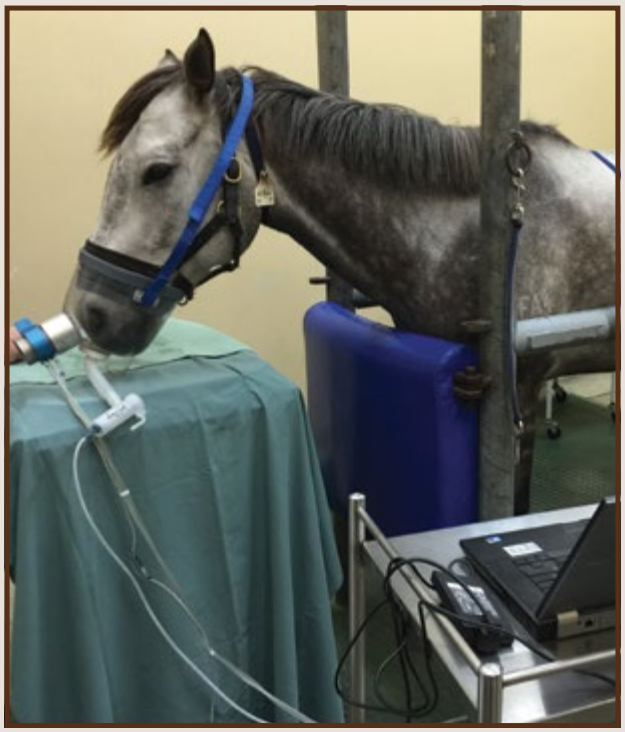
PROJECT SUMMARY

RURAL INDUSTRIES
Research & Development Corporation

Working towards improving diagnosis of equine inflammatory airway disease



Background

Inflammatory airway disease (IAD) is a common non-infectious lower airway disease of athletic horses worldwide.[1] IAD is commonly linked to poor athletic performance[2] resulting in significant cost to the racing industry in days lost to training[3] and management of the disease. The definition of IAD has been refined to include a horse of any age that displays poor performance, exercise intolerance or coughing with or without excess tracheal mucus **and** exhibits non septic inflammation on cytological examination of bronchoalveolar lavage fluid (BALF) **or** pulmonary airway dysfunction as evidenced by lower airway obstruction, airway hyperresponsiveness, or impaired blood gas exchange at rest or during exercise.[1]

Horses with IAD can have several BALF cytological profiles: a mild relative neutrophilia[4-6]; increased eosinophils[7]; relative mastocytosis[8]; or, a mixed response. There is variability in the reported distribution of cell types in BALF from normal horses, but cytological changes historically consistent with IAD commonly involve one or more of the following variants: neutrophils greater than 5%; mast cells which exceed 2%; or eosinophils 1% or greater.[9] Neutrophilic responses have been associated with coughing[10], while mastocytic[10,11] and eosinophilic[7] responses have been associated with heightened airway reactivity or hyperresponsiveness.[12]

According to the original literature neutrophilic responses were considered the most common cytological derangement in IAD of racehorses and sport horses.[4-6] More recent literature and sampling horses over a wider geographic area, suggests that mastocytic/eosinophilic responses occur with a higher prevalence than previously reported.[13] A recent Australian epidemiological study reported that mastocytosis was the most common abnormal BALF cytological profile.[14] Mastocytosis, defined as mast cells exceeding 2% of the total cell population, has been associated with airway hyperresponsiveness[10,11,] exercise intolerance[10,11] and pulmonary dysfunction[15]. There is growing debate within the literature regarding what relative percentage of mast cells constitutes a clinical mastocytosis, with some now proposing that a relative percentage of <5% is within limits of clinical normality.[16-18] This has not been supported by pulmonary function testing, and a recent epidemiological study[14] did not demonstrate any significant correlation between horse characteristics, historical signs or clinical findings and BALF cytology that would differentiate horses with mast cells greater than 5%, compared to horses with between 2 and 5% mast cells or horses with normal BALF cytology.

The clinical signs consistent with IAD may be vague, therefore non-specific pulmonary function testing (PFT) can be used to determine the functional significance of these clinical signs[19], as the majority of IAD horses are reported to display objective pulmonary dysfunction at rest or respond to provocation with histamine[12]. A number of testing modalities are available to determine equine pulmonary function with the majority not applicable to a clinical setting. One exception, flowmetric plethysmography (FP), is a non-invasive lung function testing method that has been adapted for use in horses. As the sensitivity of the FP system is similar to conventional lung mechanic testing, the test is coupled with bronchoprovocation methods (histamine challenge) to improve sensitivity.[19]

Horses with an exaggerated response to a histamine aerosol challenge have airway hyperreactivity (AHR), which is thought to be an excellent marker of small airway inflammation[20], particularly that induced by mast cells or eosinophils[1].

