Testing of milk samples fails to detect on-going *Mycoplasma bovis* infections in dairy herds

Mycoplasma bovis (M. bovis) is a small bacterium capable of causing disease in cattle of all ages. It is part of the bovine respiratory disease complex and also associated with arthritis and otitis media in calves. In cows, the usual clinical presentation is mastitis and pneumonia, while arthritis is increasingly being reported. Since the first isolation in the USA in 1961, it has spread to many countries and is now endemic in many regions, including Europe. Over the last two decades, *M. bovis* has gained more attention due to its apparently increasing prevalence, intensified severity of the clinical signs and greater antibiotic resistance in recovered *M. bovis* isolates. The traditional way of diagnosing *M. bovis* has been bacterial culture of milk or other body fluids. However, easier and less expensive diagnostic test methods are requested for cows with arthritis or other systemic clinical signs of *M. bovis*-associated disease. In Denmark, serological assays such as ELISA are frequently used for testing dairy cows for other diseases, because they are relatively inexpensive per test and convenient, especially if applied to milk samples routinely collected for other purposes.

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A study of factors influencing the *M. bovis* ELISA optical density measure (ODC%)[1], which indicates the level of antibodies directed against *M. bovis*, in bulk tank milk (BTM) found that the prevalence of test-positive lactating cows was correlated with the BTM ODC%. For each 10% increase in the prevalence of milk-test positive lactating cows, the BTM OCD% went up by 9 ODC% on average [1]. However, it became obvious that clinical signs consistent with *M. bovis* were reported by farmers even in herds during periods with low ODC%-values measured in BTM (Figure 1). To pursue explanations for the observed dynamics of BTM ODC% and associations with underlying infection patterns, investigations of the antibody responses in individual cows with different clinical signs were warranted.

This was approached through an observational longitudinal study in four dairy herds with acute outbreaks of *M. bovis*-associated disease. The cows were divided into different disease groups based on the observed clinical signs, and the pattern in antibody responses in serum and milk were associated with the time since the clinical signs were first observed. The antibody response measured by the ELISA[2] was generally very dynamic, short-lived and dependent upon the observed clinical signs. Even in systemically diseased cows, the average estimated ELISA ODC% was below the recommended cut-off at 37 ODC% 60–70 days after clinical signs were observed for the first time (Figure 2). This makes the antibody response to *M. bovis* much more dynamic than seen for other diseases and means that frequent monitoring would be necessary to detect emerging *M. bovis* infection in the herd [2].

The ODC% in serum was primarily elevated in cows with clinical signs of systemic *M. bovis*-associated disease, while the ODC% in milk was mostly elevated in cows with mastitis and *M. bovis* PCR-positive milk samples. These findings suggest that secretion of antibodies against *M. bovis* in different fluids differ depending on clinical signs, making milk samples merely useful for detecting *M. bovis* udder infections [2]. In addition, differences were found when looking at the PCR results. Despite the fact that some cows with arthritis, or with non-specific or no clinical signs, were below the recommended PCR-cut-off at Ct-value 37, the cows with mastitis were, in general, clearly further below the cut-off (i.e., more clearly test positive) (Figure 3).

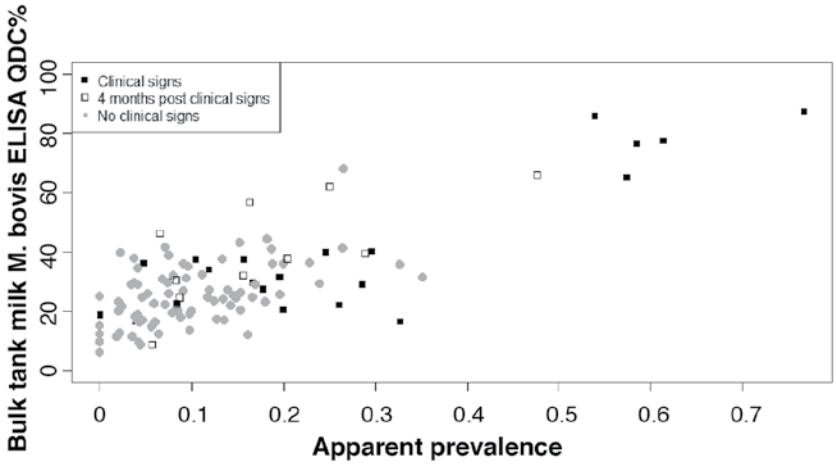

Figure 2 – Descriptive statistics showing the bulk tank milk *Mycoplasma bovis* ELISA optical density measurement (ODC%) plotted against the apparent milk prevalence of antibody-positive lactating cows. Modified from Figure 2 in Petersen et al. 2016, Factors associated with variation in bulk tank milk *Mycoplasma bovis* antibody-ELISA results in dairy herds. J. Dairy Sci. 99:3815–3823.

