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Short communication: Correlation between within-herd antibody-prevalence and bulk tank milk antibody levels to *Mycobacterium avium* ssp. *paratuberculosis* using 2 commercial immunoassays

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ABSTRACT

The objective of this study was to determine the correlation between the results obtained with the ELISA technique for antibodies to *Mycobacterium avium* ssp. *paratuberculosis* in serum and bulk tank milk at the herd level. For this purpose, 203 samples of bulk tank milk were analyzed with 2 commercial ELISA from dairy herds with a prevalence of seropositive animals that was also determined. In regard to the reference test (results in blood serum), the sensitivity of the bulk tank milk test to detect high-positive herds ($\geq$10% seroprevalence) ranged from 85.7 to 71.4%. The specificity to detect herds with no seropositive animals ranged from 70.5 to 53%. In a quantitative approach, Pearson correlation coefficients, reported as a measure of the linear association between herd seroprevalences and transformed optical density values recorded in bulk tank milk, were 0.39 and 0.54 for the studied ELISA. Although the test results were relatively fairly correlated with the within-herd prevalence, the practical utility of bulk tank milk testing for *Mycobacterium avium* ssp. *paratuberculosis* seems limited, especially regarding specificity.
Key words: dairy cattle, milk, serology, Johne's disease

Short Communication

Mycobacterium avium ssp. *paratuberculosis* (**MAP**) is the causal agent of paratuberculosis (Johne's disease), a chronic enteric granulomatous disease in ruminants; the disease seems to respond to antibiotics as symptoms weaken, but recur after antibiotics are no longer administered. Testing options for determination of herd MAP status include, mainly, individual or pooled fecal culture, culture of environmental samples, and ELISA on milk or serum (Lavers et al., 2014). Although the relative sensitivity (**Se**) of ELISA for detection of antibodies against MAP as compared with fecal culture is rather low, ELISA technology has gained an important place in herd-based testing schemes because of its low cost, fast processing time, and high-throughput potential (van Weering et al., 2007). Antibody ELISA is the most commonly used laboratory test for the detection of specific antibodies against MAP in serum and milk (Khol et al., 2013).

The adaptation of the ELISA technique for detecting antibodies in bulk tank milk (**BTM**) samples constitutes an inexpensive test, as it provides information about the status of a large group of animals from a single sample. Bulk tank milk testing is a frequently used tool for the surveillance and monitoring of several infectious diseases in dairy cattle, such as infectious bovine rhinotracheitis (**IBR**), enzootic bovine leucosis, bovine virus diarrhea (**BVD**), and salmonellosis. Bulk tank milk for BVD, IBR, and salmonellosis has been widely used to estimate the within-herd prevalence of antibody-positive cows and to monitor dairy herds (Nylen et al., 1999; Warnick et al., 2006; Diéguez et al., 2008; Eiras et al., 2012).

The utilization of BTM samples could also be a practical and cost-effective way to screen dairy herds for the presence of MAP. Milk sampling is markedly more convenient than fecal or blood sample testing for any disease of dairy cattle (Wilson et al., 2010). However, literature is scarce about the correlation between the BTM response and the within-herd prevalence for MAP infections.

The Se and specificity (**Sp**) of antibody ELISA against MAP vary widely, depending on the product

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and gold standard applied (Khol et al., 2013). When used on BTM, the ELISA tests have been shown to perform similarly to serum ELISA assays at the herd level, with an Se of 56 to 83% when fecal culture is used as a reference (Lombard et al., 2006; Wilson et al., 2010). The objective of our study was to examine the correlation between within-herd seroprevalences and BTM antibody levels against MAP in dairy cattle using 2 commercial immunoassays.

The study was performed in northwestern Spain, which is the major cattle-farming region of the country and the ninth within the European Union, accounting for 38% of milk production in Spain and 1.5% in the European Union (MAPAMA, 2015). Galicia was the first region in Spain to establish a voluntary MAP control program, which began in 2004 and remains active. The percentage of Galician herds involved increased from 4.6% in 2004 to 45% in 2013. Vaccination against MAP is not permitted, as the region is not free of tuberculosis.

