

RESEARCH COMMUNICATIONS I:
Large Animals

AIRWAY DISEASE SYMPTOMS IN PEOPLE ASSOCIATED WITH EQUINE BARNs: A CROSS SECTIONAL QUESTIONNAIRE STUDY

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Horse sports contribute \$140 billion to the yearly US GDP, and horse sports are perceived to be 'healthy'. However, organic dust exposure causes lower airway inflammation in up to 80% of stabled horses, and workers in other animal industries have a greater risk of developing chronic respiratory disease. We hypothesized that people exposed to horse barns would be more likely to report symptoms consistent with lower airway disease (LAD) than people not exposed. This questionnaire study examined one cohort (BE) exposed to an equine barn on a regular basis (n=82); the other (CTL), never exposed to equine barns (n=78). A case was designated as reporting one or more of the following: dry or productive cough, wheezing, chest tightness, shortness of breath, difficulty breathing, or a doctor diagnosis of asthma. 50% of BE reported LAD symptoms, compared with 15% of CTL. A logistic regression analysis was used to look at the association between LAD symptoms and exposure to equine barns. This model showed that BE were more likely to report LAD symptoms (crude odds ratio of 5.72, CI = 2.64 -12.41). A descending logistic regression technique was then used to determine an adjusted odds ratio of 6.07 (CI = 1.12-32.81). Confounders included age, smoking, use of a fireplace or air-conditioning, and sleeping with any feather products. All other risk factors tested (pets in household, gas cooking or heating, use of woodstove, wall-to-wall carpeting or visible areas of mold in household) were not confounders. This study is the first step towards creating an awareness of health risks associated with equine management practices and implementing changes in order to create a healthier environment for horses and humans.

EVALUATION OF RESPIRATORY HEALTH IN STABLED CLINICALLY HEALTHY HORSES

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Purpose: The purpose of the study was to evaluate the clinical status of a group of clinically healthy horses by performing resting physical examination and tracheal wash (TW) and bronchoalveolar lavage (BAL). The horses were permanently housed in the same facility and performed the same type of work.

Methods: The horses (n=28) were subjected to a clinical examination in the morning in the box, prior to the TW and BAL. The clinical examination included measuring temperature, pulse and respiration (TPR), auscultation of the lungs and heart, inspection of the nares, symmetry of the head, palpation of the mandibular lymph nodes. Before tranquilization of the horses (4 ml domosedan, 0.05ml atropine, iv) a bloodsample was collected from the jugular vein.

Lidocaine gel was applied to the nostril, and the endoscope was inserted through the left nostril. A sterile polyethylene catheter was advanced through the biopsy channel into the tracheal lumen. 30ml of pre-warmed isotonic saline was infused and the catheter was advanced into the pool of saline and aspirated. The whole procedure was videotaped for further evaluation.

The endoscope was advanced to the carina and 20ml lidocaine was instilled through the biopsy channel. The endoscope was withdrawn back to a position just rostrally to the epiglottis and the BAL catheter was inserted through the right nostril and placed in the trachea under endoscopic guidance, after that the endoscope was removed. The catheter was gently passed down the trachea until reaching the bifurcation, where a coughing reflex was triggered. The catheter was introduced down to the bronchial tree until resistance was felt and the cuff was inflated. Through the catheter 300 ml pre-warmed isotonic saline was injected, the fluid was aspirated relatively slowly to facilitate collection and to minimize mucosal and cellular trauma. The proces was repeated with another 300 ml saline.

Results: At the clinical examination none of the horses showed clinical signs of respiratory diseases. The blood samples showed a normal profile in almost all of the horses. A large percentage of the horses showed low amounts of mucus in the trachea (15/28). In the TW few or moderate numbers of neutrophils was found (17/28). Only a few horses had less than 5% neutrophil percentage in the BAL(17/28).

Conclusions: Though the horses at the clinical examination showed no signs of lower respiratory disease and no coughing was observed during the clinical observation, a large percentage of the horses had signs of subclinical inflammatory airway disease based on the TW and BAL.

