PROBLEMATIC DETECTION OF *MYCOBACTERIUM AVIUM* subsp. *PARATUBERCULOSIS*

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Introduction

Culturing of MAP takes 4 weeks - 40 months (Grant et al., 1998; Griffiths, 2002).

Culture test sensitivity is around 50% (Yayo et al., 2001). The specificity is 100% and therefore, the method is considered the "golden rule" (European Commission, 2000), but lacks standardisation.

ELISA is recommended for screening purpose because of 99% specificity. Limits are poor sensitivity (45% on average, varying widely between different stages of Johne's disease) and performance on animals >15 months (serology).

The PCR method alone shows 30% sensitivity. The lower limit of detection is estimated at 100 cfu/ml (Gerlach, 2002). The method should be used in milk only. Different detection regimens including (IS900)-PCR detect the Mycobacterium avium complex (7th ICP, Bilbao, June 2002) rather than MAP.

Results and Discussion

The analysis of MAP remains questionable due to several reasons: 1st, short culturing might not yield accurate results. 2nd, culture tests, the "golden standard" start with a decontamination procedure (hexadecylpyridinium or NaOH / oxalic acid) influencing the yield of MAP (Glanemann et al., 2004). 3rd, decontamination procedures might kill sphaeroplasts and hinder culture of MAP in Crohn's disease patients (Greger, 2001). 4th, staining is inadequate for non walled sphaeroplasts (De Clari, 1998) or bacterial cells in macrophages (might explain false negative tests; Stein, 2003) and insensitive (lower detection limit around 10'000 cfu/ml; Gerlach, 2002). 5th, the lower limit of detection of culture tests is between 10-100 cfu per ml or g (Gerlach, 2002; Yayo et al., 2001).

Besides analytical problems, our interest centres in the consequences for interpretation of analysis results:

6th, nowadays, the prevalence of infected animals can only be observed correctly by monitoring programs applying multiple testing (intermittent shedding, serology).

7th, hydrophobic clump formation would explain the observation of positive shedding at stage I (in the digestive tract accumulated MAP might be released at once), (Gerlach, 2002) and the MAP killing efficiency of turbulent flow pasteurisation (Pearce et al., 2001). These effects might be partially explained by the disruption of clusters following a third power law. A 3rd power law (volume) will distribute 1000 MAP in 550 clusters, i.e. 1 of 100 bacterial cells, 10 of 10, and so forth).

The same (model)idea might help explain the „all at once release" of MAP in early Johne's disease stages (detachment and disruption of clusters because of peristaltic movement) followed by discontinuous dissemination of MAP in the intestine (faeces) or body (macrophage activity) of animals and humans.

Conclusions

A standardised detection regime with known and high sensitivity / specificity as well as low detection limit is needed. Meanwhile, all analysis results concerning both, Johne's and Crohn's diseases, must be interpreted with great care. The difficulties for analysing MAP in food processing and dissemination might partially be explained by disruption of MAP clusters.

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