

# Comparison of thoracic ultrasonography with analysis of bronchoalveolar lavage fluid for the diagnosis of subclinical bronchopneumonia in dairy calves

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## Introduction

The pathophysiology of bronchopneumonia (BP) is such that peripheral airways fill with neutrophilic exudate, which results in pulmonary consolidation and atelectasis. This exudate increases the proportion of neutrophils in bronchoalveolar lavage fluid (BALF), with proportions ranging from 10% to 20% suggestive of BP. Pulmonary ultrasonography (US), which differentiates between aerated and nonaerated lung tissue, has good sensitivity (Se = 85%) and specificity (Sp = 98%) for detection of BP in clinically ill calves and might be useful for diagnosis of subclinical BP. The purpose of this study was to compare thoracic US with BALF as a means to diagnose subclinical BP in dairy calves, with gross postmortem examination used as the gold standard.

## Materials and Methods

One hundred twenty-eight Holstein bull calves, 3 to 6 days old, were assessed weekly for 12 weeks. During each assessment, a respiratory score was assigned and bilateral thoracic US was performed. The US involved scanning the intercostal spaces from the first to the tenth ribs with a portable 6.2-MHz variable frequency linear rectal probe. The hair was not clipped and 70% isopropyl alcohol was used as the transducing agent. Twenty-four bull calves that had not been treated with antimicrobials during the previous two weeks, that did and did not have evidence of lung consolidation, and had a normal respiratory score (RS < 5) were selected for a more intensive evaluation that consisted of BAL and postmortem examination. A total of 24 bull calves were selected in the following categories: completely normal US (n = 4), comet-tailing (5), < 1 cm lung consolidation (5), 1 to 3 cm lung consolidation (5), and > 3 cm lung consolidation (5). Each calf was sedated with xylazine (0.227 mg/lb or 0.05 mg/kg, IV) and butorphanol (0.045 mg/lb or 0.1 mg/kg, IV). A sterilized flexible fiberoptic bronchoscope was passed intranasally into the lung on the side that

the ultrasonographic lesion was visualized and BALF was collected; the lung was lavaged with 240 mL of sterile saline (0.9% NaCl) solution in two aliquots. The BALF samples used for diagnostic purposes were obtained from the last aliquot. Immediately after BALF collection, each calf was euthanized with captive bolt and exsanguination. A respiratory-system based field necropsy was performed. The BALF samples and lung tissue specimens were kept on ice water and evaluated within 4 hours of collection. All examinations were performed by the same individual.

## Results

With gross postmortem examination used as the gold standard, the sensitivity and specificity of thoracic US for detection of subclinical BP were 94% (95% confidence interval [CI], 69% to 100%) and 100% (95% CI, 64% to 100%), respectively. Results of a receiver operator characteristics curve suggested that a BALV neutrophil proportion (NP) of 4% was the optimum cut-off for detection of BP, and at that cutoff, the sensitivity and specificity of BALF for detection of subclinical BP was 81% (95% CI, 56% to 94%) and 75% (95% CI, 36% to 95%), respectively. Agreement between US and postmortem gross examination was excellent (Kappa, 0.90).

Total nucleated cell counts in BALF did not differ significantly ( $P = 0.65$ ) between calves with consolidated ( $0.52 \times 10^9$  cells/L; n = 15) and completely normal lungs ( $0.59 \times 10^9$  cells/L; 4). The NP was significantly ( $P < 0.01$ ) greater in BALF obtained from calves with consolidated lungs (12%), compared with that in BALF obtained from calves with completely normal lungs (1.25%). The NP in BALF obtained from calves with comet-tailing (2%) was significantly ( $P = 0.05$ ) less compared to BALF obtained from calves with consolidated lungs (12%), but did not differ from that in BALF obtained from calves with completely normal lungs (1.5%;  $P = 0.52$ ). The macrophage proportion in BALF obtained from calves with consolidated lungs (79%) was significantly ( $P < 0.01$ ) less than that in

BALF obtained from calves with completely normal lungs (98%). The relationship between US severity and NP was not linear once consolidation was present. Results of univariable logistic regression analysis revealed that detection of lung consolidation by US increased the odds of an increased NP in BALF (OR, 23; 95% CI, 2.6 to 198;  $P < 0.01$ ).

### **Significance**

Thoracic US offered an immediate, non-invasive method to reliably diagnose subclinical BP on dairy farms in calves < 3 months old.