EFFICACY OF A PLANT BASED FORMULATION IN THE TREATMENT OF RECURRENT AIRWAY OBSTRUCTION IN HORSES

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The purpose of this double blinded placebo controlled cross-over clinical study was to assess the ability of a plant based formulation (*Ginkgo biloba*, *Zingiber officinale*, *Chlorella pyrenosa*) to prevent or delay the onset, decrease the magnitude of response and/or speed the recovery of lung dysfunction, clinical signs of disease, airway inflammation, and pulmonary oxidative stress in horses with recurrent airway obstruction (RAO) on exposure to organic dust. The performance of the active supplement was judged on the basis of responses in lung function, clinical examination, airway inflammation and pulmonary oxidative stress following organic dust challenge compared to responses on the placebo diet. Lung dysfunction was assessed by measuring airway reactance and airway responsiveness to histamine by forced oscillation mechanics. Clinical signs were assessed by assigning scores for respiratory rate, nasal discharge, abdominal lift/expiratory effort, nasal flaring, lung sounds and cough. Airway inflammation was determined by cytological analyses of tracheal wash and bronchoalveolar lavage fluid (BALF) samples, and by measuring the concentration of hydrogen peroxide in exhaled breath condensate (EBC). Oxidative stress was assessed by measuring the concentrations of reduced ascorbic acid, dehydroascorbate (DHA, oxidised ascorbic acid), reduced glutathione and oxidised glutathione in tracheal wash and BALF. Results of statistical analyses demonstrated that BALF ascorbic acid concentrations were higher after challenge in horses when fed the active formulation compared to the placebo and that BALF, DHA and ARR (ratio of DHA to total ascorbic acid), were lower after challenge in the active supplement horses irrespective of the order of treatment allocation.

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1. Deaton CM, Marlin DJ, 2004. *Am J Vet Res*. Pulmonary epithelial lining fluid and plasma ascorbic acid concentrations in horses affected by recurrent airway obstruction. Jan;65(1):80-7.
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**RANDOMISED CLINICAL TRIAL EVIDENCE OF AEROSOL
ADMINISTRATION EFFICACY FOR THE RESOLUTION OF PULMONARY
NEUTROPHILIA IN YOUNG PERFORMANCE HORSES IN SYDNEY,
AUSTRALIA**

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Respiratory disease is one of the major causes of lost training days for horses in Australia and overseas (Rossdale, Hopes et al. 1985; Bailey 1998) and continues to be a significant contributor to wastage. Airway inflammation, diagnosed by tracheal aspirate (TA) cytology, is a highly prevalent respiratory condition in young thoroughbred racehorses and is detrimental to their respiratory function and hence performance. To date, treatment modalities for respiratory disease have been commonly based on speculative diagnosis, with survey results suggesting that antibiotics are over-prescribed by Australian veterinarians when treating coughing horses (Christley 1999). The recent availability of tailored systems for aerosol drug administration in horses facilitates mainstay therapies for the treatment of lower respiratory tract (LRT) disease, such as bronchodilators and anti-inflammatory drugs (usually corticosteroids) to be administered topically and target specifically therefore minimising undesirable side effects commonly encountered when drugs, particularly corticosteroids are administered systemically via oral or intravenous (IV) routes.

To ascertain the efficacy of stand alone steroidal inhalation therapy compared with combination steroidal and bronchodilator therapy, a Randomised Controlled Trial (RCT) was conducted on young racing horses (2.7 ± 0.7 yo) throughout Sydney metropolitan race stables over a period of 14 months. Horses were recruited as inflammatory cases following bacteriological ($<10^3$ cfu/mL) and cytological ($>20\%$ neutrophils) evaluation of TA samples in addition to mucus scoring. Following recruitment, horses were randomly assigned Treatment 1; Flixotide® (fluticasone propionate [steroid], n=21) or Treatment 2; Combination therapy Atrovent® (ipratropium bromide [bronchodilator]+ Flixotide®, n=20). Horses were administered pharmaceuticals twice daily for 14 days via a metred dose inhaler (MDI) with the Equinehaler® used as a coupling device. Subsequent endoscopy, bacteriological and cytological evaluation of TA samples and mucus scoring were performed on Day 7 and Day 14 at cessation of treatment. The results of differential TA cytology were incrementally compared between Day 0, Day 7 and Day 14 of treatment, as shown in Figure 1.

