**.01**[b]

Note: Results are expressed as median (range) or number (%) of horses. Bolded values represent significant differences between groups as single predictors.
Abbreviations: QH, Quarter Horse; TB, Thoroughbred; TW, tracheal wash; WB, warmblood.
[a]Wilcoxon rank sum for medians and chi-square or Fisher exact for proportions performed on top predictors by predictor response screening.
[b]Remained significant with Benjamini-Hochberg test for multiple comparisons.

3.9 | Treatment

Twenty-eight horses (28/46, 61%) were treated, and all were given corticosteroids (9/9 clinics), with 15 of 28 (54%) additionally being given antiviral medications (7/9, 78% of clinics). Dexamethasone was the most commonly used corticosteroid (22/28, 79%; median [range] duration, 19 [4-56] days), followed by prednisolone (6/28, 21%; median [range] duration, 15 [7-30] days). Inhaled fluticasone was added in 3 horses (3/28, 11%). Thirteen of 28 (46%) horses were treated with valacyclovir for a median (range) of 12 (2-30) days. One horse each was treated with acyclovir and ganciclovir. Information on adjunctive treatments was not collected.

3.10 | Outcome

Nineteen horses (19/46, 41%) were euthanized or died during hospitalization. A poor prognosis was the primary reason for euthanasia (10/46, 22%), followed by disease progression despite treatment (4/46, 9%). Two of 46 (4%) horses had complications of colic, diarrhea, and laminitis, leading to euthanasia. One horse developed acute respiratory distress and died. One client declined treatment and requested euthanasia, and no reason was provided in 1 case.

Of the 27 short-term survivors, 1 was lost to follow-up within 3 months, and 11 of 45 (24%) survived at least 3 months. An additional 15 of 45 horses (33%) died or were euthanized within 3 months after discharge. All deaths occurred within the first 2 months after discharge (Figure 4; median [range], 4 [1-7] weeks). One horse died suddenly 5 weeks after discharge, 11 of 45 (24%) horses were euthanized because of disease progression or treatment failure, 1 developed acute fulminant heart failure, and 1 was euthanized for other reasons at 5 weeks after discharge. Of the 11 of 45 (24%) long-term (>3 months) survivors, 1 had progressive disease and was euthanized 8 months after discharge. Another was euthanized 91 months after discharge and the reason was not recorded. The remaining 9 of 45 (20%) were alive at the last follow-up (median [range], 28 [11-676] weeks) and were reported as improved (6/45, 13%), not improved (1/45, 2%, at 3 months), or not described (2/45, 4%).

3.11 | Prognostic indicators

Predictor screening for factors associated with short-term survival identified maximal rectal temperature, respiratory rate, peripheral blood band neutrophil count, BALF neutrophil and lymphocyte percentages, corrected ultrasound score, and corticosteroid treatment as predictors. Because BALF neutrophil and lymphocyte percentages are interdependent variables, the ratio of BALF lymphocytes:neutrophils was considered in the bivariate analyses. Bivariate analyses confirmed lower maximal rectal temperature, lower band neutrophil count, higher BALF lymphocyte:neutrophil ratio, and corticosteroid treatment as predictors of short-term survival (Figure 5; Table 6). Maximal rectal temperature, band neutrophil count, BALF lymphocyte:neutrophil ratio, and corticosteroid treatment remained significant predictors of survival after correction for multiple comparisons.

Predictor response screening for factors associated with long-term survival identified institution, maximal rectal temperature, respiratory rate, blood lymphocyte count, BALF neutrophil and lymphocyte percentages, and BALF EHV-5 status as predictors (Figure 6; Table 7). Bivariate analyses confirmed that lower respiratory rates, higher blood lymphocyte counts, and higher BALF lymphocyte:neutrophil ratios

