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Non-invasive monitoring of changes in exhaled markers of airway inflammation in Thoroughbred racehorses

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What is Inflammatory Airway Disease?

- Inflammatory Airway Disease (IAD) is a multi-factorial disease associated with inflammation of the lower airways (lungs) of the horse.
- Clinical signs may not be present in horses at rest but IAD can cause **significant exercise intolerance.**
- IAD is of major significance to the Thoroughbred racehorse, with between 11-50% of young racehorses affected with the condition[1]
- Diagnosing IAD can require invasive and costly tests, including lavage of the lower airways.

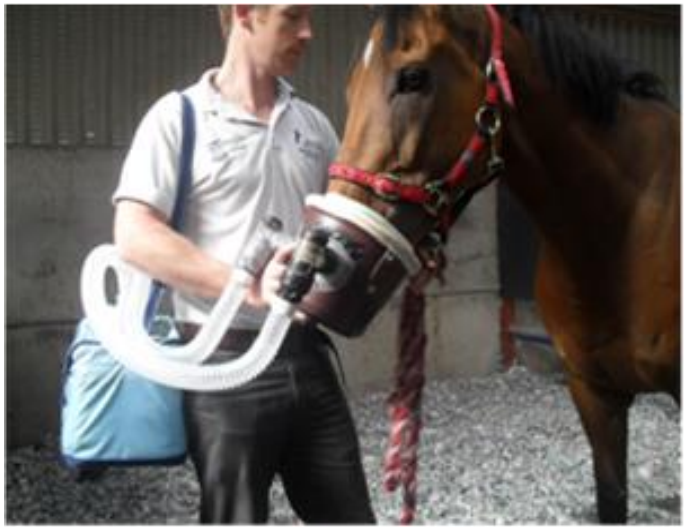
Why study IAD in racehorses?

- Developing a non-invasive modality by which IAD can diagnosed, monitored and controlled in Thoroughbred racehorses would result in;
  - Increased diagnosis and awareness of the condition
  - Better control of treatment
  - Improved performance and productivity
  - Increased welfare of the intensively trained racehorses

Exhaled Breath Analysis

- Exhaled breath analysis has been established as a means of diagnosing and monitoring airway inflammation in people, i.e. in asthmatic patients[2]

- The technique has been applied to many animals including horses, dogs, cats, cattle, sheep and even poultry[3]



Exhaled Breath Analysis

- A **biomarker** is a characteristic or substance that can be measured as an indicator of some biological state or condition.
- Various **biomarkers** can be measured in exhaled breath which can indicate the presence of inflammation in the airways. These include;
  - Gases – Nitric Oxide (NO), Carbon Monoxide (CO), Ethane
  - Soluble enzymes – Hydrogen Peroxide
  - Proteins – Isoprostanes, Leukotrienes
  - pH of breath condensate
- Many show large degrees of individual variation and can further be affected by respiratory flow rates, ambient temperature and collection techniques.

Aims of this Study

The aims of this study were to:

1. Develop and modify the methodologies for EB and EBC collection for the specific application for use in Thoroughbred racehorses.

2. Assess the influence of exercise *per se* and exercise in extremes of ambient temperature on exhaled markers of inflammation.

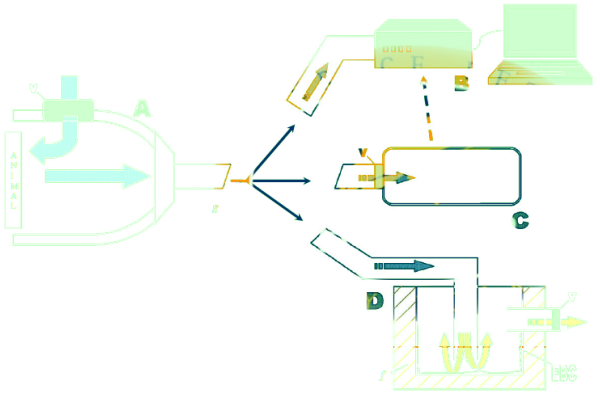
3. Investigate the association between exhaled markers of inflammation and respiratory secretion cytology in poor performing Thoroughbred racehorses.

