Investigation of paratuberculosis status based on comparative analysis of serology and faecal culture in a dairy herd in Thuringia (Germany)

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ABSTRACT
In Thuringia, a federal state of Germany, paratuberculosis is being monitored by a voluntary control programme. Within the framework of this programme, a dairy herd of about 400 cattle with clinical problems of untreatable chronic diarrhoea was tested for the first time to detect paratuberculosis.

The objective of the present investigation was to determine the status of paratuberculosis in this herd, to analyse the diagnostic significance of an absorbed ELISA and the faecal culture, and compare it with age and lactation.

Cattle older than 24 months (n=279) were included in the study. In faecal culture, 93 cows (33 %) were detected as shedders and in ELISA, 39 cows (14 %) were positive or doubtful. Of the 93 shedders, only 31 cows (33 %) were classified as positive or doubtful by the ELISA. Almost half of the shedders were in the first or second lactation period. No significant differences in age of cows between serological negative and positive shedders were observed. The average milk production of shedders was significantly reduced (p<0.001) by approximately 1000 kg per lactation period, which corresponds to 10 % of the milk production. There was a high prevalence of paratuberculosis in the herd. Faecal culture is still the most suitable method to determine the individual status of paratuberculosis in a herd as most of the shedders were not detected by ELISA.

In view of the economic losses through reduced milk production, a herd management based on testing of the individual animals by faecal culture was found necessary to institute an effective control programme for paratuberculosis in infected herds.

Key words: age, cattle, ELISA, faecal culture, lactation monitoring, paratuberculosis, prevalence, serology.

INTRODUCTION
Paratuberculosis (PTB) is an economically important infectious disease of dairy cattle, causing untreatable diarrhoea, reduced milk production and progressive weight loss (Chioldini et al., 1984). The determination of infection status at herd level is necessary to implement control measures to reduce the transmission of the infection to susceptible cattle. Although PTB is widely prevalent, there is a lack of adequate studies on prevalence at herd or national level due to the difficulty of diagnosis. Despite the fact that the bacterial culture of MAP is time consuming (12-16 weeks), it is generally considered to be the most accurate method with 100 % specificity and 38–55 % sensitivity (Sockett et al., 1992; Whitlock et al., 2000). The absorbed serum ELISA is rapid and substantially economic, but its sensitivity and specificity are in the ranges of 28–29 % and 95–100 %, respectively (Collins et al., 2005).

In Thuringia, a federal state of Germany, a voluntary control programme exists for the monitoring of paratuberculosis using ELISA und faecal culture as diagnostic tools. In the framework of this programme, a dairy herd of 400 cattle with clinical problems of untreatable diarrhoea was tested for the first time to determine the paratuberculosis status of the herd, to analyze the diagnostic significance of an absorbed ELISA and faecal culture, and to compare the results with the age, lactation and the milk yield.

MATERIALS AND METHODS
Samples: Blood and faecal samples were collected from cattle (n=279) older than 24 months.
**Faecal culture:** Culture was carried out with 3 g faeces according to the AVID method (www.dvg.net). Briefly, following 24 h decontamination with 0.75 % (w/v) hexadecylpyridinium chloride, the samples were incubated on Herrold’s egg yolk medium supplemented with mycobactin J for 12 weeks. The growth of *Mycobacterium avium* ssp. *paratuberculosis* (MAP) colonies was confirmed by amplification of the MAP specific insertion sequence IS900 by PCR (Englund et al. 1999). A sample was regarded as positive if one or more MAP colonies were detected.

**Serology:** The serological analyses were performed with EDTA plasma. The ELISA (IDEXX) included a preabsorption step with *Mycobacteria phlei* to detect specific antibodies of MAP in the serum. Classification of results was according to manufacturer instruction in negative, doubtful and positive samples. Samples classified as positive or doubtful were summarised.

