ABSTRACT
The aim of this study was to compare the culture of Mycobacterium avium subsp. paratuberculosis from 2050 individual fecal samples from Dutch cattle herds in the following two culture methods: on a conventional solid agar, using modified Löwenstein-Jensen media (LJ), and in the TREK ESP para-JEM Culture System II. All samples cultured in the ESP-system were tested by Ziehl-Neelsen staining (ZN) and IS900 PCR after 42 days incubation. LJ-cultures were read at 8, 12 and 16 weeks, and suspect colonies were tested by IS900 PCR.

Overall, 12% of the samples were positive by both ESP-system and the LJ culture method and 79% were negative by both methods. A further 4% of the samples were positive by ESP culture only whilst 5% were positive by LJ culture only. The agreement between both methods was reasonable (kappa = 0.68).

INTRODUCTION
Culture of Mycobacterium avium subsp. paratuberculosis (Map) on conventional solid agars is time-consuming due to the slow growth of Map. Therefore, systems with a reduced incubation time, such as the TREK ESP para-JEM Culture System II, may allow earlier detection of Map shedders in certification-, surveillance and control programs for Map. However, the diagnostic test characteristics of the TREK ESP para-JEM Culture System II in routine diagnostic samples from Dutch cattle herds were unknown. Therefore, the aim of this study was to compare results of the ESP culture system II (TREK diagnostic systems, para-JEM reagent liquid media) with a modified LJ media culture on routine diagnostic fecal samples from Dutch cattle herds.

MATERIALS AND METHODS
A total of 2050 individual fecal samples were tested in parallel with both a modified LJ media culture method and the ESP culture system II.

For the culture on LJ-media the samples (2 gram) were decontaminated using a 4% sodium hydroxide solution and malachite green-oxalic acid suspension in two steps. After centrifugation the sediment was treated overnight with Neomycin and amphotericin B. From each sample 4 tubes of LJ-media (with mycobactin) were inoculated with 0.2 mL from the white intermediate layer. The tubes were incubated for up to 16 weeks and inspected every four weeks. When suspect growth was observed, growth of Map was confirmed by IS900 PCR.

A modified sedimentation-centrifugation method as described by Stable et al. (1997) was used for the decontamination of samples cultured in the TREK ESP system. Briefly, the samples (2 gram) were diluted in 35 mL sterile distilled water, and after shaking and sedimentation 5 mL of the supernatant were removed. For decontamination, 25 mL 0.9% HCP-BHI (hexadecylpyridinium chloride in brain heart infusion broth) was added and incubated overnight. After centrifugation and discharging the supernatant the sediment was treated overnight with an antibiotic mixture of neomycin and amphotericin B. One flask Para-Jem (TREK diagnostic systems) was inoculated with 1mL of the samples after adding the supplements and antibiotics according to the manufacturer. The flasks were then incubated in the ESP TREK system which measures the gas pressure every 20 minutes. After 6 weeks of incubation, all samples, detected or not yet detected by ESP system, were further investigated via Ziehl-Neelsen staining and the IS900-PCR.
RESULTS
In 601 (29%) of 2050 samples, the ESP-system indicated growth of \textit{Map} based on the optical signal which indicates the reduction of gas-pressure in culture flasks. However, only 29% of these 601 samples could be confirmed using both the Ziehl-Neelsen staining of the culture material and the IS900-PCR. Moreover, in 8% of the 2050 samples the ESP-system did not indicate growth of \textit{Map} whilst the samples were Ziehl-Neelsen-positive and IS900-PCR-positive. Overall, 340 of the 2050 samples (17%) cultured in the ESP liquid culture were ZN-positive and PCR-positive.

In 352 (17%) of the 2050 samples, suspected colonies were found in the LJ-culture that were subsequently confirmed as \textit{Map} using the IS900-PCR. Approximately 60% of these samples were found positive at 8 weeks of incubation.

Overall, 254 samples (12%) were positive by both ESP liquid culture (defined as acid-fast bacilli using the ZN-staining and PCR-positive as confirmation) and the LJ solid culture method, and 1612 (79%) were negative by both methods (Table 1). A further 86 samples (4%) were positive by ESP culture only, whilst 98 samples (5%) were positive by LJ culture only. Agreement beyond chance between both methods was reasonable ($\kappa = 0.68; 0.64-0.72$).

<table>
<thead>
<tr>
<th></th>
<th>LJ positive</th>
<th>LJ negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESP positive (ZN+/PCR+)</td>
<td>254</td>
<td>86</td>
<td>340</td>
</tr>
<tr>
<td>ESP negative</td>
<td>98</td>
<td>1612</td>
<td>1710</td>
</tr>
<tr>
<td>Total</td>
<td>352</td>
<td>1698</td>
<td>2050</td>
</tr>
</tbody>
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$Kappa = 0.68 (0.64 – 0.72)$

DISCUSSION AND CONCLUSION
In total 2050 samples were investigated in-parallel with both a solid phase culture method using Löwenstein-Jensen media and the liquid media ESP TREK system. The detection rates of both methods were comparable for the detection of \textit{Map} in feces. There was a reasonable agreement between both methods. However, to obtain a comparable detection rate with the ESP system, it is necessary to test all samples after 6 weeks of incubation using the Ziehl-Neelsen staining and IS900-PCR, which adds considerably to the costs of the system. The usage of only the optical signal of the ESP system leads to both false-positive and false-negative results; therefore a further confirmation is to be advised. The advantage of the ESP culture system is a considerable lower time-to-detection then with the conventional culture method on Löwenstein-Jensen media.

REFERENCE