Case Report
J Vet Intern Med 2014;28:939–943

Pulmonary Disease Potentially Associated with *Nicoletella semolina* in 3 Young Horses

E.L. McConachie, K.A. Hart, D.D. Whelchel, E.L. Schroeder, H.C. Schott II, and S. Sanchez

Key words: Bronchopneumonia; Inflammatory airway disease; *Pasteurella*; Weanling.

Case 1

A 10-month-old, 200-kg, Arabian filly presented to the University of Georgia Veterinary Teaching Hospital for evaluation of cough, nasal discharge, and fever of 24 hours duration. Before referral to UGA, the filly had never traveled off the farm. The filly was vaccinated against EEE/WEE viruses, EHV-1, EIV, WNV, tetanus, and *Streptococcus equi* subsp. *equi* at 8 months of age. Deworming with ivermectin was performed 1 week before presentation. Several horses on the farm had a suspected viral upper respiratory tract infection 2–3 weeks before the filly became ill.

On examination, the filly was quiet, alert, and responsive. Heart rate was 60 beats per minute, respiratory rate was 20 breaths per minute, and rectal temperature was 101.3°F. There was bilateral mucopurulent nasal discharge and an intermittent spontaneous cough, and tracheal palpation elicited coughing. Pulmonary auscultation revealed normal lung sounds bilaterally at rest and during rebreathing examination. No other abnormalities were noted on physical examination.

CBC was within reference range. Percutaneous transtracheal aspirate (TTA) yielded a grossly turbid fluid classified as septic, suppurative inflammation with nondegenerate neutrophils and rare intracellular bacterial rods. PCR for the *R. equi* virulence associated protein-A (vap-A) gene and 16S rRNA on the fluid was negative. Nasopharyngeal lavage was negative for *S. equi* subspecies *equi* by PCR. Nasal swabs were PCR negative for EHV-1 and EIV viruses, as was whole blood buffy coat EHV-1 PCR. Thoracic radiographs revealed a moderate caudoventral interstitial-alveolar pattern. Transthoracic ultrasonography revealed mild bilateral cranioventral pleural roughening.

Initial treatment for presumptive bacterial bronchopneumonia consisted of broad-spectrum antimicrobial treatment with potassium penicillin G (22,000 U/kg IV q6 h) and gentamicin (6.6 mg/kg IV q 24 h), and anti-inflammatory treatment with flunixin meglumine (0.25 mg/kg IV q8 h). Within 72 hours, the filly's fever and nasal discharge resolved and her cough improved.

Aerobic bacterial culture of TTA fluid yielded heavy growth of *S. equi* subsp. *zooepidemicus* and heavy growth of a slow-growing nonreactive nonfermenting gram-negative rod with colony morphology consistent with the recently characterized *Pasteurellaceae* organism *Nicoletella semolina*[1] (Fig 1), both with broad sensitivity profiles. This organism was cultured on a chocolate-agar plate incubated in carbon dioxide and

Abbreviations:

CBC	complete blood count
EEE	Eastern equine encephalitis
EHV-1	equine herpesvirus-1
EIV	equine influenza virus
IAD	inflammatory airway disease
OU	oculus uterque (both eyes)
TMS	trimethoprim-sulfamethoxazole
TTA	transtracheal aspirate
VAP-A	virulence associated protein-A
WEE	Western equine encephalitis
WNV	West Nile virus

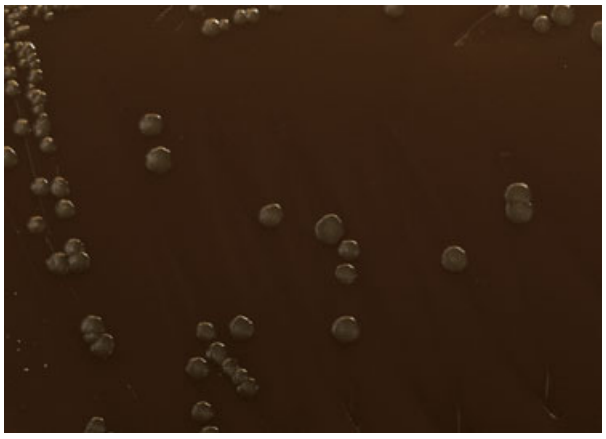

Fig 1. Typical colony morphology of *N. semolina* cultured on a chocolate-agar plate. Note the raised, waxy, dry appearance, similar to a grain of semolina wheat.