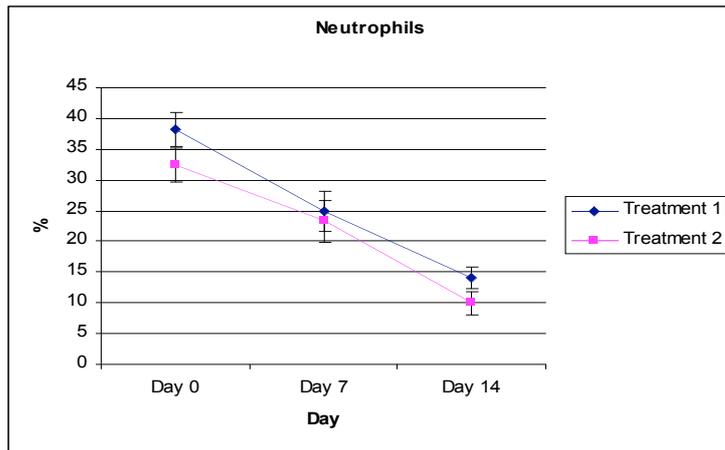


Figure 1: Interaction means of neutrophils between Day 0 and Day 14.

It can be concluded that the treatments used in this experiment are not significantly different at the 0.05 level. There is no statistical difference in neutrophil percentage between Treatment 1 and Treatment 2 ($p=0.074$) however, there is a highly significant difference between Days (Day 7-0, $p=0.001$; Day 14-7, $p=0.001$) for both treatments. In addition, no significant interaction was observed between Treatment and Day for neutrophil percentages ($p=0.675$; $p=0.694$), indicating that the Treatments respond similarly across Days, which is demonstrated by plotting the means (Figure 1). It is therefore concluded that steroidal anti-inflammatory inhalation therapy is equally efficacious as steroidal and bronchodilatory agents combined, and treatment should persist for 14 days in order to gain maximum treatment benefit.

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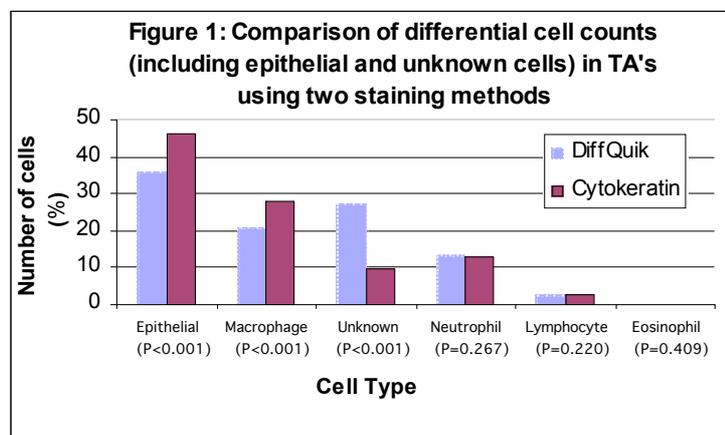
COMPARISON OF DIFFERENTIAL CELL COUNTS IN EQUINE TRACHEAL ASPIRATES USING IMMUNOCYTOCHEMISTRY AS A STAINING TECHNIQUE

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Lower airway inflammation is prevalent in young racehorses and contributes to wastage and significant economic losses. In Australia, diagnosis of lower airway inflammation is most commonly attained through cytological evaluation of tracheal aspirates (TAs), using a modified Wright-Giemsa stain (Diff Quik®). However, the interpretation of differential TA cytology for diagnosis of airway inflammation is contentious as absolute cell numbers are not routinely assessed, and therefore true shifts in specific cell populations (e.g. neutrophils) cannot be determined. Differential cell counts are difficult to perform on TAs due to an inability to accurately distinguish between cell types, particularly epithelial cells and macrophages, based on cellular morphology (McGorum and Dixon, 1994). The purpose of this study was to develop techniques for better differentiating between cell types, and subsequently compare these techniques with the routine staining methods.

