

DO BALF CYTOKINE PROFILES VARY DEPENDING ON THE SAMPLED LUNG IN HORSES WITH UNILATERAL IAD-CONSISTENT CYTOLOGY?

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Reasons for performing study: Little data on BALF cytokine profiles are available from racehorses with IAD; cytological diagnosis being most frequently made from one lung only per horse.

Objectives: To compare cytokine mRNA expressions and protein concentrations in BALF from both lungs of horses with unilateral IAD-consistent cytology.

Study design: Cross-sectional study.

Methods: As part of a larger study, 250 ml saline was randomly instilled in one lung and 500 ml in the contralateral lung of 30 clinically healthy Standardbred racehorses. This procedure was repeated 72 h later, inverting the volume per lung. Cytological cut-off values for IAD diagnosis was neutrophil proportions >10% when instilling 250 ml. For these samples, mRNA expression and concentrations of IL-1 , IL-4, IL-8, IL-10, IL-17, TNF-  and IFN-  were determined by RT-qPCR and ELISA.

Results: Eleven horses had BALF with IAD- and CTL-consistent cytology from, respectively, each lung, and were enrolled in the study (22 samples). Data were not significantly influenced by the sampling day, and BALF total cell counts or cytokine concentrations were not significantly different among lungs. Relative mRNA expression of IL-1  (3.887 ± 3.082 ; $P = 0.01$) and IL-10 (3.225 ± 1.710 ; $P = 0.005$) were significantly higher in BALF of IAD- compared to CTL consistent lungs (respectively 1.408 ± 1.118 and 1.488 ± 1.393); and also correlated to neutrophil proportions (respectively $r = 0.54$; $P = 0.01$ and $r = 0.65$; $P = 0.001$).

Conclusions: Differences in cytokine mRNA expression were associated with IAD- or CTL consistent BALF cytology in the same racehorses in training. These findings suggest that specific local immune reactions or regulation within the lower airways should be considered in IAD.

Ethical animal research: The study was approved by the Regional Animal Ethic Committee, and informed consent was provided by all horse owners. **Sources of funding:** LAB  O, CISCO-Oniris and AVEF (French Association of Equine Practitioners). **Competing interests:** None declared.

COMPARISON OF NANOPARTICULATE CpG IMMUNOTHERAPY WITH AND WITHOUT ALLERGENS IN RAO-AFFECTED HORSES

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Reasons for performing study: New therapeutic strategies to modulate immune responses in human and equine allergic airway diseases are under extensive investigation. Stimulation of Treg cells with immune modulating agents is a novel therapeutic option.

Objectives: The aim of this field study was to compare the effects of a nebulised nanoparticulate CpG immunotherapy (CpG-GNP) with and without specific allergens.

Study design: Longitudinal clinical study comparing 2 therapeutic options.

Methods: Twenty RAO-affected horses were divided into 2 treatment groups (CpG alone and CpG with allergens). Two specific allergens were selected for each horse according to anamnesis and a functional *in vitro* test. Treatments were given by nebulisation 7 times and the horses were examined 3 times: baseline (I), after the treatment course (II), and after 6 weeks later (III). Clinical parameters, indirect intrapleural measurement, arterial blood gas, amount of tracheal mucus and neutrophil percentage were evaluated.

Results: CpG alone resulted in a significant improvement in clinical parameters and a significant reduction of tracheal mucus after treatment and at 6 weeks post treatment. After CpG plus specific allergens, there was significant improvement of 70% of examined parameters. However, there were no significant differences in the results compared with CpG-GNP treatment alone.

Conclusions: There were no significant differences between treatment groups. CpG-GNP immunotherapy alone produced a potent and persistent effect on allergic and inflammatory parameters and may have potential as for treatment of equine and human allergic inflammatory airway diseases.

Ethical animal research: The study was approved by the regional legal agency for animal experiments of the Government of Bavaria, Germany (No. 55.2-1-54-2531-31-10). Owners gave informed consent for their horses' inclusion in the study. **Sources of funding:** Partly supported by the Deutsche Forschungsgemeinschaft (DFG) (Germany) (GE'2044/4-1). The AeroNeb Go[™] vibrating mesh nebuliser (Aerogen, Galway, Ireland) was sponsored by Inspiration Medical (Bochum, Germany). **Competing interests:** None declared.

ULTRASONOGRAPHIC IDENTIFICATION OF THE PULMONARY VEINS IN ADULT HORSES

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Reasons for performing study: In man, pulmonary veins (PV) are of major importance in atrial fibrillation pathophysiology as ectopy often

originates in atrial myocardial sleeves extending in the PV wall. In addition, PV size on ultrasound is used to assess left atrial volume overload. In horses, PV anatomy and ultrasound have hardly been studied.

Objectives: To study PV anatomy and establish a standard approach for echocardiographic identification of the PV from a right and left parasternal view.

Study design: Anatomical and echocardiographic description.

Methods: Firstly, *post mortem* left atrial and PV silicone corrosion casts were made in 10 horses to investigate normal PV anatomy. Secondly, left and right parasternal ultrasound images and silicone casts were made in 3 horses, to compare images and casts.