From the Department of Large Animal Medicine, University of Georgia College of Veterinary Medicine, Athens, GA (McConachie, Hart, Whelchel); the Department of Large Animal Clinical Sciences, Michigan State University College of Veterinary Medicine, East Lansing, MI (Schroeder, Schott); the Department of Infectious Diseases, University of Georgia College of Veterinary Medicine, Athens, GA (Sanchez); and Dr Whelchel is presently affiliated with Southern Equine Service, Aiken, SC.

Corresponding author: K.A. Hart, DVM PhD DACVIM (LAIM), Department of Large Animal Medicine, 501 DW Brooks Drive, University of Georgia College of Veterinary Medicine, Athens, GA 30602; e-mail: khart4@uga.edu

Submitted September 18, 2013; Revised January 11, 2014; Accepted February 19, 2014.

DOI: 10.1111/jvim.12349

grew within 24 hours, and 16S rDNA sequencing revealed 99% homology with *N. semolina*[1] (Genebank number KJ405450). On day 8, antimicrobial treatment was changed to trimethoprim-sulfamethoxazole (TMS, 20 mg/kg, PO, q12 h), and the filly was discharged on day 10 for continued on-farm treatment.

On reevaluation on day 19, the filly was clinically normal except for an occasional cough and a persistent mild caudoventral interstitial-alveolar pattern evident on radiographs. TMS treatment was continued until day 40. At this time, an infrequent cough was noted, and CBC revealed a mild leukocytosis (total white blood cell count: $12.9 \times 10^3/\mu L$ reference range 5.7–$11.7 \times 10^3/\mu L$) characterized by a mild lymphocytosis ($5.547 \times 10^3/\mu L$ reference range 1.16–$5.1 \times 10^3/\mu L$). Thoracic radiographs were improved except for a persistent peri-hilar bronchial pattern. Percutaneous TTA cytology revealed mild aseptic suppurative inflammation, consistent with postpneumonic inflammatory airway disease (IAD). The filly was discharged with instructions for environmental management to minimize dust exposure. TTA fluid culture yielded growth of 2 coagulase-negative isolates of *Staphylococcus* species (suspected pharyngeal contaminants because of excessive coughing during the TTA), so TMS was discontinued. On telephone follow-up 1 and 6 months later, the filly was reportedly normal.

Case 2

A 6-month-old, 206-kg, Missouri Fox Trotter colt presented to the UGA VTH for postpurchase examination and evaluation of cough and nasal discharge. After weaning 1 month previously, the colt developed nasal discharge, cough, and fever that persisted despite 10 days of administration of TMS. The colt was evaluated at a local referral hospital, and findings supported a diagnosis of allergic bronchitis and bacterial pneumonia with TTA culture yielding *Bordetella bronchiseptica* resistant to TMS. Treatment with oral doxycycline (10 mg/kg PO q12 h) resulted in improvement, so the colt was sold and transported from Missouri to Georgia while still being treated. Vaccination and deworming history before purchase was unknown.

On presentation to the UGA VTH 2 days after this trip, the colt was bright, alert, and responsive with mild bilateral mucopurulent nasal discharge and mild serous ocular discharge OU. Vital signs were normal except for mild tachypnea (32 breaths per minute) with normal respiratory effort. Cardiopulmonary auscultation was normal at rest. Rebreathing examination elicited diffuse wheezes bilaterally and crackles caudodorsally on the left, coughing, and distress. All other findings on physical examination were normal.