To evaluate the correlation between the levels of antibodies in a sample of BTM and the within-herd prevalence, 203 samples of BTM were collected from 203 dairy herds taking part in the voluntary control program. The seroprevalence in each herd was also determined. For this purpose, serum from every animal that was lactating at the time of serum collection was gathered less than 1 wk before BTM collection. Blood samples were obtained by tail vein venipuncture using Venoject tubes (Terumo Europe, Leuven, Belgium) without anticoagulant.

The milk samples were skimmed by centrifugation (3,000 × *g*, 20 min, room temperature) and frozen at −21°C. The serum samples were frozen after blood clotting and stored with milk samples until tested. The samples were tested for antibodies against MAP with 2 commercial available indirect ELISA. The ELISA were designated as ELISA A (Idexx Paratuberculosis Screening Ab Test, Westbrook, ME; based in technology of Pourquier Institute) and ELISA B (*Mycobacterium paratuberculosis* test kit Parachek 2, Prionics AG, Zurich, Switzerland). All analyses were performed by following the recommendations of the manufacturer. All samples were preabsorbed with sonicates of environmental *Mycobacterium phlei*.

For both ELISA, optical density (**OD**) values were expressed as sample-to-positive ratio (S/P), calculated as follows:

$$(\text{OD value of the sample} - \text{the OD value of the negative control})/(\text{OD value of the positive control} - \text{the OD value of the negative control}) \times 100.$$

Serum samples were considered positive at an S/P ratio greater than or equal to 55 (ELISA A) or 15 (ELISA B).

For analysis, herds were initially categorized based on the detected seroprevalence of MAP-specific antibodies as negative (0% within-herd seroprevalence), positive (>0–<10%), and high positive (≥10%). To estimate the accuracy of BTM samples to correctly classify negative, positive, and high-positive herds, threshold values of transformed OD were calculated for each of the mentioned groups for both ELISA. For this purpose, the mean values of transformed OD resulting from analysis of BTM samples were estimated for each of the 3 seroprevalence groups. From these estimated mean values and their corresponding confidence interval, threshold values that allow the prediction of herd seroprevalence status from BTM were established. This was carried out by simple (linear) interpolation, as the straight line between the upper limit of the confidence interval of one prevalence group and the lower limit of the next one.

The Se of the BTM test with respect to the serum samples serving as reference was evaluated in terms of the proportion of positive and high-positive herds which the BTM test classified as positive and highly positive, respectively. Specificity was evaluated in terms of the proportion of negative herds (0% seropositive animals), which the BTM test classified as negative. In addition, the correlation between within-herd seroprevalence and antibody levels in BTM was evaluated in a quantitative approach, using the Pearson correlation coefficients (ρ). All analysis was done with SPSS version 12.0 (SPSS Inc., Chicago, IL).

The mean herd size of the surveyed herds was 51.1 cows (SD = 33.4; 45.5 for negative herds; 64.1 for positive herds and 55.2 for highly positive herds). Of the 203 farms used in the study, 132 had 0% seroprevalence, 57 had >0 to <10% seropositive animals, and 14 had ≥10%. The mean values of transformed OD resulting from analysis of BTM samples for each of the seroprevalence groups are summarized in Table 1.

The cut-off points, calculated to establish the intervals of transformed OD values that enabled classification of a herd within a corresponding seroprevalence group from a BTM sample, are summarized in Table 2. Using these cut-off points, with respect the reference test (results in blood serum), the Se of BTM ELISA to correctly classify high-positive herds (≥10% seroprevalence) was 85.7% (12/14) for ELISA A and 71.4% (10/14) for ELSIA B. The BTM test correctly classified inside the >0 to <10% group 36.8 (21/57) and 43.9% (25/57) of the herds with ELISA A and B, respectively (Table 3). Considering jointly positive and highly posi-