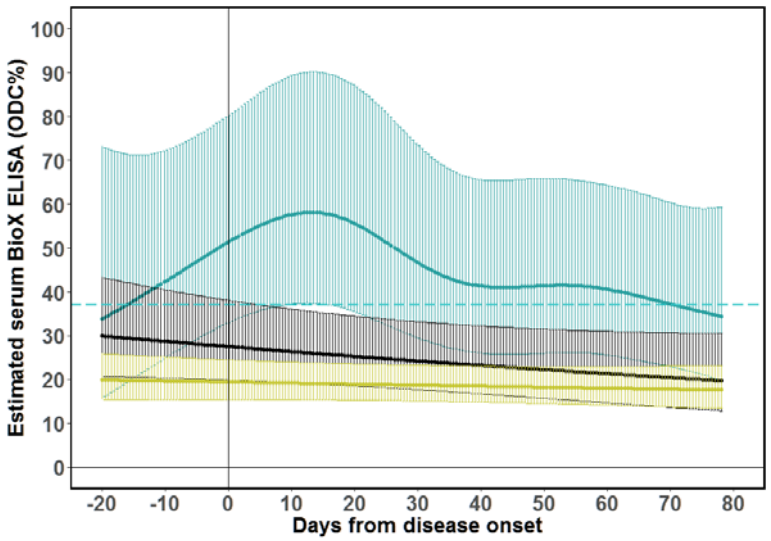

Figure 2 – Estimated mean antibody response in serum (solid line) and 95% confidence intervals (shaded area) as measured by the BioX ELISA Bio K302. Red represents the 'Systemic' group, blue is the 'None' group and black is the 'Non-specific' group. The dotted red line shows the recommended ELISA cut-off (37 ODC%). Modified from Figure 3 in Petersen et al. 2018: A longitudinal observational study of the dynamics of *Mycoplasma bovis* antibodies in naturally exposed and diseased dairy cows. J. Dairy Sci. https://doi.org/10.3168/jds.2017–14340.

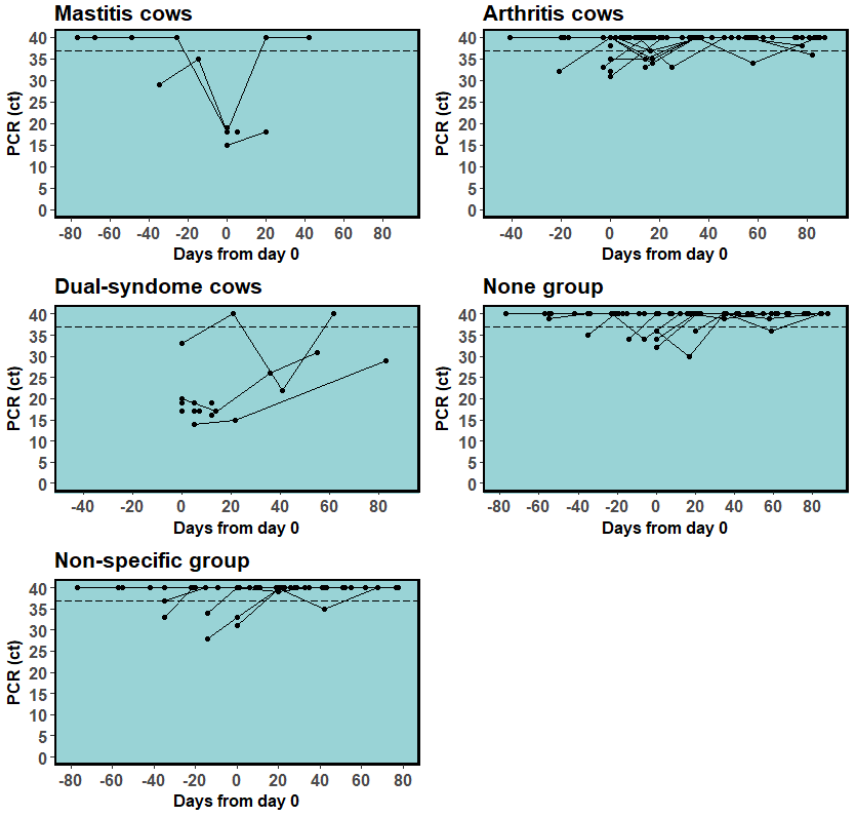

Figure 3 – Distribution of milk PCR cycle threshold (ct) values for *Mycoplasma bovis* divided into five disease groups of dairy cows from four Danish herds. Horizontal dotted lines show the recommended PCR cut-off (37 ct) under which a sample result is considered test-positive. Results from the same cow are linked by lines. 'Dual-syndrome' cows: cows with clinical signs of both arthritis and mastitis. 'None-group' cows: no clinical signs which are likely to be associated with *M. bovis*. 'Non-specific cows': clinical signs which are not typical for *M. bovis*, but where *M. bovis* could not be excluded.

This indicates that PCR in milk samples are also primarily suitable for detecting *M. bovis* udder infections and not *M. bovis*-associated disease or infection in general.

The findings of these studies are important to the dairy sector because they highlight the difficulties in diagnosing *M. bovis* in dairy herds, especially when using primarily milk samples. If only relying on milk samples, many cases are likely to be overlooked, because both individual and BTM samples reflect the presence of *M. bovis* udder infections among the cows and not all other clinical syndromes in cows. Hence, diagnosing *M. bovis* in dairy herds often requires assessment of udder infections as well as systemic infected animals, from e.g., antibody measurements in serum. The very dynamic nature of the antibody response to *M. bovis* and the clear difference related to different clinical signs were previously unclear and demonstrate the value of basic longitudinal studies. If a basic understanding of the diagnostic material or test is lacking, interpretation in different contexts and for different purposes challenging, and recommendations based on these might be incomplete or in the worst case, will be misleading.

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REFERENCES

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2. Petersen, M.B., Pedersen, J., Holm, D.L., Denwood, M., Nielsen, L.R. (2018) A longitudinal observational study of the dynamics of *Mycoplasma bovis* antibodies in naturally exposed and diseased dairy cows. Journal of Dairy Science. In pressdoi.org/10.3168/jds.2017–14340.

FOOTNOTES

[1] BioX Bio K 302 ELISA kit, BioX Diagnostics, Belgium

[2] PathoProof Major-3 PCR kit, Thermofischer Scientific, USA