CBC was within reference range, and transthoracic ultrasonography showed moderate caudodorsal pleural roughening bilaterally. Thoracic radiography revealed a moderate-to-severe peri-hilar and caudodorsal bronchointerstitial pattern, with mural thickening in the distal trachea and mainstem bronchi (Fig 2A). Cytologic analysis of percutaneous TTA fluid revealed

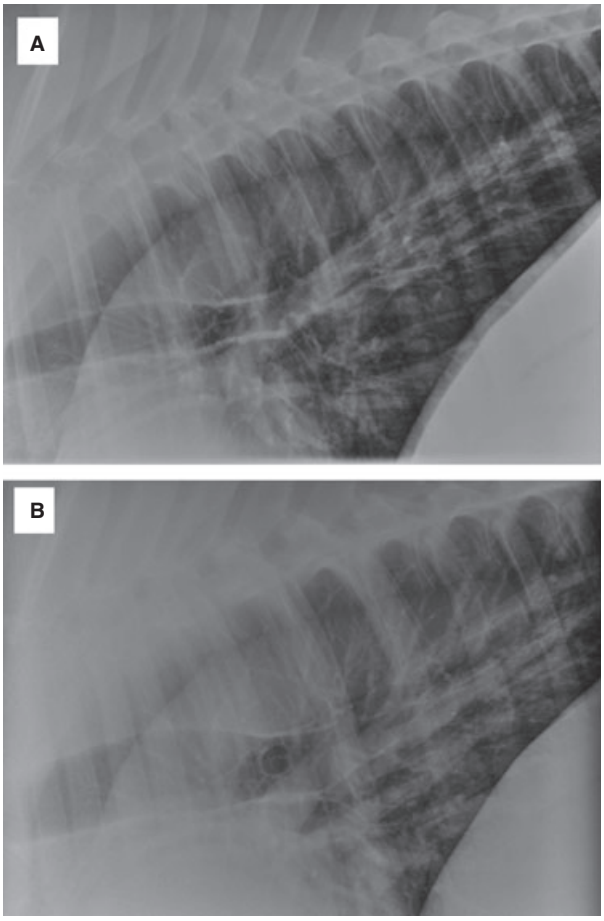

Fig 2. Thoracic radiographs from 1 weanling (Case 2) with *N. semolina*-associated pulmonary disease. (**A**) Right-to-left lateral radiograph from hospital admission, illustrating a moderate-to-severe peri-hilar and caudodorsal bronchointerstitial pattern with mural thickening of the distal trachea and mainstem bronchi, consistent with concurrent bronchopneumonia and chronic airway inflammation. (**B**) Right-to-left lateral radiograph from day 169 illustrating resolution of previous radiographic abnormalities. This radiograph was taken after resolution of clinical signs after oral and inhaled antimicrobial treatment and anti-inflammatory corticosteroid treatment.

septic, suppurative-to-mixed inflammation characterized by neutrophils, macrophages, eosinophils, and intracellular and extracellular rods and cocci. Viral diagnostics were not performed in this case because of the chronicity of signs and previous diagnosis of bacterial pneumonia.

Clinical findings supported a diagnosis of bacterial pneumonia with presumptive concurrent IAD. The colt was discharged with instructions to minimize environmental dust exposure and to continue antimicrobial treatment with doxycycline whereas TTA culture results were pending.

Aerobic culture of TTA fluid yielded heavy growth of a nonreactive, nonfermenting gram-negative rod with colony morphology consistent with *N. semolina*[1] (Fig 1), as well as light growth of both alpha-*Streptococcus* sp. and *Bacillus* spp. All bacterial organisms

were sensitive to doxycycline, so above treatment was continued. 16S rDNA sequencing of the gram-negative isolate revealed 99% homology to *N. semolina*[1] as in case 1 (Genebank number KJ405449).