Immunocytochemistry is a technique that permits specific labeling of various cell types. In order to better differentiate between cell types, protocols for immunocytochemistry were developed for equine TAs using the Dako EnVision™ system with an epithelial cell marker (mouse anti-human pan-cytokeratin AE1/AE3 (Serotec®)) as the primary antibody (Hirayama *et al*, 2002). A study was subsequently conducted with the aim of comparing differential cell counts of TAs following immunocytochemical staining with differential counts obtained using the routine staining method (Diff Quik®). Tracheal aspirates were collected from 16 horses at the Faculty Horse Unit, University of Sydney, and the University Vet Centre Camden (UVCC). Two cytopsin preparations were made from each sample, then immunocytochemistry and Diff Quick staining was performed on each preparation. A differential count of 300 cells was performed blindly on each slide which included epithelial cells, macrophages, neutrophils, lymphocytes, eosinophils and unknown cells. The two staining methods were compared using a chi-squared (χ^2) analysis then nominal logistic regression to account for horse to horse variation. Results are tabulated in Fig 1.



Results demonstrated a highly significant difference between the two staining methods ($\chi^2 = 536.9$, $df = 5$, $P < 0.001$) (Fig 1) and suggests that the method of staining of tracheal aspirates has a significant effect on cell types identified in a differential cell count. When individual cell types were compared, the Diff Quik® stains underestimated the proportions of macrophages ($P < 0.001$) and epithelial cells ($P < 0.001$). Concurrently, the cytokeratin stain demonstrated significantly fewer of the unknown cell types ($P < 0.001$). However, there was no significant difference between proportions of neutrophils, lymphocytes and eosinophils when the two stains were compared. These results suggest the cytokeratin stain is superior for evaluating the epithelial cell contribution to total cells in TAs. In addition, although Diff Quik® staining adequately identified neutrophils, the shift of individual cell types within the total cell populations is better assessed using the cytokeratin stain and therefore will more accurately diagnose lower airway inflammation.

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LUNG SURFACTANT AND SEVERITY OF DISEASE IN HORSES WITH RECURRENT AIRWAY OBSTRUCTION (RAO)

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Rationale: Pulmonary surfactant dysfunction and altered phospholipid composition contribute to the severity of airway obstruction in human asthma patients. Surfactant alterations have been reported in horses clinically affected with recurrent airway obstruction (RAO).

Hypothesis: Surfactant alterations in RAO-affected horses are related to disease severity.

Objective: To compare surfactant content and composition between RAO-affected horses (at different disease stages) and control horses.

Methods: Eight horses with confirmed RAO and 8 healthy control horses were examined in pairs (RAO/control). Horses were studied while RAO horses were in remission (on pasture), clinical crisis (in challenge environment), and recovery (on pasture). Evaluations included: clinical scoring, lung function testing, airway endoscopy, and cytology of bronchoalveolar lavage fluid (BALF). Crude surfactant pellets (CSP) were isolated from BALF using ultracentrifugation. Phospholipids (PL) from CSP were extracted and analyzed for PL content using the Bligh/Dyer and Bartlett method. PL composition was determined by HPLC with an evaporative light scatter detector. A mixed effects repeated measures analysis of variance was used for statistical analysis.

Results: PL content was significantly lower in CSP from RAO horses compared to control horses at the 3 sampling times. Compared to remission, CSP PL content during crisis decreased 38% in RAO horses and 11% in control horses. In RAO horses CSP content in phosphatidylcholine (PC) and phosphatidylglycerol (PG), 2 of the major surfactant PLs, was significantly decreased in crisis compared to remission.