**Milk yield:** Total milk yield was calculated based on monthly measurement.

**Statistic:** For statistical analysis an unpaired, two-tailed t-test was used.

**RESULTS AND DISCUSSION**

**Faecal culture and serology**

In faecal culture, 93 of 279 tested cattle were detected as shedders equivalent to 33 % of the tested cattle. In comparison to the cultural confirmation of MAP, specific MAP antibodies were detected by ELISA in samples of 39 cattle equivalent to 14 % of tested cattle. The results of faecal culture and serology in relation to average age and milk yield are shown in Table 1. Of the 93 shedders, only 31 cows (33 %) were classified as positive or doubtful by the ELISA. Sixty two shedders were not detected by ELISA and 8 serologically positive or doubtful tested cattle (2.9 %) were cultural negative. The percentage of serological positive results in faecal culture with more than 100 CFU/tube was much higher for serological positive cattle (48.5 %) in comparison to serological negative cattle (14.5 %) (Fig. 1a).

**Table 1.** Diagnostic results of faecal culture and serology related to average age and milk production (Mean ± Standard deviation)

<table>
<thead>
<tr>
<th>Serology</th>
<th>Culture negative</th>
<th>Culture positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Positive/ Doubtful</td>
</tr>
<tr>
<td>Numbers of cattle tested</td>
<td>178</td>
<td>8</td>
</tr>
<tr>
<td>Age (months)</td>
<td>50.5 ±18.8</td>
<td>55.4 ± 14.2</td>
</tr>
<tr>
<td>Milk yield (kg/ lactation period)</td>
<td>9741 ±1542</td>
<td>9983 ± 652</td>
</tr>
</tbody>
</table>

**Fig. 1.**

*a*) Percentage of faecal samples with high CFU-amount concerning serological results,

*b*) Fractions of shedders of every age group in relation to total tested cattle
The cultural method detected more cattle positive in comparison to ELISA, which might be due to the facts that animals either did not seroconvert or might have transplacental infections leading to a lack of immunological recognition of paratuberculosis infection (Seitz et al., 1989). It is also possible that the culture method detected animals in the early stage of the infection with low antibody response. A decrease in the “cut-off” value of ELISA might have increased the proportion detected. However this was not assessed in the present study.

The false positive results of ELISA might be due to shedding of bacteria below the detection limit at the time of sampling. It is widely known that the sensitivity of faecal culture is limited due to intermitting shedding, inhomogeneous distribution of MAP in faeces and losses during decontamination process.

**Age**

The evaluation of data concerning age of tested cattle showed no significant differences (p > 0.1) between serological negative and positive shedders (46.0 and 50.6 months, respectively, see Table 1). Almost half of the shedders were between two and three years old and hence were in their first or second lactation period (Fig. 1b).

The presence of more shedders during first or second lactation period indicated the onset of the clinical disease at a young age disproving the old concept of occurrence of clinical disease at later stages of life.

**Milk yield**

The mean milk production of shedders was significantly reduced (p<0.001) by approximately 1000 kg per lactation period (8719 kg compared to 9749 kg of non-shedders), which corresponded to 10 % of the milk yield (Fig. 2). A reduced lactation was also observed when comparing milk yield of serologically positive or doubtful cattle (8802 kg) with serologically negative cattle (9481 kg).

Significant impact of paratuberculosis infection on milk yield has been widely reported (McKenna et al., 2006). Non-significant influence of serological status on milk yield might be due to lower number of serological positive subjects in the present study.
CONCLUSIONS
There was a high prevalence of paratuberculosis in the herd. Faecal culture is still the most suitable method to determine the individual status of paratuberculosis in a herd as most of the shedders were not detected by ELISA. In view of the economic losses through reduced milk production, a herd management based on testing of the individual animals by faecal culture was found necessary to institute an effective control programme for paratuberculosis in infected herds with high prevalence.

REFERENCES
www.dvg.net, AVID (Arbeitskreis Veterinärmedizinische Infektionsdiagnostik).