Reevaluation of the colt at 30–60 day intervals revealed resolution of fever, lethargy, and tachypnea, though intermittent cough persisted. A mild bronchointerstitial pattern and diffuse pleural roughening persisted on serial thoracic imaging, and CBCs revealed a persistent lymphocytosis ($6.6 \times 10^3/\mu L$ to $9.4 \times 10^3/\mu L$, reference range: $1.16–5.1 \times 10^3/\mu L$). TTA culture persistently recovered *N.semolina,* with occasional concurrent light to moderate growth of other organisms (alpha-*Streptococcus* sp., *Bacillus* spp., *Actinobacillus* spp., *Enterobacter* spp., *Pantoea agglomerans, Leclercia* spp.). Thus, the colt was readmitted to the UGA VTH on day 120 for additional antimicrobial treatment with aerosolized gentamicin[2] once daily for 5 days. Anti-inflammatory treatment for IAD with prednisolone (1 mg/kg PO q24 h, tapering over 6 weeks) was also initiated at this time. Environmental management and doxycycline were continued as above, and the foal was rotationally and larvacidally dewormed by the owner after discharge on day 125 as per farm protocol.

On day 169, the colt was clinically normal with no coughing reported. Thoracic radiographs were normal (Fig 2B), and TTA fluid cytology was improved with mild mixed cell inflammation and mucus. Aerobic culture of TTA fluid yielded no growth. Medication was discontinued, and the colt was discharged with instructions to continue to minimize dust exposure and to pursue additional diagnostics[3,4] for IAD if respiratory signs recurred. At telephone follow-up 6 months and 2 years later, the colt was reportedly normal and in training.

Case 3

A 23-month-old, 380-kg, Quarter horse gelding presented to the Michigan State University Veterinary Teaching Hospital for evaluation of intermittent cough, nasal discharge, and exercise intolerance of several months' duration. Five months previously, after transportation from Texas to Michigan, the gelding and 7/8 other horses on the farm developed cough and nasal discharge. The other horses recovered within 2 weeks without treatment, but because of persistence of signs at that time the gelding was treated with TMS for 14 days. Mild nasal discharge and intermittent cough persisted after this treatment. Four months later, after transportation to a training facility, nasal discharge and cough increased in severity, and lethargy, inappetance, and exercise intolerance developed for 1–2 weeks, prompting referral to MSU VTH.

At examination, the gelding was bright, alert, and responsive and vital signs were normal except for mild pyrexia (101.1°F). Mild bilateral mucopurulent nasal discharge, serous ocular discharge OU, and submandibular lymphadenomegaly were present. Pulmonary auscultation at rest revealed diffusely increased lung sounds bilaterally, and rebreathing examination revealed tracheal rattles and elicited mild distress and coughing. A grade 4/5 right forelimb lameness with focal metacarpal swelling, presumably because of recent trauma, was apparent. No other abnormalities were noted on physical examination.

Hematologic abnormalities included a mild leukocytosis (WBC: $13.43 \times 10^3/\mu L$, reference range $5.1–13.21 \times 10^3/\mu L$) characterized by a mature neutrophilia ($7.58 \times 10^3/\mu L$, reference range $1.94–7.4 \times 10^3/\mu L$) and monocytosis ($1.03 \times 10^3/\mu L$, reference range $0.01–0.35 \times 10^3/\mu L$). Nasal swabs for PCR detection of EHV-1, EHV-4, and EIV were negative. Transthoracic ultrasonography revealed mild bilateral diffuse pleural roughening. Thoracic radiographs and upper airway endoscopy were normal, with the exception of mild tracheal mucus accumulation and moderate bilateral retropharyngeal lymphadenomegaly. Guttural pouch lavage and culture resulted in moderate growth of an alpha-hemolytic *Streptococcus* spp., *Actinobacillus* spp. and heavy growth of a nonreactive nonfermenting gram-negative rod with colony morphology consistent with *N. semolina* (Fig 1).[1] Transendoscopic TTA was performed with a guarded catheter, and cytological analysis revealed suppurative inflammation with extracellular bacteria, increased macrophages, and abundant mucus. Bacterial culture of TTA fluid yielded light growth of *S. equi* subsp. *zooepidemicus*, an alpha-hemolytic *Streptococcus* spp., and moderate growth of a nonreactive nonfermenting gram-negative rod with colony morphology (Fig 1) and 16S DNA sequencing consistent with *N. semolina*[1] as in Cases 1 and 2.

