J. Dairy Sci. 101:1–14
https://doi.org/10.3168/jds.2017-14340

A longitudinal observational study of the dynamics of *Mycoplasma bovis* antibodies in naturally exposed and diseased dairy cows

Mette B. Petersen,[1] Jeanette Pedersen,[2] Dinah L. Holm,[3] Matthew Denwood, and Liza R. Nielsen
Department of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, 1870 Frederiksberg C, Denmark

ABSTRACT

Mycoplasma bovis is an important pathogen causing disease and substantial economic losses in cattle. However, knowledge of the dynamics of antibody responses in individual cows in the face of an outbreak is currently extremely limited. The use of commercial antibody tests to support clinical decision-making and for surveillance purposes is therefore challenging. Our objective was to describe the dynamics of *M. bovis* antibody responses in 4 Danish dairy herds experiencing an acute outbreak of *M. bovis*-associated disease, and to compare the antibody dynamics between dairy cows with different disease manifestations. A total of 120 cows were examined using a standardized clinical protocol and categorized into 4 disease groups: "mastitis," "systemic," "nonspecific," and "none." Paired blood and milk samples were collected and tested using a commercial *M. bovis* antibody–detecting ELISA. Plots of raw data and generalized additive mixed models with cow and herd as random effects were used to describe serum and milk antibody dynamics relative to the estimated time of onset of clinical disease. Cows with mastitis had high optical density measurement (ODC%) of antibodies in both milk and serum at disease onset. The estimated mean ODC% in milk was below the manufacturer's cut-off for the other groups for the entire study period. The estimated mean serum ODC% in the "systemic" group was high at onset of disease and stayed above the cut-off until 65 d after disease onset. However, the lower 95% confidence interval (CI) for the mean ODC% was only above the manufacturer's cut-off between 7 and 17 d after onset of disease. The CI of the "systemic" and "none" groups did not overlap at any time between the day of disease onset and 65 d after disease onset, and the estimated mean ODC% for both the "nonspecific" and "none" groups were generally below the cut-off for the majority of the study period. In conclusion, the serum antibody responses were highly dynamic and showed a high level of variation between individual cows. This strongly suggests that serology is unlikely to be useful for individual diagnosis of *M. bovis*-associated disease in dairy cows. However, it might still be useful for herd- or group-level diagnosis. Antibodies in milk were only increased in cows with *M. bovis* mastitis, indicating that milk antibody measurements only have diagnostic utility for cows with mastitis.

Key words: *Mycoplasma bovis*, ELISA, antibody, BioX Bio K 302, dairy cow

INTRODUCTION

Mycoplasma bovis is an infectious disease of cattle that is associated with a diverse spectrum of clinical signs and substantial production losses worldwide (Nicholas, 2011). In cows, *M. bovis* typically causes mastitis, arthritis, and pneumonia (Maunsell et al., 2011), whereas pneumonia, otitis media, and arthritis are more commonly seen in calves (Maunsell and Donovan, 2009). The preferred method of diagnosing *M. bovis* has historically been bacteriological culture of body fluids (e.g., milk and joint fluids) or swabs (e.g., eye and nasal swabs) from individual animals, as well as bacteriological culture of bulk tank milk for herd-level diagnosis. Even though PCR is becoming more frequently used due to reductions in cost and processing time (Wawegama and Browning, 2017), these methods can have low sensitivity in practice due to intermittent shedding patterns, and it may be difficult to obtain the best sample materials from infected animals on which to apply these tests. Therefore, there is increasing interest in using serology for *M. bovis* diagnostics.

In Denmark, serological assays such as ELISA are frequently used for testing dairy cows, because they are inexpensive and convenient, especially if applicable to milk samples routinely collected for other purposes. Two commercial ELISA kits are available from BioX

Received December 22, 2017.
Accepted April 6, 2018.
[1] Corresponding author: mbp@sund.ku.dk
[2] Present affiliation: Øster Jølby Dyreklinik, Udvejen 3, 7950 Erslev, Denmark.
[3] Present affiliation: Dyrlæge Center Vest, Herningvej 74 A, 6950 Ringkøbing, Denmark.

