Poster #1

Effects of incubation temperature, decontamination method and regular shaking on diagnostic performance of a new liquid culture method, the TREK ESP® Culture System II and para-JEM® Broth, for detection of *Mycobacterium avium subsp. paratuberculosis* in fecal samples

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INTRODUCTION

At the Animal Health Service in the Netherlands fecal culture for *M. paratuberculosis* is performed on a relatively large scale (600 samples per week). Routinely, fecal samples are decontaminated, and subsequently cultured on solid Löwenstein-Jenssen (LJ) medium in the presence of mycobactin (AHS standard method). Cultures are inspected every four weeks up to 16 weeks if necessary. When suspect growth is observed, a Ziehl-Neelsen stain is performed. Ziehl-Neelsen positive cultures are confirmed by *IS900* PCR.

In previous studies, presented at the AAVLD meeting 2003, we evaluated the performance of a new liquid culture method, the TREK ESP *para*-JEM Culture System II, as compared with our standard method. In the first experiments, we found equivalent results for both culture systems with a strong reduction in time to detection for ESP. In a subsequent comparison between both culture techniques on 260 bovine fecal samples the LJ system demonstrated a higher sensitivity for detection of low shedders. In another experiment, however, weekly shaking of liquid cultures enhanced growth resulting in a better sensitivity and reduction of time to detection by about a week. In the present study effects of incubation temperature, decontamination method and shaking were investigated. Finally, the optimized liquid culture was again compared with solid culture using a panel of bovine fecal samples submitted for routine diagnosis.

MATERIALS & METHODS

In Experiment I, fresh bovine fecal samples were taken from – predominantly light – shedders (n=40) and from cattle (n=48) originating from herds with a long history of being culture negative and were cultured on LJ agar slants. In parallel, samples were also cultured in ESP *para*-JEM culture bottles with *para*-JEM Blue either after decontamination as prescribed by the manufacturer (Cornell double incubation method), or the AHS in-house method (1), or a double incubation method as described by Stabel *et. al.* (2). For the shedder panel, additionally a duplicate series of each decontamination method was shaken vigorously once a week and placed back in the system. Using only the Cornell double incubation decontamination method prescribed by the manufacturer, another two series from the shedder panel, with and without weekly shaking, were cultured offline (out of the instrument) at 37°C. All samples detected or not yet detected by ESP at the conclusion of the experiment (49 days) were further investigated via ZN and PCR methodologies.

In experiment II, fresh fecal samples were taken from equivalent numbers of known shedders and up to then previously recognized culture negative cattle originating from four different infected herds (n=116). Samples were cultured in parallel on LJ agar slants and in ESP *para*-JEM culture bottles with *para*-JEM Blue after decontamination as described by Stabel *et. al.* (2). Also pooled samples (n=5 per pool, 13 pools) from one infected herd were investigated by both liquid and solid culture methods.

RESULTS

In experiment I, 23/40 fecal samples from previously culture positive cattle were positive on LJ, whereas 21 to 26 samples were detected by the ESP system as confirmed by PCR and ZN. Additionally a few PCR positive samples were found that were not detected by ESP. For the prescribed Cornell double incubation decontamination method we confirmed in this study the previously observed reduction in Time To Detection (TTD) by weekly shaking. For the in-house and Stabel decontamination methods no differences in TTD were observed between stationary incubation and weekly shaking. Mean TTD was lowest for the in-house decontamination method (22 days), intermediate for the Stabel decontamination method with and without shaking (27 days) and highest for the prescribed decontamination method with stationary incubation (35 days). Offline incubation at 37°C with and without shaking yielded an equivalent number of positives (n=27). The panel from culture negative herds (n=48) yielded surprising results for the in-house decontamination method. After decontamination according to the manufacturer's prescribed method or the Stabel method, in each series six samples were detected by ESP, but all were ZN and PCR negative. However, after decontamination according to the in-house method 26 samples were detected by the system with 19/26 ZN positive but no samples PCR positive. False-positive results clustered strongly in one herd, and the identity of these acid-fast bacteria is still unclear.

In experiment II, 48/116 individual bovine fecal samples scored positive either in the ESP system or the LJ system. Both culture systems scored 40 samples positive with 32 samples in common. The agreement beyond chance – the kappa-value – was 0.70. The mean TTD for the ESP system (39.1 days) was significantly lower than for the LJ system (75.6 days). In the ESP system when combined with the Stabel decontamination method, false-positive signals were observed for 21/116 samples. However, these signals were almost all found within the first 12 days of incubation with atypical graphs, and were probably caused by a *Bacilli*-like species, visible as non-acid fast bacteria in the ZN stain. Both culture methods scored 7/13 pooled samples from one infected herd positive with complete agreement of results. Related to the individual LJ culture results of all samples involved, one pool was found positive in both culture systems with all individual samples scored negative, and one pool was found negative in both culture systems with 1/5 individual samples scored low-positive (1-10 CFU).

CONCLUSIONS

- In Experiment I the TREK ESP *para*-JEM Culture System II with the poising buffer *para*-JEM Blue yielded equivalent or better results than the standard LJ system.
- A reduction in TTD after weekly shaking was confirmed for the prescribed decontamination method.
- Surprisingly, this reduction in TTD was not repeated for the two other decontamination methods.
- The AHS in-house decontamination method yielded an unexpectedly high numbers of false-positive reactions in the ESP system.
- The decontamination method according to Stabel *et. al.* (2) when combined with the ESP system had the advantages of relatively low numbers of false-positive reactions with a low TTD after – the more feasible – stationary incubation.
- Incubation at 37°C did not improve culture results.
- For fresh fecal samples from - predominantly light - shedders the ESP system combined with the Stabel decontamination method and the standard LJ system yielded equivalent numbers of positive results, whereas the TTD was twice as long for the LJ system.
- In experiment II frequent false-positive signals were found in the ESP system when used in combination with the Stabel decontamination method. However, considering low TTDs and graph reviewed most of these signals can be ignored.
- For pooled samples from one infected herd a good agreement was found between both culture systems and individual LJ culture results.

REFERENCES

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