CONTROL OF PARATUBERCULOSIS IN THE CLOSED HERD OF CATTLE BY REAL TIME PCR: DOES PASSIVE SHEDDING REALLY EXISTS?

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Introduction
The aim of this study was to investigate, whether it is possible to control paratuberculosis by quantitative real time PCR (qPCR) in the closed cattle herd with known history of paratuberculosis and to determine general rules of the qPCR results interpretation. For this purpose, a closed herd of Limousine cattle monitored for 2 years (6 collections) for the presence of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) in faeces by culture and IS900qPCR.

Materials and methods

Herd status and sampling
- 40 cows and 1 breeding bull of Limousine beef cattle included in the Paratuberculosis control programme based on faecal culture examination.
- 3 last culture examinations were negative, the 4th (Month 0) was done in parallel with IS900qPCR.
- The herd is stabled in a shed during winter and unsheltered on the pasture during summer. Calves remained with their dams until the age of 8 to 9 months, when heifers were moved to another location to prevent inbreeding and young bulls were transferred to feedlot for the fattening.
- Individual faecal sample were collected directly from the rectum using a disposable glove.
- Tissue samples (intestine or mesenterial lymph nodes) from slaughtered animals were collected where possible and served as the final confirmation of infection.

Sample analysis
- For culture, 5 g of faeces or 1 g of tissue (mesenterial lymph nodes) was decontaminated in HPC, seeded on HEYM slants with 2 µg/ml of Mycobactin J and incubated at 37°C for 3 months (Pavlik et al., 2000).
- DNA from faeces was isolated by modified protocol of QIAamp DNA Stool Mini Kit (Kralik et al., 2011). The detection and quantification of MAP was performed by IS900qPCR with internal amplification control. Quantification was done according to the plasmid gradient (Slana et al., 2008).
- Blood sera were investigated for the presence of antibodies against MAP by ID Screen Paratuberculosis Indirect ELISA kit (ID Vet, Montpellier, France).

Results
- In the first collection two cows (4.9%) were culture positive and 25 (61.0%) were positive by IS900qPCR. Two cows with more than $10^4$ MAP cells in 1 g of faeces were culled. When after 4 months the examination was repeated portion of positive animals dropped to 42.1% (16 out of 38) with no culture positive individuals. After additional 5 months, only 6.1% (only 2 cows out of 33 remaining) were positive by IS900qPCR. This status remained more or less unchanged until the end of the monitoring.
- During the course of monitoring 14 cows were slaughtered for different reasons. IS900qPCR found ten of them at least once positive in faeces. Only 2 of them were positive by faecal culture and 4 by culture of their tissues. Both cows positive in faeces by culture were low shedders.

Conclusion
- Analysis of the closed herd revealed that MAP is shed by animals in very low amounts undetectable by culture.
- Data confirmed previous study (Kralik et al., 2011) that the detection limit of culture is approx. $10^3$ MAP cells per g of faeces.
- Removal of two high shedders at the beginning of experiment led to the statistically highly significant decrease of IS900qPCR positivity within 9 months.

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» Animal shedding low amounts of MAP in faeces in more than two consecutive samples should be considered as highly suspected.

» The data suggest that passive shedding likely does not exist on the level of culture examination of faeces (Pradhan et al., 2011), but it is likely that it exists on the level of qPCR.

» Sporadic occurrence of animals slightly positive for MAP in faeces by qPCR is probably unavoidable in herds with history of paratuberculosis.

References


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