How did we do this?

- A safe, portable and enclosed collection system was developed which could be used in a field setting.
- The condensing device was rigorously tested and modified to ensure it did not cause undue interference with the horse's normal respiratory patterns

- All techniques and study designs were approved by the ethics & welfare committee at the University of Glasgow

Exhaled breath (EB) and exhaled breath condensate (EBC) collection



The subject breaths through a facemask (**A**) which has one-way valves (**v**) in place to ensure that the full exhalation is directed into the conducting tube (*g)*. The conducting tube diameter should be equivalent to that of the animal's trachea and should be as short as possible to minimise air flow resistance during exhalation.

Direct on-line measurement of exhaled gases via chemiluminescence is achieved by directing the exhaled breath (or a sample thereof) into dedicated analysers. Alternatively a sample of exhaled breath can be collected into a non-permeable bag (Tedlar bag, **C**) and stored for off-line analysis.

Collection of EBC is achieved by directing the exhaled breath through a condensing chamber (**D**).

The condensing surface is creating by placing a removable inert plastic container into a cooling agent (*f)*. One-way valves (**v**) are also incorporated into the sampling devices to ensure no re-breathing of EB and to prevent contamination of the EBC chamber with ambient air.

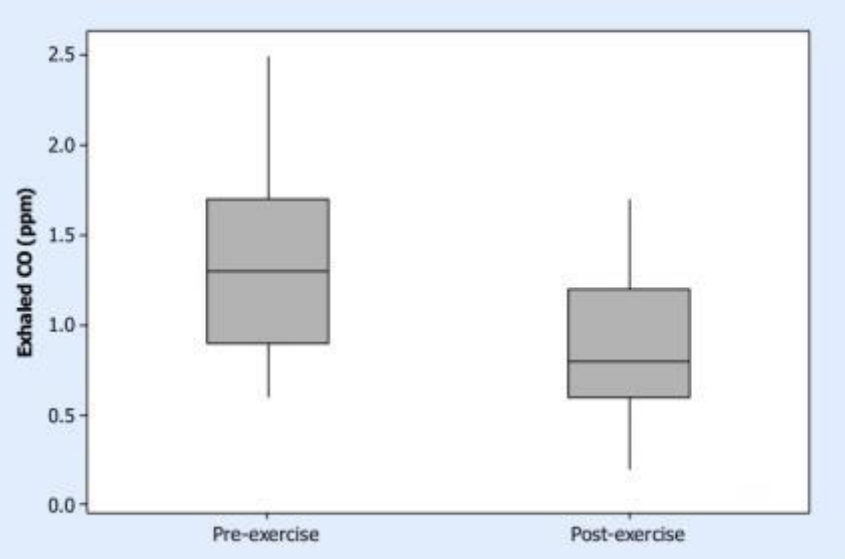
Presented, with permission, from Cathcart et al. 2012[3]

Influence of Exercise & Ambient Temperature?

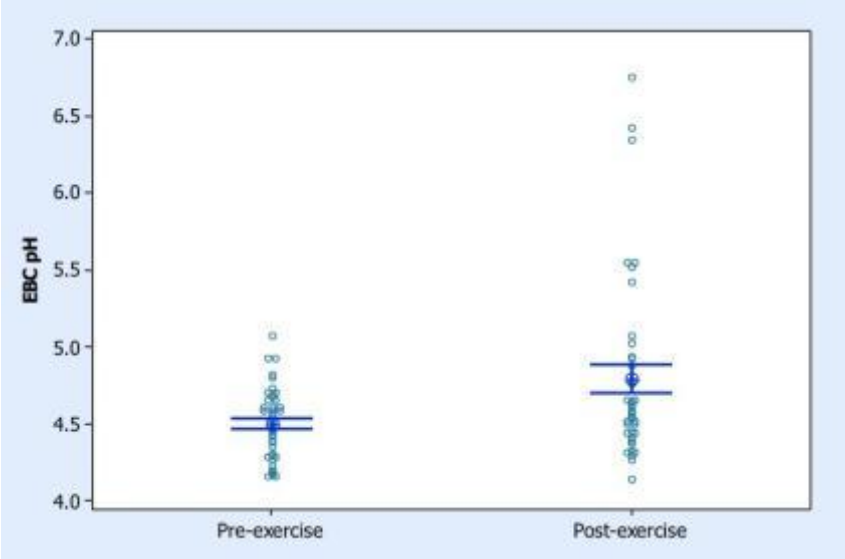
- 42 Thoroughbred racehorses were sampled, prior to and following standard training exercise
- 23 horses were sampled in summer and 19 were sampled in winter

