

Detection of *Mycobacterium avium* subsp. *paratuberculosis* in bovine semen by real-time PCR

Conclusion: Real-time PCR was shown to be a reliable method for detection of *Mycobacterium* subsp. *paratuberculosis* (*M. ptb*) in spiked bovine semen.

Introduction: Paratuberculosis, a chronic enteric disease in ruminants also known as Johne's disease, is caused by *M. ptb*. Clinical symptoms include diarrhoea, emaciation and eventually death. Control of the disease is complicated by the fact that symptoms develop at a very late stage and bacteriological cultures may need up to 16 weeks to form visible colonies. For breeding purposes, there is a need for a sensitive method to test semen for *M. ptb*. However, PCR-inhibiting components in semen may constitute a problem for PCR-analysis.

Aim: Evaluation of beadbeating and real-time PCR as methods to lyse and detect *M. ptb* in bovine semen.

Materials and methods: Samples of diluted (10-20-fold) *M. ptb*-free semen were spiked with serially diluted *M. ptb*, previously washed from free DNA to avoid overestimating the sensitivity of the test. Since CFU-count usually underestimates the number of detectable genomes, the bacteria were counted in a Bürker chamber. Incubation with lysisbuffer and proteinase K followed by mechanical disruption by beadbeating released the DNA which was recovered with phenol/chloroform extraction. Real-time PCR with a fluorescent probe targeting the insertion element IS900 was used for detection. PCR-inhibition was monitored

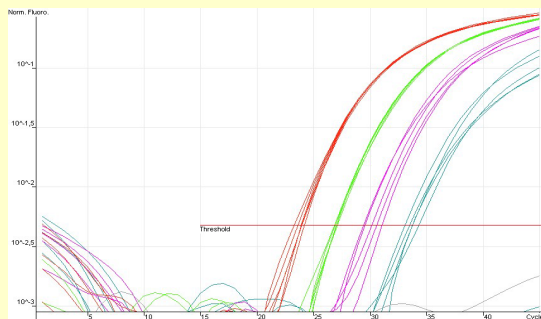


Figure 1. Real-time PCR (Rotorgene 3000) on *M. ptb* isolated from bovine semen. Clusters of normalized curves from left to right: 10⁴, 10³, 10², 10 bacteria per sample of 100 µl. Blanks and PCR-controls not shown.



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by the addition of a mimic molecule, targeted by a differently labelled probe, to the PCR.

Results: As few as 10 *M. ptb* per sample of 100 µl semen could be detected (figure 1). The extraction method was enough to remove PCR-inhibiting components.

Discussion: As 10 manually counted *M. ptb* corresponded to less than 1 CFU (viability ~1%, according to our results), this method is more sensitive than would be expected of semen-culture.

Although IS900 is unique to *M. ptb*, it is impossible to find a short sequence on the IS900 that alone is specific for *M. ptb*, since there exist similar IS-elements in other *Mycobacteria*. Verification with other real-time PCR systems should be performed.

References:

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