Antimicrobial treatment for bacterial respiratory tract infection was initiated with potassium penicillin G (22,000 IU/kg IV q6 h) based on suspicion of *S. equi* subsp. *equi* infection before results of diagnostic testing. Treatment was changed to ceftiofur sodium (2.2 mg/kg IV q12 h) after culture results were obtained on day 3. Phenylbutazone (3 mg/kg PO q12 h for 4 days) was administered to address inflammation associated with the forelimb lameness and respiratory tract. The gelding's appetite improved and the lameness resolved, but an intermittent cough persisted. Repeat upper airway endoscopy on day 5 revealed resolution of retropharyngeal lymphadenopathy and persistent tracheal mucus. On day 6, the colt was discharged on doxycycline (10 mg/kg PO q12 h) with instructions to minimize environmental dust exposure. One week later, the colt was clinically normal so doxycycline was discontinued. At telephone follow-up 1 year later, the gelding was reported to have returned to training without further respiratory issues.

Discussion

We report of isolation of *N. semolina* from the respiratory tract of horses with respiratory disease in North America. All 3 animals in this report had some clinical signs consistent with infectious respiratory disease and isolation of *N. semolina* in conjunction with other common equine airway flora from lower airway secretions at hospital admission. Differences in case

chronicity, severity, and clinician preference resulted in different antimicrobial choices and duration of treatment among the 3 cases, but the *N. semolina* organism isolated appeared sensitive to a broad range of commonly available antimicrobials and clinical improvement was observed with antimicrobial treatment in all cases. *N. semolina* might have directly contributed to infectious pulmonary disease in these 3 cases, but a causative role for this organism as a primary pathogen was not definitively demonstrated as the prevalence of *N. semolina* in airway secretions from normal horses in North America is not currently known. Further, the chronic duration of signs and evidence of lower airway inflammation in all 3 cases, and the necessary addition of anti-inflammatory corticosteroid treatment for complete resolution of signs and elimination of *N. semolina* isolation from TTA fluid in case 2 suggests that concurrent IAD might also have played a role in clinical disease in these young horses.

N. semolina is a relatively recently described member of the *Pasteurellaceae* family and is distinct from the 9 other genera in this family based on genetic sequence, morphology, and biologic characteristics.[1] *N. semolina* has been isolated from TTA fluid in similar proportions of healthy horses (3%) and horses with respiratory tract disease (1.8–5%) in Europe.[5,6] It remains unclear if *N. semolina* is a normal component of equine airway flora that has gone unrecognized because of slow growth in culture, or if it is an emerging equine pathogen in Europe and North America.

In the cases described herein, *N. semolina* was isolated from TTA fluid in association with ≥1 other common equine respiratory tract organism—concurrently with *S. equi* subspecies *zooepidemicus* in cases 1 and 3, and shortly after primary isolation of *B. bronchiseptica* in case 2. In all cases, other organisms such as alpha-*Streptococcus* spp. and *Bacillus* spp. that are generally considered nonpathogenic were also isolated. The relative contribution of each of these organisms to the clinical signs remains unclear, but *N. semolina* was a predominant organism isolated with moderate to heavy growth at one or more times in each case. Thus, *N. semolina* might contribute to infectious pulmonary disease in some young horses in North America, or it might be a component of normal airway flora that is found as an "innocent bystander" in pulmonary disease caused by other infectious or inflammatory stimuli. Further study is needed to characterize a causative role—if any—for this organism in such cases.

All 3 cases in this report had clinical evidence of both bacterial bronchopneumonia (fever, malaise, radiographic abnormalities, improvement with antimicrobial treatment) and chronic airway inflammation (nondegenerate neutrophils and other inflammatory cells in TTA fluid, abundant mucus, bronchial thickening, persistent cough/exercise intolerance). It is difficult to determine a specific temporal relationship between the presence of noninfectious lower airway inflammation and lower airway infection, as all cases had signs of both bronchopneumonia and IAD at presentation to the referral centers.