Results: Four major PV ostia were identified on the casts, draining specific lung areas. From a right parasternal longitudinal view, ostium III was identified between the right atrial wall and right pulmonary artery, and ostium II left-dorsal to it. From a left parasternal oblique short-axis view, ostium III, its antrum and side-branches were used as anatomical landmarks (adjacent to the right atrial wall). Slightly more caudal, ostium II was visualised with, in one horse, its antrum and 2 branches. Ostium I was only seen in one horse, slightly ventral to ostium II. Ostium IV was always identified in cross-section on a left short-axis view, left to the pulmonary artery bifurcation.

Conclusions: A standard echocardiographic approach allows identification of pulmonary veins in adult horses. Left oblique and short axis views were superior although right long-axis views also showed ostiums II and III. These data allow reference values to be established for pulmonary venous size and flow, and to investigate new treatment strategies for atrial fibrillation.

Ethical animal research: *Post mortem* study with owner's informed consent; ultrasound with owner's informed consent. Study performed following the local ethical guidelines. **Source of funding:** Ghent University (institutional). **Competing interests:** None declared.

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CAUSES OF PLEURAL EFFUSION IN HORSES IN THE UK

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Reasons for performing study: Pleural effusion (PE) is reported to occur most commonly secondary to bacterial pneumonia or lung abscesses, with neoplastic effusions contributing the minority of cases. The majority of these reports originate from America and Australia, where long distance transport of horses, a recognised risk factor, appears to occur more frequently. Anecdotally, neoplastic PE is more commonly diagnosed in the UK.

Objectives: To describe the causes of PE in horses resident in the UK, and to identify potential markers that can help differentiate between infectious and neoplastic causes of PE.

Study design: Retrospective clinical study.

Methods: Medical records from 4 referral hospitals in southern England were searched for horses diagnosed with PE. Information gathered from medical records included signalment, diagnosis (infectious vs. neoplastic), admission physical examination and biochemical findings, and characteristics of the effusion (volume, cell count, total protein [TP] concentration). Statistical comparisons were made between the neoplastic and infectious group using appropriate testing.

Results: Seventy horses were identified, of which 28 (40%) were neoplastic and 42 were infectious. Horses with infectious effusions were

significantly younger (median 7 vs. 13 years; $P = 0.002$) and had significantly smaller volumes of pleural fluid drained at admission (9.8 vs. 32.3 l; $P < 0.001$). Horses with infectious PE had a significantly higher rectal temperature (38.6 vs. 38.2°C; $P = 0.03$), fibrinogen concentration (7.8 vs. 5.7 g/l; $P = 0.02$) and serum amyloid A concentration (223 vs. 104 mg/l; $P = 0.02$). Pleural fluid characteristics identified a significantly greater cell count and TP concentration in horses with infectious PE (47 x 10⁹/l vs. 3.4 x 10⁹/l; $P < 0.001$; 54 vs. 31 g/l; $P = 0.001$).

Conclusions: These results suggest that in the UK neoplastic effusions account for a greater proportion of PE than previously reported. A large volume of PE in an older horse with a low cell count and TP concentration should increase the index of suspicion of neoplasia.

Ethical animal research: This was a retrospective study of clinical cases. Explicit owner informed consent for inclusion of animals in this study was not stated. **Source of funding:** None. **Competing interests:** None declared.

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POST EXERCISE CARDIAC TROPONIN I RELEASE AND CLEARANCE IN NORMAL STANDARDBRED RACEHORSES

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Reasons for performing study: There are currently no studies detailing cardiac troponin I (cTnI) release and clearance in normal horses post exercise using an analytically validated assay. These data are essential for selecting appropriate sampling times in equine athletes with suspected myocardial damage.

Objective: To plot the magnitude and time course of cTnI release and clearance, using a validated cTnI assay, after maximal effort.

Study design: Descriptive longitudinal study.

Methods: Five clinically normal Standardbred racehorses in full race training were included in the study. Physical examinations were performed on subjects and blood samples were taken via jugular venipuncture pre-exercise. Horses were exercised in harness at race intensity in groups on a training track. A second blood sample was taken immediately post exercise and an intravenous catheter was then placed in a jugular vein. Hourly blood samples were taken for 24 h. All samples were collected in red top serum Vacutainer® tubes and allowed to clot for 30 min before being centrifuged and serum harvested. Serum samples were stored at -80°C until analysis. All samples were analysed using the Abbott ARCHITECT STAT High Sensitivity cTnI® assay.

Results: Mean resting cTnI level was 1.33 ± 0.6 ng/l (range, 0.82–2.33 ng/l). All horses exhibited an increase in cTnI level after exercise with peak elevation occurring 2–6 h post exercise (mean, 4.6 ± 1.7 h). Mean peak increase in cTnI level was 11.96 ± 9.41 ng/l (range, 1.72–23.76 ng/l). All horses returned to baseline levels within 24 h.

Conclusions: All horses experienced an increase in cTnI post exercise, with the peak occurring 2–6 h post exercise. Further studies are needed to determine the significance of these increases.

Ethical animal research: The study protocol was approved by the University's Animal Care Committee. Explicit informed consent was