Factors associated with pulmonary inflammation in calves as determined by cytology on non-endoscopic broncho-alveolar lavage samples

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Objective

Pulmonary inflammation in calves can be due to infectious and non-infectious causes, with possible interactions. Non-endoscopic broncho-alveolar lavage (BAL) is a practical and cheap method currently used for pathogen identification in cattle. Cytological analysis of these BAL samples can provide insights in the level of pulmonary inflammation in calves. The objective of the present study was to determine which clinical signs, lung ultrasound findings and BAL characteristics are associated with total nuclear cell count and the differential count of pulmonary leukocytes.

Materials and methods

A cross-sectional study was conducted on 332 calves (Holstein Friesian (n=177) and Belgian Blue (n=155)) from 59 conveniently selected herds between January and April . Animal selection criteria were judgment as clinically healthy by the farmer, no previous antimicrobial use, age between 1 and 6 months and indoor group housing. Animals were clinically examined and lung ultrasound performed. Broncho-alveolar lavage fluid (BALF) was collected using a non-endoscopic method and instillation of 40 mL of saline. Bacterial culture was performed and species confirmation was done by Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS). The total nucleated cell count (TNC) of the recovered lavage fluid was determined manually using a haemocytometer. Cytocentrifuge preparations of BALF (1200 rpm for 10 minutes) were evaluated to determine the 400 cell differential cell count. A mixed model with herd as a random factor was used to identify factors influencing BALF total and differential cell counts.

Results

Of the calves, 49.1% (163/332) demonstrated lung consolidation on ultrasound. Pathogen isolation rates were 33.7% (112/332) for *Pasteurella multocida*, 15.4% (51/332) for *Mannheimia haemolytica*, 3.6% (12/332) for *Histophilus somni* and 3.3% (11/332) for *Mycoplasma bovis*. Isolation rates were not influenced by clinical signs or ultrasonographic findings. Mean TNC was 1,9 x 10^9 cells/L (standard deviation 1.8; range 0.0-13.7). Of the calves, 63.0% (209/332) had an increased TNC (> 1.0 x 10^9 cells/L), but only from 61.2% (128/209) of these a pathogen could be isolated. In contrast, from 37.4% (46/123) of the low TNC's a pathogen could be isolated. In

the final multivariable model TNC's were associated with isolation of *P. multocida* (P< 0.01), volume of recovered lavage fluid (P< 0.001) and presence of erythrocytes (P< 0.01).

Mean differential cell count (%) of leukocytes in BALF were 42.8% macrophages (standard deviation 19.1; range 2.4-92.3), 36.6% neutrophils (standard deviation 23.9; range 0.0-97.4) and 5.5% lymphocytes (standard deviation 5.3; range 0.0-45.8). In the final multivariable model isolation of *P. multocida* (*P*< 0.01), increased respiratory rate (*P*< 0.05) and a positive trachea reflex (*P*< 0.05) were positively associated with BALF neutrophil percentage. Macrophage percentage in BALF was inversely associated with the recovered volume of BALF (*P*< 0.05) and *P. multocida* (*P*< 0.05) isolation and was significantly higher in calves maintaining sternal recumbency (*P*< 0.01). The lymphocyte percentage in BALF increased with an increasing age (*P*< 0.05) and the presence of erythrocytes in BALF (*P*< 0.05).

Conclusions

Subclinical bronchitis and pneumonia are widespread in group-housed calves. Observed neutrophil percentages are markedly higher than reported in experimental studies in healthy calves using endoscopic lavage. Clinical signs, ultrasonography and bacterial culture explained very little of the variation in total and differential leukocyte counts in BALF from calves, suggesting that other factors like respiratory viruses or non-infectious environmental factors affect pulmonary inflammation.