Studies reporting associations between BALF cytology and PFT are limited. Significant correlations between BALF mast cell and/or eosinophil percentage and AHR have been reported.[7,10,11] Horses with AHR are expected to have abnormal BALF, however the phenomenon of normal pulmonary function, as determined by PFT, and abnormal BALF cytology is reported to occur less than 20% of the time.[19]

Preliminary data from our geographic region have demonstrated that horses with BALF mast cell relative percentages exceeding 2% are common, and occur with or without overt signs of respiratory tract disease. An investigation of the effect of BALF mastocytosis on airway reactivity was warranted.

Aims/objectives

The primary objective of this project was to examine the relationship between BALF cytology and PFT findings in a group of sedentary horses maintained in a consistent environment. The first aim was to determine if cytological changes within BALF, particularly the relative mast cell percentage, was correlated with AHR, as determined by PFT. A secondary aim was to determine if mast cell tryptase (MCT), a marker of mast cell degranulation correlated with cytological changes within BALF or PFT in this geographical location.

The second objective was to examine the relationship between BALF cytology and PFT in horses presented for evaluation of poor performance that required a respiratory tract evaluation. This was investigated using similar aims and methods to that described for the primary study objective with the exception that two geographic regions were included.

Summary of methods

Objective 1

Thirty-eight healthy adult horses from a university owned herd were used. All animal testing was performed with the approval of Murdoch University Animal Ethics Committee (R2423/11). Subjects consisted of 18 Thoroughbreds, 19 Standardbreds and one Arabian, aged between 4 and 27 years. Management of the horses was consistent and all were housed in a large pasture for a minimum of 4 months before and throughout the study.

Pulmonary function testing and histamine bronchoprovocation was performed using a commercial flowmetric plethysmography system (Open Pleth ™) as recommended[21] in an indoor room with adequate ventilation (figure 1 and 2). The test was discontinued when a maximum concentration of 32mg/ml of histamine diphosphate was reached or airway impedance (as measured by delta flow) had increased by at least 50%. The concentration of histamine that caused a 35% change in delta flow (PC35) was determined using a histamine dose response curve. Airway hyperreactivity was expressed as a PC35 value and categorised as per manufacturer's recommendations.[21]

Reproducibility of the technique in this environment was determined on a subset of 10 horses (3 observations per horse over 2 weeks) and deemed to be acceptable with an intra class correlation coefficient of 0.655.

Bronchoalveolar lavage was performed as previously described[22], in horses <24 hours after PFT. Total nucleated cells counts and relative cellular percentages of cells were determined and in a group of 24 horses MCT was measured from the BALF supernatant using a commercial ELISA (EIAab Inc).

Objective 2

Over a period of 2 years horses were enrolled to participate in the study. Clinical respiratory tract evaluation was performed in all cases. Upper airway endoscopy was performed at the primary clinicians discretion as it was not considered a definitive test for IAD and was an additional expense. All horses had a PFT and BAL performed as described above. Data from horses in another geographical location, where both a PFT and BAL were performed, were included in the data analyses.

Data analyses

Variables of interest included the relative cell percentages, PC35 and MCT concentration. Non-parametric data analysis was used, as the data were not normally distributed. For continuous variables correlations were determined using a Spearman's rank correlation coefficient. Data were then categorised and a Chi-squared test for independence or a Fisher's exact test was used to investigate the level of association between categories. Direct comparisons between categories were undertaken using a Mann-Whitney U test.