All cases in this report had a history of transportation or presumptive viral respiratory disease on the farm or both before development of respiratory signs, suggesting that such factors might have caused an initial pulmonary insult. *N. semolina* appears to be a component of normal equine respiratory tract flora in adult horses,[5,6] so opportunistic infection with or overgrowth of *N. semolina* after another primary viral infection, environmental respiratory insult, or both seems possible in these cases. In addition, *N. semolina* overgrowth could occur in conjunction with bacterial bronchopneumonia because of another organism, such as *S. equi* subspecies *zooepidemicus* in cases 1 and 3. In case 3, the animal had a several month history of intermittent pulmonary signs that worsened substantially after shipping to a new training facility 2 weeks before referral, so it also possible that the colt acquired a new infection—*N. semolina* or another viral or bacterial pathogen or both—upon arrival at that facility. Unfortunately, serial viral PCRs or isolation or serology for all relevant respiratory viral pathogens (e.g. equine herpesvirus-2,4, or 5, rhinoviruses, adenovirus) were not performed in the animals described herein because of financial constraints (case 1) or clinician suspicion they would be low yield because of the chronicity of signs (cases 2 and 3). In addition, temporal delays between development of clinical signs and diagnostic testing in cases 2 and 3 could have resulted in failure to identify the primary initial pathogen in these cases.

Pulmonary ascarid migration is also common in foals and young horses and could result in an airway insult permitting secondary *N. semolina* invasion. The presence of eosinophils in TTA fluid in cases 1 and 2 supports this possibility, though deworming histories varied and fecal diagnostics for parasites were not performed in these cases. Recent evidence suggests that eosinophilia in equine lower airway secretions can be transient and resolve without specific treatment other than deworming.[7] Finally, immunologic immaturity can contribute to impaired pulmonary immune responses that limit bacterial clearance in younger animals and people,[8–10] and might also have contributed to the development of disease in these young horses. It is possible that bacterial infection and the associated immune response triggered airway inflammation, leading to development of secondary postpneumonia IAD. Proinflammatory cytokines released from airway immune cells ignite a cascade of changes in the respiratory epithelium and small bronchioles, resulting in bronchoconstriction and excessive and abnormal mucus production,[11] which can produce clinical signs of IAD even after the infection resolves.

Alternatively, it is possible that these foals had underlying primary noninfectious/allergic IAD, which resulted in impaired mucociliary clearance[12] and permitted secondary infection with *N. semolina* or other bacterial organisms or both. The persistent isolation of *N. semolina* from TTA fluid in Case 2 until after anti-inflammatory corticosteroid treatment supports this theory. Isolation of *N. semolina* from equine TTA fluid

has been associated with a significant increase in TTA fluid neutrophils, suggesting that this organism is associated with increased lower airway inflammation.[6] *N. semolina* was isolated from 1.8% (19/1,054) equine TTA samples collected by equine practitioners for routine bacteriologic diagnostics in clinical cases with respiratory signs, and was significantly associated with a higher neutrophil percentage on TTA cytology in *N. semolina* positive samples (median 87%) as compared to *N. semolina* negative samples (median 52%).[6] It is not clear, however, if this increase in neutrophils is because of primary IAD, primary bronchopneumonia because of *N. semolina* or other organisms, both, or neither. Interestingly, *N. semolina* has previously been isolated in conjunction with common equine respiratory tract flora such as *S. equi* subspecies *zooepidemicus,*[6] as was also found in the 3 cases described herein.