Conclusion: RAO at all disease stages was characterized by a low surfactant PL content. RAO crisis induced a decrease in PC and PG content. Further studies are needed to investigate the role of surfactant in the pathophysiology of RAO.

DNA BINDING ACTIVITY OF TRANSCRIPTION FACTORS IN BRONCHIAL CELLS OF HORSES WITH RECURRENT AIRWAY OBSTRUCTION

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Horses with recurrent airway obstruction (RAO) present many similarities with human asthmatics including airway inflammation, hyperresponsiveness, reversible obstruction, and increased NF- κ B expression. Studies in experimental asthma models have shown that transcription factors such as activator protein-1 (AP-1), GATA-3, cyclic AMP response element binding protein (CREB) and CAAT/enhancer binding protein (C/EBP) may also play an important role in airway inflammation. The purpose of this study was to measure DNA binding activity of these transcription factors in the airways of horses with RAO and to compare it to pulmonary function and bronchoalveolar lavage fluid (BALF) cytology.

Seven horses with RAO and six control animals were studied during a moldy hay challenge and after 2 months at pasture. Pulmonary function, BALF cytology and transcription factors' activities in bronchial brushings were measured during hay and pasture exposures.

During moldy hay challenge, RAO-affected horses developed severe airway obstruction and inflammation and a significantly higher airway AP-1 binding activity than in controls. After 2 months on pasture, pulmonary function and airway AP-1 binding activity were not different between RAO and control horses. The DNA binding activity of CREB in airways of RAO-affected horses increased significantly after 2 months at pasture and became higher than in controls. A significant positive correlation was detected between AP-1 binding activity and indicators of airway obstruction and inflammation. Airway GATA-3, CEBP and CREB binding activities were negatively correlated with indices of airway obstruction. However, contrarily to CREB binding activity, GATA-3 and CEBP binding activities were not different between RAO and control horses and were unaffected by changes in environment.

In conclusion, the results of the present study suggest that the effect of moldy hay exposure on RAO-affected horses is at least partly mediated by an increase in AP-1 binding activity in the airways. Prolonged allergen evicton results in upregulation of CREB and down regulation of AP-1. Future therapies for RAO could target transcription factors involved in modulation of airway inflammation.

DETECTION OF *RHODOCOCCUS EQUI* IN SAMPLES OF TRACHEOBRONCHIAL SECRETIONS OF FOALS BY MICROBIOLOGICAL CULTURE AND BY POLYMERASE CHAIN REACTION

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The goal of the present study was to investigate whether new PCR-methods would improve diagnostic of *Rhodococcus(R.) equi*. In a first step, sensitivity and specificity of the PCR-methods in respect to the “gold standard” microbiologic culture was determined. Second, sensitivity and specificity of both microbiologic methods were evaluated in respect to the clinical diagnosis.

The tracheobronchial secretions of 48 foals with clinical suspected and sonographically revealed pulmonary abscesses and of 37 healthy foals were evaluated by bacteriological culture as well as by four PCR-methods: *aceA*-, *ideR*-, *vapA*- and VP-PCR.

In respect to the “gold standard” microbiological culture, most PCR methods had sensitivities between 63.9 and 69.4% except the *vapA*-PCR (27.8%). The specificity of all PCR methods in this comparison was between 98 to 100%. In this analysis, clinical diagnosis had a low sensitivity (66.7%) and a low specificity (51.0%).

In respect to the clinical diagnosis, microbiologic culture’s sensitivity was 50.0% and specificity 67.7%. In this analysis, sensitivities of most PCR methods were between 33.3 and 37.5% except for the *vapA*-PCR (10.4%). The specificity of all PCR methods was between 78.4 and 86.5%.

In conclusion, these results show that the diagnostic potential of both microbiologic methods is different and that for the diagnosis of *R. equi*-pneumonia in foals the combination of microbiologic culture with PCR should be used for examination of samples of the airways of foals.