6 - Identification of Mycoplasma bovis directly from bronchoalveolar lavage fluid with MALDI-TOF MS

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Topic	Infectuous Diseases

Mycoplasma bovis is a leading cause of pneumonia in modern calf rearing. Fast identification is essential to ensure appropriate antimicrobial therapy, and to timely initiate control and prevention measures at farm level. Current diagnostic methods show issues, as they are either not able to differentiate between *Mycoplasma* spp., too time-consuming or expensive. Therefore, the objective of this study was to develop a rapid culture-based protocol to identify *M. bovis* directly from bronchoalveolar lavage fluid (BALF) with MALDI-TOF MS and to compare this method with other diagnostic tools.

BALF was obtained from 67 calves (8 farms). Presence of *M. bovis* was determined using three culture-based methods: (1) standard culture and lipase activity; (2) direct transfer method with MALDI-TOF MS from modified pleuropneumonia-like organisms agar and (3) direct MALDI-TOF MS detection after an enrichment procedure of BALF. After 24, 48 and 72 hours of incubation, protein extraction was performed and analyzed by MALDI-TOF MS. Also, a triplex real-time PCR for *M. bovis*, *M. bovirhinis* and *M. dispar* was performed. Results were analyzed with a Bayesian latent class model.

The model estimated the prevalence of *M. bovis* in the dataset at 10.9% (95% credibility interval (CI): 4.5-21.3). Method 1 and 2 showed detection of *M. bovis* in 8% and 15% of the samples after 5-10 days of incubation, respectively. For method 3, 1%, 19% and 22% of the samples were positive for *M. bovis* after 24, 48 and 72h of incubation, respectively. Sensitivity and specificity were 87% (CI: 49-100) and 97% (90-100) for method 1, 73% (36-97) and 99% (94-100) for method 2 and 88% (54-99) and 90% (80-96) for method 3. The results of statistical comparison of these methods compared to PCR, will be presented at the EBC.

In conclusion, direct identification of *M. bovis* from BALF is a promising rapid identification method with potential to massively improve timely initiation of effective therapy, control and prevention.