

## 8 Summary

### Effects of an experimentally induced infection with *Mannheimia haemolytica* A1 on pulmonary functions in calves

#### Aims

The aim of this study was to evaluate the complexity of pathophysiological mechanisms of an infection caused by *Mannheimia haemolytica* A1 in calves - with respect to pulmonary functions and resulting consequences for the total organism. Therefore, variables of lung function, blood gases, acid-base-status, red and white blood cells, and parameters of an acute inflammation in the peripheral blood were quantified before and after an experimentally induced challenge. Accompanying patho-morphological findings were recorded *post mortem*.

#### Methods

From a total number of 24 calves (age: 3 to 15 days, body weight: 31 to 47 kg, both sexes) 20 calves were included after a quarantine period of 6 weeks confirming clinically healthiness. For experimental challenge, 10 ml of a culture containing *Mannheimia haemolytica* A1 ( $1.5$  to  $2.0 \times 10^9$  cfu/ml) were injected intra-tracheally twice in each animal with an interval of 30 hours. At the end of the study, all calves were necropsied either due to spontaneous death or after euthanasia 5 days after infection.

During the total observation period of 45 days, a daily clinical examination was performed in each calf. To control the presence of *Mannheimia haemolytica* A1, both nasal swabs (to detect antigen by culture) and blood samples (to detect antibodies) were collected once per week ante infectionem (*a.i.*) and daily post infectionem (*p.i.*). For lung function testing, the Impulse-Oscillometry-System (IOS) was used. Variables of ventilation and respiratory mechanics were measured twice *a.i.* (-2 weeks, -1 week) as well as 3 days and 4 days after infection. *Via* a catheter that was placed in *Aorta abdominalis* arterial blood was sampled in 12 of the 20 calves at following time points: one day and immediately *a.i.*, and 3, 6, 12, and 24 hours (h) as well as 2, 3, 4, and 5 days (d) after infection. At these defined time points, variables of blood gas analysis, acid base status, and haemoglobin spectra were measured in parallel in both arterial and venous blood. Furthermore, in the venous blood red and white blood cells as well as acute phase proteins (haptoglobin and fibrinogen) were analysed. At dissection, patho-morphological changes in lungs were registered, and tissue was sampled at five locations within each lung for further bacteriological investigation.

#### Results

**Clinical observations:** After challenge, clinical symptoms were characterised by dyspnoea and fever (respiratory rate started to increase 3 h *p.i.*, and rectal temperature 6 h *p.i.*). Furthermore, a higher intensity of cough and nasal discharge was observed.

**Ventilation and respiratory mechanics:** Three and 4 d *p.i.*, both tidal volume ( $V_T$ ) and minute ventilation ( $V_{min}$ ) were significantly decreased while the respiratory rate was significantly increased. In addition, the following IOS-variables decreased significantly: respiratory resistance at 3 Hz, lung compliance, and coherence at 3 and 5 Hz.

**Blood gas analysis, acid base status and haemoglobin spectra:** After challenge, both partial pressure of  $O_2$  ( $PO_2$ ) and saturation of haemoglobin ( $SO_2$ ) decreased significantly in arterial as well as in venous blood samples indicating severe hypoxemia. Furthermore, the partial pressure of  $CO_2$  ( $PCO_2$ ) decreased significantly in both arterial and venous blood at

days 1 and 2 *p.i.* indicating hypocapnia. In addition, the alveolar-arterial oxygen difference (A-aDO<sub>2</sub>), the pulmonary shunt, and the arterial-venous difference in oxygen (a-vDO<sub>2</sub>) were significantly increased after infection. These findings were accompanied by a shift of the oxygen bound graph to the right (i.e. P<sub>50</sub> increased significantly). Hemoxymetry results were characterised by a significantly reduced percentage of oxygenated and a significantly increased percentage of deoxygenated haemoglobin.

Analysis of acid base variables resulted in significant decreases in standard base excess (SBE) and actual base excess (ABE) in both arterial and venous blood, after challenge. Furthermore, significant decreases of standard bicarbonate (SBC) and actual bicarbonate (HCO<sub>3</sub><sup>-</sup>) were analysed in both arterial and venous blood samples after infection.

**Peripheral blood parameters:** Related to red blood cells, the post challenge period was characterised by significant decreases in haemoglobin concentration, haematocrit, and the total number of erythrocytes. The indexes of erythrocytes MCV and MCH, however, remained unchanged whereas MCHC showed a tendency to increase (non-significant). No significant change was observed in the number of thrombocytes. Results related to white blood cells inclusive their differentiation indicated significant increases in total leukocyte number as well as in neutrophils with segmental nucleus, neutrophils with rod-shaped nucleus, and monocytes. Neither basophiles nor eosinophils were changed by experimental infection. An observed decrease in lymphocytes (3 h *p.i.*) could not be secured statistically. Markers of acute inflammation, i.e. concentrations of haptoglobin and fibrinogen in the venous blood, were found to be significantly increased in the post challenge period compared to baseline values before infection.

**Pathology:** Within the group of 20 calves, the extension of pneumonic lesions varied between 0.5 – 55.4 % of the total lung surface. In general, a large heterogeneity was found with respect to both quantity and quality of lung injuries.

### **Conclusions**

An experimentally induced challenge with *Mannheimia haemolytica* A1 in calves led to severe deteriorations in pulmonary functions and consequently to a strong impairment of oxygen transfer from alveoli into blood. Since no increase in respiratory resistance was measured and because CO<sub>2</sub> could be eliminated from the body (hypocapnia), there were no indications for the presence of obstructive disturbances of ventilation. However, restrictive disturbances in ventilation did occur and were measured by a diminution of lung compliance. Results of blood-gas-analysis (hypoxemia, increased A-aDO<sub>2</sub>, and increased pulmonary shunt) allow the conclusion that lung function disturbances were mainly related to an impaired diffusion capacity of the lung and to deterioration in lung perfusion.

In addition to damages within the respiratory system, systemic reactions of acute inflammation were predominantly reflected by parameters of white blood cells and by strong increases in the concentrations of haptoglobin and fibrinogen in the blood.

Since clinical symptoms (fever, dyspnoea, nasal discharge, and cough) and pathological findings (acute pneumonia of varying extend) were found to be non-specific, all pathogenetic mechanisms as evaluated in this study should be taken into account when *Mannheimia haemolytica* A1 has been found to be involved in respiratory diseases. Under field conditions, one must assume the presence of severe pulmonary dysfunctions at the same time when clinical symptoms occur. Consequently, multiple therapeutic components are necessary (in addition to antibiotic treatment) to improve pulmonary function and gas exchange and to reduce consequences of acute inflammation.