Finally, the 3 cases described in this report also share some clinical features described in 12 weanling-aged foals with chronic interstitial pneumonia, all of which recovered fully after treatment with broad-spectrum antimicrobials and corticosteroids.[13] TTA cultures failed to identify a common pathogen in those cases, suggesting that airway inflammation rather than a specific bacterial pathogen was a key factor in the clinical disease.[13] Such airway inflammation also likely played a role in the cases described in this report, as respiratory signs or radiographic abnormalities or both attributed to concurrent IAD persisted in at least 2 cases for weeks to months after clinical resolution of signs of infection. In case 2, the continued isolation of *N. semolina* from TTA fluid for 4 months until after a course of anti-inflammatory corticosteroid treatment provides further support for a primary inflammatory insult resulting in *N. semolina* overgrowth in some horses. However, corticosteroid treatment was not necessary for resolution of signs in 2/3 cases, suggesting that associated airway inflammation can be self-limiting after infectious respiratory tract disease resolves.

In sum, *N. semolina* might play a role in infectious or inflammatory pulmonary disease or both in some young horses in North America. If isolated from animals with signs consistent with bronchopneumonia, it might be a contributing infectious organism and appropriate antimicrobial treatment should be considered. As the cases described herein had evidence of both infectious bronchopneumonia and chronic lower airway inflammation, concurrent IAD should be considered as a predisposing or complicating factor in *N. semolina*-associated pulmonary disease in young horses, which might warrant specific anti-inflammatory treatment. Specific culture techniques are necessary to recover this organism, but unique colony morphology makes identification straightforward. Further prospective and experimental study is needed to better understand the relative prevalence of *N. semolina* in respiratory tract flora in normal horses in North America to determine if the organism plays a role in the pathogenesis of pulmonary disease in young horses.

Acknowledgment

Conflict of Interest Declaration: Authors disclose no conflict of interest.

References

1. Kuhnert P, Korczak B, Falsen E, et al. *Nicoletella semolina* gen. nov., sp. nov., a new member of Pasteurellaceae isolated from horses with airway disease. J Clin Microbiol 2004;42:5542–5548.

2. McKenzie HC 3rd, Murray MJ. Concentrations of gentamicin in serum and bronchial lavage fluid after once-daily aerosol administration to horses for seven days. Am J Vet Res 2004;65:173–178.

3. Bedenice D, Mazan MR, Hoffman AM. Association between cough and cytology of bronchoalveolar lavage fluid and pulmonary function in horses diagnosed with inflammatory airway disease. J Vet Intern Med 2008;22:1022–1028.

4. Couetil LL, Hoffman AM, Hodgson J, et al. Inflammatory airway disease of horses. J Vet Intern Med 2007;21:356–361.

5. Hansson I, Johansson K, Persson M, et al. The clinical significance of *Nicoletella semolina* in horses with respiratory disorders and a screening of the bacterial flora in the airways of horses. Vet Microbiol 2013;162:695–699.

6. Maillard K, Richard E, Kuhnert P, et al. Isolation of *Nicoletella semolina* from equine tracheal washes. J Equine Vet Sci 2013;33:561–564.

7. Riihimaki M, Lilliehook I, Raine A, et al. Clinical alterations and mRNA levels of IL-4 and IL-5 in bronchoalveolar cells of horses with transient pulmonary eosinophilia. Res Vet Sci 2008;85:52–55.

8. Heier I, Malmstrom K, Sajantilia A, et al. Characterisation of bronchus-associated lymphoid tissue and antigen-presenting cells in central airway mucosa of children. Thorax 2011;66:151–156.

9. Martin T, Ruzinski J, Rubens C, et al. The effect of type-specific polysaccharide capsule on the clearance of group B streptococci from the lungs of infant and adult rats. J Infect Dis 1992;165:306–314.

10. Renz H, Brandtzaeg P, Hornef M. The impact of perinatal immune development on mucosal homeostasis and chronic inflammation. Nat Rev Immunol 2011;12:9–23.

11. Fahy J, Dickey B. Airway mucus function and dysfunction. N Engl J Med 2010;363:2233–2247.

12. Turgut K, Sasse H. Influence of clenbuterol on mucociliary transport in healthy horses and horses with chronic obstructive pulmonary disease. Vet Rec 1989;125:526–530.

13. Nout Y, Hinchcliff K, Samii V, et al. Chronic pulmonary disease with radiographic interstitial opacity (interstitial pneumonia) in foals. Equine Vet J 2002;34:542–548.