- Exhaled Carbon Monoxide (CO), Nitric Oxide (NO) and EBC pH were analysed in all samples

Influence of exercise & temperature - Results



Exhaled CO decreased significantly after exercise



EBC pH significantly increased after exercise

- The decrease in exhaled CO was greater in horses exercised in warm ambient conditions

Cathcart et al. 2013 [5]

Poor Performance Study

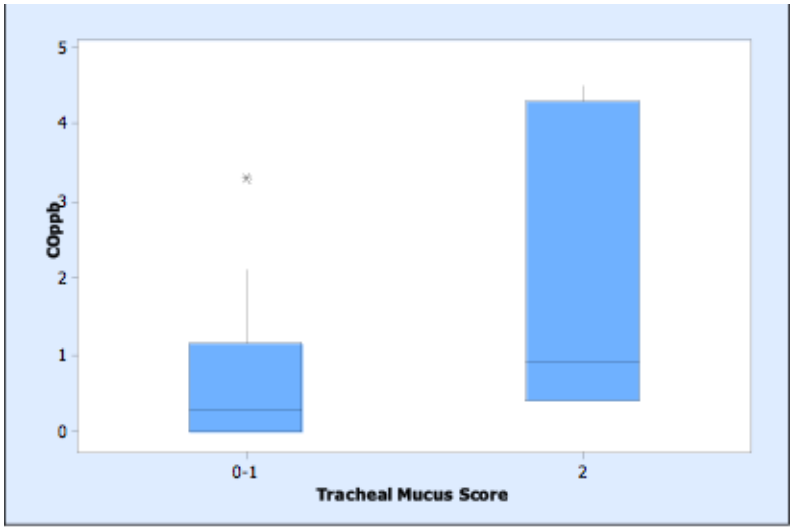
- 25 Thoroughbred racehorses were sampled during assessment for poor performance

- Concurrent broncho-alveolar lavage (BAL) samples were obtained as part of the normal clinical assessment
  - The BAL procedure involves collecting cells lining the airways deep in the lung using an endoscope. It is very accurate but requires the horse to be sedated so potentially interrupts racing schedules

- BAL results were compared to the exhaled CO, NO and EBC pH to investigated the presence of any associations.

Poor Performance Study

- Horses with higher tracheal mucus scores had significantly higher concentrations of exhaled carbon monoxide



- No other associations were found between exhaled biomarkers and cytological indicators of inflammation in the lower airways

Unpublished Data

Conclusions

- Exhaled breath analysis is applicable to Thoroughbred racehorses in a field setting
- Carbon Monoxide can consistently be measured in the breath of Thoroughbreds
- Exhaled CO significantly changes in response to exercise, changes in ambient temperature and in the presence of mucus accumulation in the trachea
- Nitric Oxide is not a reliable exhaled biomarker in horses
- pH reflects respiratory function but not disease status

Future Prospects

- Refining the sample collection technique would be required before conducting larger scale studies
- Portable analysers for exhaled markers of inflammation would greatly enhance the logistics of conducted wider-scale studies
- Exhaled carbon monoxide holds promise as an easy-to-measure exhaled biomarker
- A larger scale study investigating the link between exhaled CO and tracheal mucus score would be beneficial corroborate the results of this study

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