CONCORDANCE BETWEEN *MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS* ELISA RESULTS IN SERA AND MILK FROM DAIRY GOATS

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1. Introduction

Paratuberculosis is a chronic infection mainly of cattle, sheep, goats and other ruminants, which is caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP). The disease causes decreased productivity and suboptimal productive life and, thus, substantial economic losses to the farming industry (Clarke, 1997). To control MAP-infection, there is a need of an accurate, rapid and low cost disease screening technique. Screening of milk samples for the detection of antibodies to MAP is appealing due to the low cost and ease of sample collection and the minimal additional work load required by the farmer compared to the collection of serological samples. Studies in dairy cattle showed that the antibody titre, against MAP-infection, in milk varies with the lactation stage (Nielsen et al., 2002). There are no similar studies in dairy sheep and goats. Therefore, our objective was to estimate the correlation between the results of an enzyme-linked immunosorbent assay (ELISA) to detect MAP-infection in paired sera and milk samples obtained from Greek dairy goats at different stages of lactation.

2. Materials and Methods

Two hundred and twenty-five goats from a dairy goat flock with 250 animals, with known history of clinical paratuberculosis, which kidded in the period from December 2008 to March 2010, were studied. Paired sera and milk samples were obtained from the animals four consecutive times: during the first 24 hr after kidding, two (early lactation) and four (mid-lactation) months later and at the end of their seven-month-long lactation period. After clotting the sera were harvested and stored at minus 21^{o}C. The colostrum/milk samples were centrifuged, skimmed and stored at the same temperature as sera. In order to avoid day-variability, paired milk and sera were thawed and tested simultaneously (Nielsen, 2002) with a commercially available ELISA kit ((Pourquier® ELISA Paratuberculosis), using the manufacturer's protocol. For each stage of lactation, ELISA results were interpreted at the cut-offs (S/P ratio 0.45 for sera and 0.2 for milk) recommended by the manufacturer and at cut-offs reduced by 50% (Kostoulas et al., 2006). The dichotomized results of paired sera and milk samples were cross-classified in two separate two-by-two tables. Differences in the proportions positive were evaluated for significance by McNemar's χ^2 test for symmetry. Additionally, we estimated the concordance correlation coefficient (r_{ccc}) between the S/P ratios of the paired sera and colostrum/milk samples, by lactation stage, in order to measure the overall level of agreement between the two sets of test results regardless of cut-off selection. All analyses were carried out using Stata v. 10 (StataCorp, 2007).

3. Results

For the recommended cut-offs, the ELISA results did not differ between milk and sera samples obtained at kidding (McNemar's χ^2 test = 1.8, p=0.38), early (1.0, p=1.00) and mid-lactation (2.0, p=0.50) but differed at late lactation (12.00, p=0.0005). The same relationship was found at the 50% lowered cut offs for kidding (0.2, p=1.00), early (0.00, p=1.00), mid (0.67, p=0.69) and late lactation (7.14, p=0.01). These are in Table 1.

The estimated r_{ccc}'s were high in early, mid and late lactation but were low-to-moderate at kidding (Table 2).

Table 1
McNemar's test for symmetry in the proportions between *Mycobacterium avium* subsp. *paratuberculosis* (MAP) indirect enzyme-linked immunosorbent assay (ELISA) results in milk and sera. Results were interpreted at the manufacturer's recommended cut-offs for sera and milk and at cut-offs reduced by 50%

Stage of lactation	**Recommended cut-offs McNemar's χ^2 (p-value)**	**50% reduced cut-offs McNemar's χ^2 (p-value)**
Kidding	01.80 (0.3750)	00.20(1.0000)
Early	01.00 (1.0000)	00.00(1.0000)
Mid	02.00 (0.5000)	00.67(0.6875)
Late	12.00 (0.0005)[§]	07.14(0.0129)[§]

§ indicates significant results at $p<0.05$.

Table 2
Concordance correlation coefficients (r_{ccc}) and 95% confidence intervals (CIs) for the S/P ratios of paired milk and sera samples by lactation stage

Stage of lactation	r_{ccc}	CIs
Kidding	0.488	0.399 - 0.577
Early	0.887	0.866 - 0.909
Mid	0.805	0.744 - 0.866
Late	0.892	0.861 - 0.923

4. Discussion

This is the first study to assess the concordance between paired milk and sera samples in dairy goats. The proportions of ELISA-positive milk/colostrum samples did not differ from those in sera throughout lactation; however, they differed at late lactation. A similar pattern was reported in dairy cattle (Nielsen et al. 2002b). Interestingly, the r_{ccc} were high throughout lactation but low-to-moderate at kidding. Recently, Moreno-Indias et al. (2011) reported that the IgG concentration in goat colostrum declines rapidly in the first 10 hr after kidding. Currently we are performing additional analyses, using latent-class mixture models (Choi et al. 2006), in order to compare the overall discriminatory power of milk and serum ELISA in dairy goats. We anticipate that these results will further elucidate on the utility of milk ELISA testing and its interpretation on a lactation-stage specific basis as a potential tool in future control efforts in dairy goat flocks.

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