Diagnostic opportunities for use of *Mycoplasma bovis* antibody measurements in serum and milk

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Background: *Mycoplasma bovis* (*M. bovis*) causes disease and substantial economic losses in cattle. In Denmark outbreaks in dairy herds are occasionally seen, as well as on going disease problems among calves, both in dairy and slaughter calf herds. Despite this, there is currently a lack of affordable and convenient diagnostic tests for use in clinical work and for surveillance of infection with *M. bovis*.

To develop diagnostic guidelines for antibody testing against *M. bovis* we aimed to describe and compare the dynamics of *M. bovis* antibody responses in dairy cows and calves. Four Danish dairy herds experiencing an acute outbreak of *M. bovis*-associated disease were visited 5 times. A total of 120 cows and 83 calves were examined using a standardized clinical protocol and sampled with paired blood and milk samples, which were analysed for antibodies against *M. bovis* using the commercial ELISA kit BioX Bio K 302 at Eurofins-Steins Laboratory in Brørup. All animals were divided into disease groups based on the findings of the clinical examinations.

Results: The antibody responses in serum were highly dynamic and varied significantly between individual cows. The cows with mastitis generally had high antibody levels in both serum and milk at disease onset, while the cows with arthritis only had high antibody levels in serum. The duration of high antibody levels seems to be shorter than for other diseases, as the estimated mean was already below the manufacturer's cut-off at 37 ODC% 65 days after disease onset. The antibody levels in the cows with no *M. bovis*-associated disease were below the cut-off throughout the study period in most of the cows.

The interpretation of serology in calves differs from that in adult cows. The BioX ELISA response was below the 37 ODC% cut-off for the entire study period for many of the calves, even when they were clearly ill from *M. bovis*. The estimated mean ODC% slowly rose but did not reach the recommended individual animal cut-off at any time in three of the four herds. The tendency was that the highest ODC% was not reached until the calf was 60 days old and the pattern did not differ between disease status groups.

Conclusion: Due to the large individual variations, serology does not appear to be very useful for individual diagnosis of *M. bovis*-associated disease in dairy cows and calves. However, it might still be useful for herd or group level diagnosis. Antibodies in milk were only detected in cows with *M. bovis* mastitis, making antibodies in milk useful only for differential diagnosis of mastitis. We also conclude that the BioX ELISA is not a suitable test for diagnosing *M. bovis* in individual calves aged less than four months due to low sensitivity and the test cannot be used to differentiate between calves with arthritis and/or otitis media, and respiratory disease.