CHARACTERIZATION OF T-HELPER LYMPHOCYTES IN PERIPHERAL BLOOD AND BRONCHO-ALVEOLAR LAVAGE OF HEALTHY HORSES AND IN HORSES WITH RECURRENT AIRWAY OBSTRUCTION

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INTRODUCTION
Heaves, also known as recurrent airway obstruction (RAO), is a respiratory disease of horses that is characterized by lower airway neutrophilic inflammation and airway obstruction caused by hypersensitivity of susceptible horses to inhaled organic dust. The immunological processes underlying RAO remain unclear.

OBJECTIVE
The objective of this project was to characterize the nature of the adaptive immune response in affected horses. We hypothesized that the immune response in RAO affected horses is predominantly a Th17 type response with a Th17/Treg imbalance.

MATERIAL AND METHODS
• Horses: 4 horses with a history of RAO (19.8±2.2 years, 473.3±65.2kg) and 3 healthy horses (13.0±5.0 years, 559.0±52.0 kg)
• Recruited after being kept in pasture for at least one month
• Blood and broncho-alveolar lavage (BAL) were sampled at day D0, and D10, & D20 after exposure of horses to hay and straw (environmental challenge) (BAL sampling was not achievable in 1 of the 4 RAO-horses) (figure 1)
• Extraction of CD4+ lymphocytes by Magnetic-Activated Cell Sorting
• Study of gene expression profile of cytokines Th1 (IFN-γ), Th2 (IL-4), Th17 (IL-17), Treg (IL10) and transcription factors Th1 (T-bet), Th2 (GATA3), Th17 (RORγT),Treg (FOXP3) by quantitative polymerase chain reaction (qPCR)
• Statistical analysis : Mixed model, level of significance p<0.05

RESULTS
We did not observe significant differences in gene expressions between healthy and RAO-affected horses at D0, neither in the blood nor in the BAL. IL-4 and RORy expression were significantly up-regulated during the environmental challenge in BAL of both healthy and RAO-horses (figure 2a). The expression of GATA3 was significantly up-regulated in BAL cells from heaves-affected horses compared to healthy horses at the end of the environmental challenge (figure 2b). Furthermore, FOXP3 expression in the BAL cells was significantly up-regulated during the challenge in healthy horses but not in RAO-affected horses (figure 2b). In the blood, a significant down-regulation of IFNγ, IL-4, IL-10, IL-17, FOXP3 and RORy expression was observed as well as a significant up-regulation of GATA3 expression during the environmental challenge in RAO-affected horses. In contrary, healthy horses showed a significant up-regulation of IL-4, IL-10, IL-17, T-bet and RORy expression and a significant down-regulation of FOXP3 expression during the environmental challenge.

CONCLUSION
These results allowed neither to establish precisely the type of immune response implemented in equine heaves nor to prove an involvement of a predominant Th17 response in RAO-affected horses. Nevertheless, these data suggest that (1) a less pronounced regulatory immune response and (2) a Th2 response may have an involvement in the immunopathologic mechanisms of heaves.