Diagnostics (Rochefort, Belgium), and one (BioX Bio K 260) has been shown to have little correlation with the occurrence of disease or with PCR and bacterial culture results (Szacawa et al., 2015, 2016). However, these studies aimed mainly to compare antibody measurements to other diagnostic tests using cross-sectional study designs that are not suitable for the assessment of dynamics and persistence of antibodies. Nielsen et al. (2015) found that for the BioX Bio K 302 ELISA, it might be beneficial to raise the cut-off for herd-level diagnosis to an optical density coefficient (**ODC%**) of 50 in bulk tank milk to increase the specificity, but no field study evaluations of cut-off values at the animal level have been published. Therefore, a cut-off of 37 ODC%, as recommended by the manufacturer, is used in practice despite the lack of substantial documentation for the validity of this threshold.

Appropriate interpretation of ELISA test results requires knowledge of the dynamics and duration of excretion of antibodies against *M. bovis* relative to the time of infection and onset of associated disease. However, as recently pointed out by Hazelton et al. (2018), there is currently a limited understanding of the dynamics of antibodies directed against *M. bovis* in terms of time to seroconversion and longevity in naturally exposed cattle. The antibody response has been shown to remain high in both milk and serum for up to 20 wk after either experimental inoculation in the udder or naturally occurring *M. bovis*-associated mastitis (Boothby et al., 1987; Byrne et al., 2000). However, the *M. bovis* antibody response to systemic disease such as arthritis has not yet been fully described. Work in calves vaccinated against *M. bovis* at 3 wk of age with an experimental vaccine showed that the animals appeared to seroconvert within 14 d and that a high IgG level was maintained for at least 42 d in serum (Nicholas et al., 2002). Apart from this work in individual animals, serology has been suggested to be useful for herd-level diagnostics (Martin et al., 1990; Le Grand et al., 2002).

Further investigation of ELISA test result patterns in milk and serum from naturally exposed and diseased dairy cows is therefore warranted, in particular to understand how the ELISA response can be expected to develop over time in animals with varying clinical signs of *M. bovis*-associated disease compared with exposed animals without overt clinical signs. The objective of this study was therefore to describe the temporal dynamics of antibody responses to *M. bovis* in serum and milk taken from individual dairy cows in herds experiencing an *M. bovis* disease outbreak, with a particular emphasis on differences in these patterns between groups of animals exhibiting different disease manifestations.

MATERIALS AND METHODS

Study Design

This study was a longitudinal observational study in Danish dairy cattle herds. To fulfill the objective of describing dynamics of antibody responses in exposed animals, only herds experiencing acute outbreaks of *M. bovis*-associated disease within the study period were eligible for inclusion. This was based on the likely presence of *M. bovis*-associated disease as diagnosed by the herd advisory veterinarian based on positive PCR or ELISA test results, and an outbreak was defined as having several animals with clinical signs of mastitis (Maunsell et al., 2011), arthritis, subcutaneous swelling of the limbs (Henderson and Ball, 1999; Wilson et al., 2007), pneumonia (Maunsell et al., 2011), or combinations thereof, and positive *M. bovis* ELISA or PCR tests.

Four herds were identified as matching these criteria and permission was obtained from each herd to undertake an outbreak investigation. Each herd was visited 5 times, at approximately 3-wk intervals, during the period from July 1, 2015, to April 5, 2016. The herd visits were initiated as closely as possible following the presumed date of onset of the disease outbreak. At all visits, the aim was to assess the clinical status using a standard protocol and to collect paired blood and milk samples from selected individual cows. Where possible, the same animals were sampled at each visit. As many repeated samples as possible were obtained from as many individual animals as the project budget would allow. When it was not possible to resample the same animals, new animals were sampled.

Study Population

All 4 of the identified herds had a history of sudden onset of *M. bovis*-related clinical signs in cows or calves, and several strongly positive ELISA or PCR test results for *M. bovis* (Table 1). During the study period, one or more cows from all study herds tested positive at least once by ELISA or PCR. Detailed farm information obtained before and after enrollment is given in Table 1.

Although bacterial cultures were not included in the study design due to financial constraints, a few calves were euthanized due to severe disease and autopsies were performed. A calf from herd 2 had chronic degenerative arthrosis in several joints and bronchopneumonia with overlying pleuritis. *Mycoplasma bovis* was cultured from joint fluid, and joint fluid and lung tissue were found positive for *M. bovis* by PCR. Two calves autopsied from herd 4 had chronic omphalitis, bronchopneumonia, synovitis in several joints, and bilateral