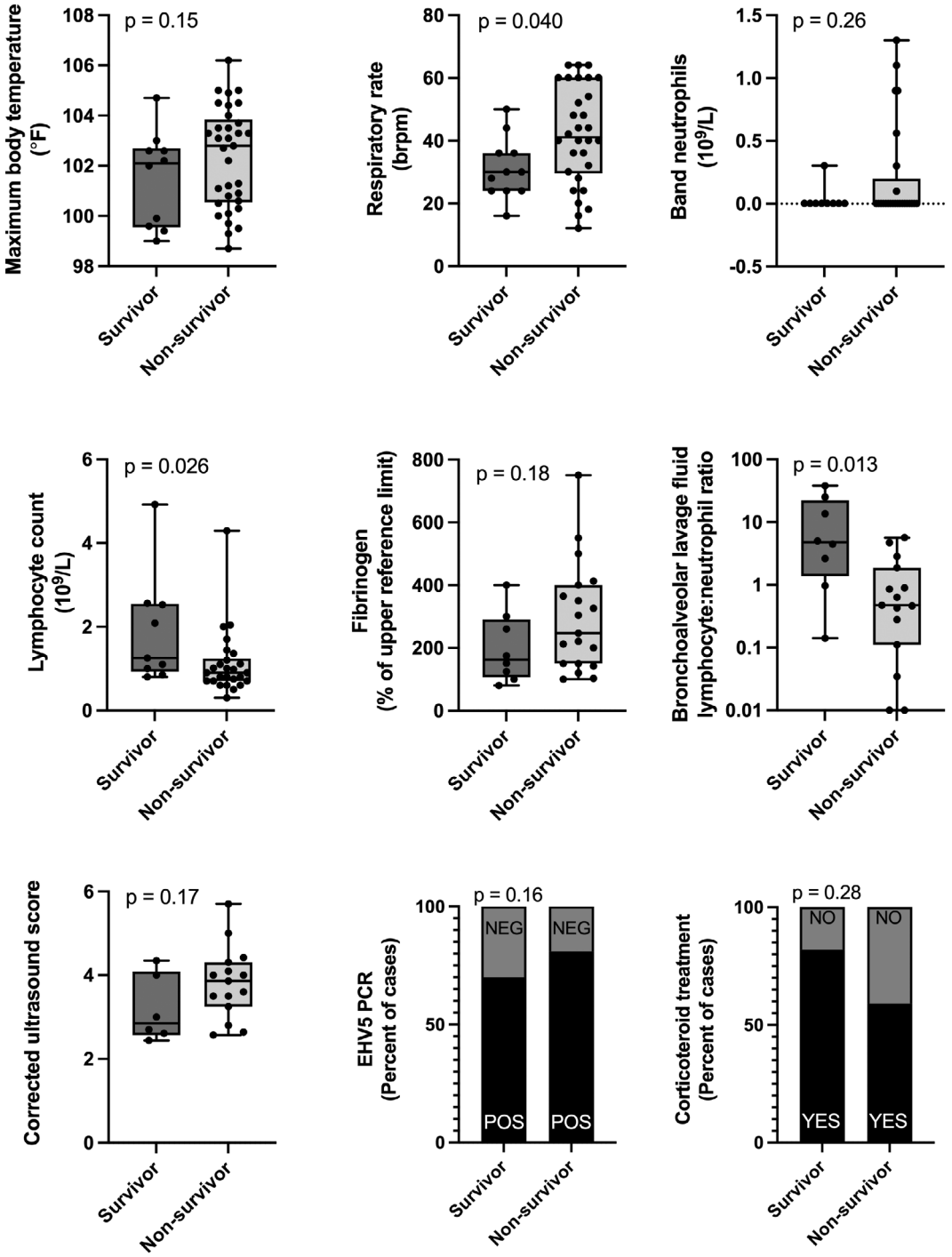

FIGURE 6 Selected test results as prognostic predictors for long-term survival in 45 horses with equine multinodular pulmonary fibrosis and 3-month follow-up. No predictor remained significant after application of the Benjamini-Hochberg test for multiple comparisons.

were associated with long-term survival, but significance was lost after correction for multiple comparisons (Table 7).

4 | DISCUSSION

Our study expands upon many previously published descriptions of EMPF, which were mostly individual case reports[4,5,10,27-33] with only a few case series of 3 to 25 horses.[1-3,11,13] Clinical signs, clinicopathologic findings, and imaging results associated with EMPF are emphasized, and the poor prognosis associated with EMPF is affirmed. Weight loss, fever, inflammatory CBC, or some combination of these should increase the suspicion of EMPF over asthma, although these findings were not consistently present. When BALF is performed, macrophage atypia should prompt the clinician to expand the differential diagnosis list beyond asthma and to consider additional testing for EMPF.

TABLE 7 Prognostic predictors for long-term survival (>3 months) in 45 horses with equine pulmonary nodular fibrosis with bivariate *P* values.