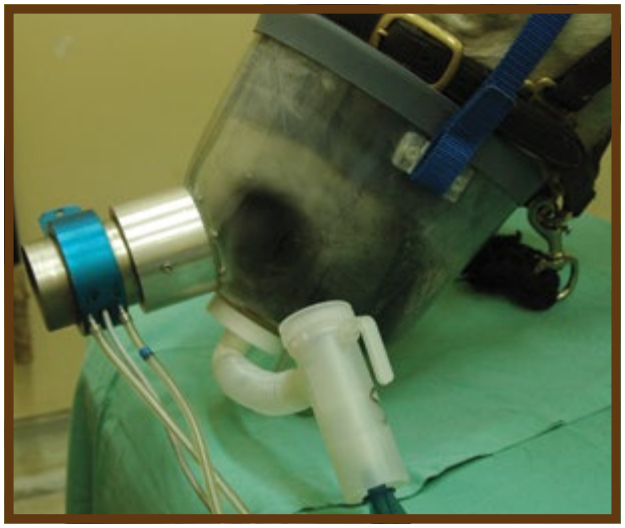


Figure 1 – The Open Pleth ™ mask and pneumotach fitted to a horse

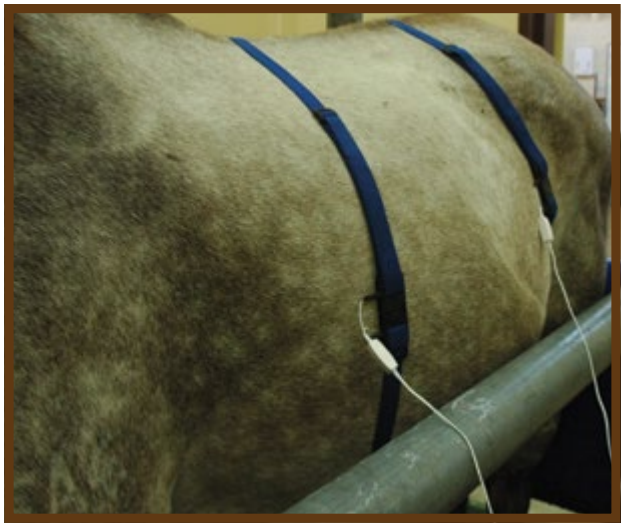


Figure 2 – The Open Pleth ™ inductance bands fitted to a horse

Results/key findings

Objective 1

No clinical signs of respiratory tract disease were present in any of the 38 sedentary animals. Airway hyperreactivity (PC35 < 8mg/ml) was demonstrated in 52% of horses. On the basis of published normal values for BALF cytology[9] 95% of horses had IAD, with a majority (75%) having a mixed inflammatory cell profile. No correlation was found between relative BALF cell percentages or total BALF cell concentration of mast cells, eosinophils or neutrophils and airway reactivity, as determined by PC35. When data were categorised there was no correlation between IAD subtypes and airway reactivity.

Within the sub-group of horses where MCT concentration was evaluated, MCT concentration was not correlated with relative mast percentage or total mast cell count. When data were categorised, MCT concentration was significantly greater in in the mastocytosis group (>2%), than mast cells ≤ 2%, but was not significantly different between the mast cell 2-5% group and the mast cell>5% group. Mast cell tryptase concentration was positively correlated with relative eosinophil percentage and the total eosinophil cell concentration, and negatively correlated with relative neutrophil percentage. When the data was categorized as eosinophils ≥ 1% or as neutrophils >5% as indicative of IAD, MCT was not significantly different between groups. Those horses that were categorised as having a combined mixed mast cell, eosinophilic, neutrophilic response had a significantly higher MCT than all other responses. Mast cell tryptase was significantly correlated with airway reactivity, but when data were categorised as being airway hyperreactive (PC35 <8mg/ml histamine) or non reactive (PC35 >8mg/ml histamine), MCT was not significantly different across groups.

Objective 2

There were 62 clinical cases available for analysis (26 from Western Australia and 36 from Queensland). Sixty nine percent of horses displayed AHR and 68% were categorised as having cytological changes consistent with IAD.[9] Fourteen percent had a sole neutrophilic response, 18% had a sole mast cell response, 2% had a sole eosinophilic response and 34% had a mixed response. Mastocytosis was the most common cytological abnormality.

No correlation was found between relative percentages of mast cells, eosinophils or neutrophils and the indices of airway reactivity PC35. When data were categorised, horses with a mixed inflammatory cell response were significantly more likely to display AHR than those horses with a sole inflammatory cell response or no inflammatory cell response.

Implications for relevant stakeholders

Inflammatory airway disease, as defined by increases in the relative percentages of key inflammatory cells, occurs commonly within Australian horses, independent of clinical signs. Mastocytosis (>2%) was a common finding across groups. Increases in the relative percentage of inflammatory cells within BALF without changes in pulmonary function, as measured by flowmetric plethysmography may occur with much greater frequency than previously reported. Relative cell percentages of mast cells and eosinophils may occur independently of AHR in some geographical locations. The finding that mixed cell responses are more likely to result in AHR, compared to sole responses, supports the notion that mixed responses represent an increased expression of inflammatory mediators within the BALF with subsequent recruitment of multiple inflammatory cell types. This is reinforced by a significant positive correlation between MCT and AHR, with MCT magnitudes greatest in mixed cell responses. The direct measurement of inflammatory cell mediators within BALF may be a more accurate reflection of dynamic airway function.

Recommendations for veterinarians and researchers

Given the often vague clinical signs of equine IAD, care should be taken in using existing global cytological definitions of BALF cytology as the sole method of confirming the diagnosis of disease in Australia. Further investigation of inflammatory cell mediators within BALF, their relationship with relative cell percentages, and their role in dynamic lower respiratory tract function is required to deepen understanding of the disease. This would allow veterinarians to interpret BALF cytology with greater certainty. Longitudinal investigations are warranted to determine if BALF cytology is programmed to an individual horse or is a product of environmental exposure, both past and present.

Intended publications from this project

Bronchoalveolar mast cell tryptase and its relationship to pulmonary function testing and bronchoalveolar fluid cytology in a group of Western Australian horses.
C Secombe, G Lester, P Stumbles, I Robertson, A Cullimore

Airway hyperreactivity occurs more commonly in horses with bronchoalveolar mixed inflammatory cell responses.
C Secombe, A van Eps, G Lester, I Robertson

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See references overleaf

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