Letters to the Editor

Effects of Shipping and Storage Conditions of Fecal Samples on Viability of *Mycobacterium paratuberculosis*\(^\text{\textregistered}\)

Johne’s disease is characterized by chronic granulomatous enteritis of ruminants caused by *Mycobacterium paratuberculosis* infection. Early diagnosis of infected animals is necessary to reduce contamination of the environment and to control disease spread through feces (3, 6). About 75% of positive animals are either low or very low shedders (7, 8). Diagnostic samples for *M. paratuberculosis* identification are generally collected, shipped, and stored under various conditions that may influence the viability of *M. paratuberculosis* (2, 4, 5), thus changing the true status of the infected animal; in fact, the culture status especially of individual low or very low shedder animals could change from positive to negative.

The aim of the present study was to mimic the most relevant conditions of shipment and storage of clinical samples obtained from naturally infected cattle and to study the effects of these conditions on the detection of *M. paratuberculosis* by standard bacteriological culture and real-time PCR. Fecal samples were collected from 11 cows confirmed to have Johne’s disease; samples were transported on ice and stored as described in Table 1. Fecal samples were tested for colony counts on Herrold’s egg yolk agar slant (three tubes each for three dilutions with mycobactin J and one without mycobactin J) (9) and by immunomagnetic bead capture of *M. paratuberculosis* from feces and extraction of genomic DNA from captured *M. paratuberculosis*, followed by PCR (1).

Several of the first- and second-dilution tubes had an *M. tuberculosis* organism too-numerous-to-count status; thus, data analyses were restricted to dilution 3. The mean colony counts at week 16 were compared across treatments, using analysis of variance (ANOVA) (SPSS version 13.0 software for Windows; SPSS Inc., Chicago, IL). Pairwise comparisons of treatment means between treatment groups were performed with the Tukey correction for multiple comparisons. Results for each tube were dichotomized into positive or negative, based on the presence of at least one colony. Fisher’s exact test (intercooled STATA version 9.2; STATA, College Station, TX) was used to compare the proportions for positive tubes and cattle. Significance was defined as a *P* value of <0.05 for all analyses.

Pairwise comparisons (ANOVA) between treatment means identified a significantly higher number of colonies with treatment 5 than with treatment 3 (*P* = 0.04). Treatments 1, 2, 4, 5, and 6 had a significantly higher proportion of positive tubes than treatment 3. At the individual animal level, significantly larger proportions of cattle were classified as positive for treat-

### TABLE 1. Pairwise comparison of treatment group transport and storage conditions of clinical fecal specimens

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Experimental conditions</th>
<th>Conditions mimicked</th>
<th>Proportion of positive result(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Temp and time</td>
<td>Handling</td>
<td>Tubes (no. of tubes), significant differences</td>
</tr>
<tr>
<td>1</td>
<td>4°C for 48 h</td>
<td>Samples collected and shipped under refrigeration and processed immediately</td>
<td>Large-capacity laboratory</td>
</tr>
<tr>
<td>2</td>
<td>4°C for 48 h, then 4°C for 1 wk</td>
<td>Samples collected and shipped under refrigeration, stored, and then processed</td>
<td>Moderate-capacity laboratory</td>
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<td>3</td>
<td>4°C for 48 h, then −20°C for 1 wk</td>
<td>Samples collected and stored under refrigeration for few days, stored in a freezer before shipping, and then processed</td>
<td>Practices by a producer or a veterinarian</td>
</tr>
<tr>
<td>4</td>
<td>4°C for 48 h, then −70°C for 1 wk</td>
<td>Samples collected and shipped under refrigeration, stored for few days, and then processed</td>
<td>Low-capacity laboratory</td>
</tr>
<tr>
<td>5</td>
<td>4°C for 48 h, then −70°C for 3 wk</td>
<td>Samples collected and shipped under refrigeration, stored for a few wk, and then processed</td>
<td>Low-capacity laboratory or large-size-herd testing</td>
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<tr>
<td>6</td>
<td>4°C for 48 h, then −70°C for 3 mo</td>
<td>Samples collected and shipped under refrigeration, stored for more than 1 mo, and then processed</td>
<td>Research-and-demonstration-herd project</td>
</tr>
</tbody>
</table>

\(^a\) The proportion of *M. paratuberculosis* culture tubes was defined as positive by having >1 colony present, and the proportion of animals was defined as positive by having at least 1 colony on 1 of 3 tubes for 6 different treatments. Differing letter designations (a, b, and c) represent significant differences (*P* < 0.05) between proportions.
ments 1, 5, and 6 than for treatment 3. All of the real-time PCR results were positive for the presence of *M. paratuberculosis* under all of the conditions tested (treatments 1, 3, and 6). While samples from treatments 1 and 6 were found to be positive using both of the detection methods, only 5 of 11 tubes were positive for fecal culture for treatment 3, and all samples were found to be positive by using real-time PCR.

Our study indicates that the storage of the samples at −20°C had adverse effects on the viability of *M. paratuberculosis* (5 of 11 samples were culture positive in treatment 3). Although all samples were positive by real-time PCR, the higher cycle threshold value in treatment 3 indicates the loss of viable bacteria and/or DNA quality. Treatment 3 may often be used by producers or veterinarians when they are sampling small populations or in individual suspected cases of Johne’s disease. Thus, DNA-based PCR could be an alternative for situations where intermediate or long-term storage is necessary. Short-term storage of fecal samples at 4°C and longer term storage at −70°C appear to have no deleterious effects on *M. paratuberculosis* viability, but short-term storage at −20°C should be avoided as it substantially reduces the viability of the bacteria in the sample.

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REFERENCES


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