Test result	Non-survivor (n = 34)	Survivor (n = 11)	*P* value[a]
Clinical and demographic findings			
Institution	A—2/34, 6%	A—0/11, 0%	.25
	B—8/34, 24%	B—2/11, 18%	
	C—1/34, 3%	C—1/11, 9%	
	D—7/34, 21%	D—3/11, 27%	
	E—1/34, 3%	E—0/11, 0%	
	F—1/34, 3%	F—0/11, 0%	
	G—0/34, 0%	G—2/11, 18%	
	H—3/34, 9%	H—1/11, 9%	
	I—11/34, 32%	I—2/11, 18%	
Age (years)	15 (8-26)	14 (5-23)	
Breed	WB—15/34, 44%	WB—4/11, 36%	
	TB—9/34, 26%	TB—3/11, 27%	
	QH—4/34, 12%	QH—0/11, 0%	
	Other—6/34, 18%	Other—4/11, 36%	
Sex	Female—19/34, 56%	Female—5/11, 45%	
	Gelding—15/34, 44%	Gelding—5/11, 45%	
	Stallion—0/34, 0%	Stallion—1/11, 9%	
Duration of clinical signs (days)	28 (5-210)	36 (7-360)	
Historical fever	19/34, 56%	7/11, 63%	
Fever at admission	14/34, 41%	4/11, 36%	
Maximum rectal temperature	102.8 (98.7-106.2)	102.1 (99-104.7)	.15
Weight loss	28/34, 82%	7/11, 64%	
Nasal discharge	11/34, 32%	5/11, 45%	
Increased respiratory effort	26/34, 76%	8/11, 73%	
Respiratory distress	6/34, 18%	0/11, 0%	
Respiratory rate (brpm)	**41 (12-64)**	**30 (16-50)**	**.04**
Hematology			
Total leukocytes ($\times 10^9$/L)	11 (3-35.8)	8.4 (4.46-20.5)	
Neutrophils ($\times 10^9$/L)	8.95 (2.2-30.8)	7.87 (3.5-15.3)	
Band neutrophils ($\times 10^9$/L)	0 (0-1.3)	0 (0-0.3)	
Lymphocytes ($\times 10^9$/L)	**0.9 (0.3-4.29)**	**1.25 (0.8-4.92)**	**.03**
Monocytes ($\times 10^9$/L)	0.30 (0-1.4)	0.20 (0-0.96)	
Eosinophils ($\times 10^9$/L)	0.05 (0-0.6)	0.04 (0-0.8)	
Basophils ($\times 10^9$/L)	0 (0-0.9)	0.04 (0-0.21)	
Fibrinogen (% of upper reference limit)	247 (100-750)	163 (80-400)	.18
BALF differential count			
Neutrophils (%)	37 (7-87)	9.5 (1-85)	
Lymphocytes (%)	18.5 (0-62)	39 (12-75)	
Macrophages (%)	35 (10-88)	30.5 (3-70)	
Mast cells (%)	1 (0-3)	1.25 (0-3)	
Lymphocyte:neutrophil ratio	**0.47 (0-5.6)**	**4.7 (0.14-38)**	**.01**
Virology			
EHV-5 PCR+ BALF, TW, or biopsy	17/20, 85%	7/10, 70%	
EHV-5 PCR+ BALF	10/12, 83%	4/8, 50%	.16
EHV-5 PCR+ lung	4/8, 50%	3/5, 60%	

TABLE 7 (Continued)

Test result	Non-survivor (n = 34)	Survivor (n = 11)	*P* value[a]
Imaging			
Nodular pattern radiographs	21/29, 72%	7/11, 64%	
Miliary pattern radiographs	5/29, 17%	1/11, 9%	
Interstitial pattern radiographs	22/29, 73%	9/11, 82%	
Bronchointerstitial pattern radiographs	17/29, 59%	5/11, 45%	
Radiograph score with lymph nodes	15 (6-25)	16 (2-31)	
Radiograph score corrected	4.88 (1.5-8.5)	5.33 (0.5-7.75)	
Nodular pattern ultrasound	15/23, 65%	8/10, 80%	
Ultrasound score	26 (4-86)	35.5 (3-113)	
Ultrasound score corrected	3.86 (2.57-5.7)	2.85 (2.44-4.35)	
Treatment			
Antiviral	11/32, 34%	4/11, 36%	
Corticosteroid	19/32, 59%	9/11, 82%	

Note: Results are expressed as median (range) and number (%) of horses. Bolded values represent significant differences between groups as single predictors.
Abbreviations: BALF, bronchoalveolar fluid; QH, Quarter Horse; TB, Thoroughbred; TW, tracheal wash; WB, warmblood.
[a]Wilcoxon rank sum for medians and chi-square or Fisher exact for proportions performed on top predictors by predictor response screening. None remained significant after application of the Benjamini-Hochberg test for multiple comparisons.

Although horses with asthma and EMPF can be presented with respiratory signs of nasal discharge, increased respiratory effort or distress or both, and tachypnea, fever is considered a useful clinical criterion for distinguishing EMPF from asthma.[1,11,15] However, fever was only reported in 57% of the horses in our study, with 39% being febrile at admission, indicating that a lack of fever is not reliable in distinguishing between the 2 syndromes. Weight loss, seen in this (78% of horses) and a previous[11] study, should prompt consideration of EMPF as a differential diagnosis, although there are anecdotal reports of weight loss in severely asthmatic horses. Because systemic inflammation was a consistent finding in our study and other reports,[1,4,11,13,33] but is uncommon in asthma,[34-36] acute phase proteins, such as plasma fibrinogen and serum amyloid A concentrations, and a CBC could be valuable first-line tests to determine which horses with asthma-like clinical signs could benefit from additional diagnostic testing for EMPF. Another useful hematologic result was lymphopenia, which was the most frequently observed hematologic abnormality in EMPF horses, but not commonly described in asthmatic horses (many of which lack CBC data).[36,37]

