DETECTION OF *Mycobacterium avium* subsp. *paratuberculosis* IN CAPRINE FECES; VALIDATION USING ADIAVET® PARATB REAL TIME KIT

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Abstract: A commercial real time PCR kit for detection of *Mycobacterium avium* subsp. *paratuberculosis* (*Map*) was tested for use in goat feces under Norwegian conditions. The study was initiated as a part of a project; “Healthier Goats, project for eradication of CAE, CLA and Johne’s disease in Norwegian goats”, aiming to eradicate diseases of great importance for the goat industry, including paratuberculosis. For eradication of the disease in a herd, rapid diagnosis of paratuberculosis is essential. PCR was performed with Adiavet®Paratb real time kit (Adiagène) using the company’s new protocol for DNA isolation, where 3-10 g of feces is analyzed. The results showed that the real time PCR was rapid, reliable and more sensitive than culture. None of the negative control samples were positive for PCR, confirming the specificity of the test. In the future, analysis with the Adiavet®Paratb real time kit might be implemented as a standard test in the Norwegian disease control program.

Introduction: *Mycobacterium avium* subsp. *paratuberculosis* (*Map*) is the causal agent of paratuberculosis or Johne’s disease (JD), a chronic granulomatous enteritis affecting ruminants. In Norway, the disease has been a problem in the goat industry. The clinical picture in goats is different from what is seen in cattle, as diarrhoea usually is not prominent. The typical picture is emaciation and reduced production yield[1].

The study was initiated as a part of a project; “Healthier Goats, project for eradication of CAE, CLA and Johne’s disease in Norwegian goats”, aiming to eradicate diseases of great importance for the goat industry, including paratuberculosis (http://geithelse.tine.no/English). The project is based on “snaching”goat kids, that is to remove the kids from the mother and the rest of the herd directly after birth. The newborn kids are then fed cows colostrum, housed separately and regularly tested for paratuberculosis. A problem for the follow-up investigation the long time needed for culture of *Map*, there is therefore a need for rapid diagnosis. The aim of this study was to validate a commercial real time PCR kit for detection of *Map* in goat feces under Norwegian conditions.

Material and methods: Faecal samples from goats were analysed by real time PCR and culture. Twenty samples collected over five days from four goats naturally infected with *Map* and 60 samples from goats in paratuberculosis free areas of Norway were sampled. Additionally, stored fecal samples from 24 previously culture positive and 18 previously culture negative samples were analysed by real time PCR. The stored samples originated from 2008 to 2010.

PCR was performed with Adiavet®Paratb real time kit (Adiagène, Saint-Brieuc, France), using the company’s new protocol for DNA isolation, where 3-10 g of feces can be analysed, increasing the sensitivity of the method. In short, 3 ± 0,2 g of feces was suspended in 20 mL sterile distilled water using a stomacher and left overnight for rehydration as recommended by Adiagène. The supernatant was filtrated using chemfilter (Adiafilter, Adiagène) for removal of PCR inhibiting substances, the pellet redissolved and the bacterial cells mechanically disrupted by bead-beating. DNA was extracted using the QIAamp® DNA Mini Kit (Qiagen, Hilden, Germany) as recommended by the manufacturer. Real time PCR was performed with the Adiavet®Paratb real time kit (Adiagène) as recommended using the EPC-Extraction internal control. The PARA positive control supplied with the kit was run for quality control of each assay, and Milli-q water was run as negative control. Real-time PCR was performed using Stratagene Mx3005P (Stratagene, La Jolla, CA, USA).
Results and discussion: The real time PCR from Adiagène worked well for analysis of goat feces in Norway (Table 1). The specificity was confirmed as 60 samples from goats in known paratuberculosis free areas were negative for both culture and PCR. The real time PCR was able to detect Map in all 20 samples from the four naturally infected goats. By culture, only 15 of these samples were positive (Table 1). The real time PCR was able to detect Map in 22 out of 24 previously culture positive samples. These samples had been stored at -20°C in up to three years, something that can explain the lower sensitivity compared to culture for these samples. 18 previously culture negative samples from positive herds were also analyzed, and Map was detected in three samples.

Table 1: Real time PCR and culture of 122 caprine fecal samples.

<table>
<thead>
<tr>
<th>Material</th>
<th>PCR +</th>
<th>PCR -</th>
<th>Culture +</th>
<th>Culture -</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naturally infected goats*</td>
<td>20</td>
<td>0</td>
<td>15</td>
<td>5</td>
<td>20</td>
</tr>
<tr>
<td>Goats from paratuberculosis free areas</td>
<td>0</td>
<td>60</td>
<td>0</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>Previously culture positive samples</td>
<td>22</td>
<td>2</td>
<td>24</td>
<td>0</td>
<td>24</td>
</tr>
<tr>
<td>Previously culture negative samples, known positive herds**</td>
<td>3</td>
<td>15</td>
<td>0</td>
<td>18</td>
<td>18</td>
</tr>
</tbody>
</table>

*Four goats naturally infected with Map, sampled five times each.
**Stored at -20°C, originating from 2008-2010.

Conclusions: The Adiavet®Paratb real time kit (Adiagène) worked well for analysis of goat feces under Norwegian conditions. The sensitivity was better that culture in the tested material, but more testing is required for statistical interpretation. None of the samples from paratuberculosis free herds were positive in the real time PCR, confirming the specificity. Even though culture still is the gold standard for diagnosis of Map, real time PCR decreases the time of diagnosis in goats from 12-16 weeks to two days. This is of great importance in an eradication programme like “Healthier Goats.”

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