Comparison of PCR and qPCR for the detection of *Mycobacterium avium* subsp. *paratuberculosis* in feces and milk of seropositive dairy cows in Uruguay: a longitudinal study

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Objectives: Paratuberculosis (PTBC) is a chronic infectious disease of ruminants caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP), with an important economic impact in the dairy sector. This study aimed at evaluating and comparing the performance of two molecular diagnostic tests (PCR and qPCR) for the detection of MAP shedding in feces and milk of seropositive dairy cows across time.

Materials and Methods: One dairy farm with PTBC seroprevalence of 7%, cattle with clinical history (chronic diarrhea, weight loss), and molecular confirmation of MAP was selected. Thirteen cows with serum anti-MAP IgG detected by a commercial ELISA were included. Fecal and milk samples were collected at 4 different time points (T1, T2, T3 and T4) with 15-day intervals and processed for the detection of MAP by PCR and quantitative real-time PCR (qPCR) targeting the IS900 sequence. The agreement between test by sample type was assessed by the kappa statistic, using R software. Values of kappa were interpreted as follows: 0–0.2 = no agreement; 0.21–0.39 = minimal; 0.40–0.59 = weak; 0.60–0.79 = moderate; 0.8–0.9 = strong, and >0.9 = almost perfect agreement. Fisher´s exact test was used assess differences in the proportion of positive cows by PCR and qPCR.

Results: The % of positive cows by PCR and qPCR in feces, respectively, were 54.5% (6/11) and 90.9% (10/11) at T1, 30.8% (4/13) and 76,9% (10/13) at T2, 46.2% (6/13) and 84.6% (11/13) at T3, and 46.2% (6/13) and 76.9% (10/13) at T4. Consistently across all sampling times, the % of positive cows detected by qPCR was higher than for PCR. The overall % of positivity was significantly higher ($p<0.05$) for qPCR (41/50, 82%) than for PCR (22/50, 44%). All PCR-positive samples were also positive by qPCR. There was no to weak agreement between both test (kappa=0.29, 95% CI 0.12–0.47).

The % of positive cows by qPCR in milk at T1, T2, T3 and T4, respectively, was 27.3% (3/11), 30.8% (4/13), 38.5% (5/13), and 30.8% (4/13). All milk samples were negative by PCR, resulting in no agreement between tests (kappa=0). Fourteen of the 15 cows with a positive qPCR result in milk across all sampling times, also showed a concurrent positive qPCR result in feces, while in 1 cow MAP was detected by qPCR in milk but not in feces collected concurrently. MAP shedding in milk was intermittent.

Conclusions: The qPCR presented higher sensitivity than PCR to detect MAP in both types of samples, but especially in milk. Due to intermittent shedding in milk, care must be taken when using this type of sample for diagnosis. The highest % of positive cows was detected by qPCR in feces. Despite the no to weak agreement between tests, qPCR consistently detected as positive all PCR-positive fecal samples, suggesting that this agreement is likely due to a significantly higher sensitivity of the qPCR. In this context, the qPCR used in this study could be valuable in disease control programs in dairy herds, although further validation studies and determination of the specificity of the qPCR are necessary.