Our study provides the first detailed analysis of BALF and TW fluid results in horses with EMPF and identifies certain BALF results that were prognostic or might be useful in differentiating between EMPF and asthma on cytologic analysis. A previous EMPF case series described cytologic findings in BALF as neutrophilia without quantification or morphologic descriptions.[14] In a 3-case series and 2 case reports, TW fluids were described as having 37% to 94% neutrophils.[10,13,33] Although there was overlap in the BALF numerical results in EMPF and asthmatic horses in our study, cytologic findings of epithelial necrosis, prominent cytophagia, and macrophage atypia in BALF smears are not typical in asthma and should raise suspicion for other conditions. Given the low case numbers, none of these cytologic findings are likely to be specific for EMPF or EHV-5 infection, but their presence might warrant additional testing for EMPF and EHV-5, particularly in horses with compatible clinical signs and neutrophilic inflammation in the BALF. A lower BALF lymphocyte:neutrophil ratio was associated with poor prognosis for both short- and long-term survival, showing that BALF analysis might be useful for prognostication. The results also indicate that BALF is preferred over TW samples for identifying cytologic abnormalities associated with EMPF. Concurrent bacterial infection was common in EMPF horses and TW culture is recommended, particularly in horses with evidence of systemic inflammation. A limitation of our study is that asthma was the only comparison group for cytologic analysis and respiratory secretions from horses with pneumonia were not assessed. Horses with pneumonia can have similar morphologic abnormalities to those seen in EMPF, including epithelial necrosis, macrophage phagocytic activity and atypia, emphasizing the need to combine BALF results with clinical, other laboratory, and imaging findings. In addition, a single clinical pathologist evaluated all archived samples of BALF and TW in our study, decreasing inherent variability in this test, particularly the subjective assessment of morphologic features, such as the degree and type of macrophage vacuolation. Several features were subtle or overlapping (eg, degree of vacuolation) and could be missed or over-interpreted on cytologic evaluation.

As with a previous study,[11] we did not identify EHV-5 infection or imaging findings as prognostic indicators, and we found that corticosteroid treatment was associated with improved short-term but not long-term survival. Our inability to identify viral- or image-related prognostic factors or factors predictive of long-term survival could be partially because of the case numbers and inherent bias in

retrospective studies. Testing for EHV-5 was performed on different sample types and was not done on all horses. In addition, EHV-5 infection is not found in all horses with EMPF.[10,11] Imaging was also subject to selection bias. Most cases only had sonographic images saved from ICS with abnormalities, potentially inflating the sonographic scores. Radiographs might only have been performed in selected horses based on abnormal sonographic findings. Rapid death occurred in 3 horses with EMPF and concurrent pulmonary hypertension,[1,32] suggesting the latter could be a prognostic indicator, but this finding was rarely evaluated in the horses in our study.

One limitation of our study was the inclusion of horses with a presumptive diagnosis of EMPF, without histologic confirmation of the disease. These cases were included because the diagnosis of EMPF is often clinical and made in practice without histologic confirmation. This situation likely is because of the morbidity and cost associated with lung biopsies and lack of discrete nodules on the pleural surface to biopsy, resulting in a diagnostic bias toward having histopathologic confirmation only in more severely affected animals. However, despite relaxing the inclusion criteria for our study, we showed similar short- and long-term survival as a smaller prior study that did use histologic confirmation for inclusion of cases.[11]

In summary, although the prognosis for EMPF remains poor, we conclude that fever has low sensitivity for discriminating EMPF from asthma but that weight loss might be a useful supportive clinical finding. Cytologic results on BALF could be helpful for identifying features that would warrant additional testing for EMPF or underlying viral infection. In addition, markers of acute inflammation and lymphopenia are potential early screening tools to identify horses with increased respiratory rate and effort that should be targeted for additional diagnostic testing for EMPF. Given the current long duration between recognition of clinical signs and definitive diagnosis, we are hopeful that earlier diagnosis and intervention might improve short- and long-term outcomes in affected horses.

ACKNOWLEDGMENT

No funding was received for this study. The authors thank Dr. Ignacio Corradini for contributing a case.

CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Authors declare no IACUC or other approval was needed.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Craven A, Todd-Donato A, Stokol T, et al. Clinical findings and outcome predictors for multinodular pulmonary fibrosis in horses: 46 cases (2009-2019). *J Vet Intern Med*. 2024;38(3):1842-1857. doi:10.1111/jvim.17084