PREVALENCE OF PARATUBERCULOSIS IN DANISH DAIRY HERDS

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Paratuberculosis is a chronic infectious disease in bovids and other ruminants in particular caused by *Mycobacterium avium* subsp. *paratuberculosis*[1]. Paratuberculosis in dairy cattle is widespread in Danish dairy herds[2] with significant economic losses as a sequela, both in herds with clinical cases of the disease and in herds with subclinical cases[3]. Still, the disease is often ignored by farmers and veterinarians either because of legislative circumstances restricting free trade between herds, or simply because clinical cases of the disease never occurs in some infected herds.

The aim of this study was: a) to estimate the prevalence of dairy herds and dairy cows infected with *Mycobacterium avium* subsp. *paratuberculosis* in a selected region; and 2) to use the prevalence estimates to increase the likelihood of a farmer to become interested in controlling the disease.

Materials & Methods

The study was carried out as a part of the multi-disease-project, "Integrated Cattle Health and Milk Quality Project"[4]. Of a total of 280 dairy herds in this region in the Southern part of Jutland, 109 agreed to participate in studies on infectious diseases, including paratuberculosis. All lactating cows in the 109 herds were sampled once in the period August 1999 till February 2000. The size and composition of these herds are shown in Table 1. The milk samples were collected from the herds via the Danish milk-recording scheme. These samples were preserved with bronopol and methyleneblue. At arrival to the laboratory[a], the samples were centrifuged for four minutes with subsequent removal of the fat fraction. The samples were then frozen at -21 ^{o}C until testing on an indirect ELISA.

Table 1. Size and composition of the 109 study herds.

Number of lactating cows per herd						
Mean	Median	25 percentile	75 percentile	Minimum	Maximum	Total
74	66	55	89	21	229	8071

The ELISA used a commercially available antigen[b]. The wells of Polysorp® microtiter plates[c] were coated with 2.00 ìl antigen /ml 0.05 M carbonate-

[a] Cattle Health Laboratory, Danish Dairy Board, Brørup, Denmark
[b] Allied Monitor, Fayette, Missouri, USA
[c] Nunc, Roskilde, Denmark

bicarbonate buffer (pH 9.6) and were incubated at 4 ^{o}C for 2 days. In the meantime, samples were thawed and absorbed overnight at 4 ^{o}C with a suspension of *M. phlei*[b] in a concentration of 0.25 mg/ml. The plates were then washed 5 times with phosphate buffered saline with 0.05% $Tween_{20}$, pH 6.8 ($PBST_{20}$) and shaken dry. The absorbed samples were added to the coated plates in duplicate and incubated overnight at 4 ^{o}C. Plates were washed again before 100 ìl horseradish-peroxidase labelled goat-anti bovine IgG (H+L)[d] at 1:2,000 in $PBST_{20}$ was added each well. A solution of ortho-phenylene diamine[e] (OPD) substrate diluted in citrate buffer (pH 5.0) to 300 µg/ml was made. One hundred ml of this OPD solution was supplemented with 80 µl of H_2O_2. The plates were given a final wash before 100 µl of the OPD substrate was added each well. Plates were left at room temperature and the reaction was stopped using 0.5 M H_2SO_4 before the negative standard sample exceed an optical density (OD) of 0.300. The OD-values were read using an ELISA reader at 492 nm with a 620 nm reference filter.

For each sample, a corrected OD-value (OD_C) was calculated by subtracting the OD of the negative standard sample.

To estimate the prevalence we used two different methods to determine the accuracy of the ELISA. These methods were called Method A and Method C elsewhere in these proceedings[5]. The sensitivity and specificity at OD_C=0.040 are given in Table 2. The true prevalence was then calculated for each herd from the apparent prevalence:

Table 2. Sensitivity (Se) and specificity (Sp) of the ELISA at cut-off OD_C=0.040

	Se	Sp
Method A	0.87	1.00
Method C	0.558	0.912

$$p(D+) = \frac{p(T+) + Sp - 1}{Sp + Se - 1}$$

where p(D+) is the true prevalence (TP), p(T+) is the apparent test prevalence (AP) and Se and Sp are the sensitivity and specificity of the applied test.

Results

Of the 8071 cows 885 reacted positive giving an apparent prevalence (AP) of 11.0%. Five herds had no reactors at all, whereas the rest had at least one reactor. Calculation of True Prevalence (TP) using Method A and Method C gives TP = 12.6% and TP = 4.6%, respectively. The prevalence estimates based on individual herds are summarised in Table 3. Calculation of True Prevalence for each herd gave no changes compared to the grand mean True Prevalence, and only 5 herds had a TP = 0% using evaluation method A, whereas 55 herds (50.5%) had a TP=0 when using method C.

Table 3. Size and composition of herd estimates of Apparent Prevalence (AP) and True Prevalence (TP) for each of the methods A and C.

	Percent positive						
	Mean	Median	25percentile	75percentile	Minimum	Maximum	Total
AP	11.1	8.6	4.6	14.0	0	47.2	11.0
TP, Method A	12.8	9.9	5.3	16.0	0	54.3	12.6
TP, Method C	4.9	0	0	11.0	0	81.7	4.9

[d] Kirkegaard and Perry Laboratories, Gaithersburg, Maryland, USA
[e] Kem-En-Tec, Roskilde, Denmark

Discussion

The accuracy of various diagnostic tests for paratuberculosis is essential for provision of appropriate prevalence estimates. The overall prevalence estimates shows TP of 4.6-12.6% individuals and 50-95% of herds. The herd prevalence estimate is not surprising compared to our previous estimate on bulk-tank milk, where Southern Jutland showed a True Prevalence of 89%[2]. Previous estimates of individual cow prevalence were 2-3%[6, 7], but these were apparent prevalences where the accuracy of the diagnostic tests were not taken into account. Additionally, these investigations were carried out many years ago and are likely to have changed. Therefore, estimates of individual cow prevalence of more than 10% infected animals are very likely. Method C probably underestimates the prevalence. In total the cow prevalence estimates is probably between 5% and 13%.

The structure of the "Integrated Cattle Health and Milk Quality Project" provides possible selection bias due to farmers' self-selection in the region. The intensive focus on paratuberculosis may have had some effect on the culling practices in the herds.

Based on the present material it can be assumed, that most farms in the region are infected with *M. avium* subsp. *paratuberculosis*. Therefore it is more likely individual farmers will accept a positive status, and dare to discuss it with others. Even the more conservative farmers might take part in this discussion. This may add to a debate about the possibility of conducting control and eradication programs for paratuberculosis. Such programs are conducted only in a few countries including Australia, The Netherlands and the United States. Discussions of these preliminary prevalence estimates with the farmers in the study region strongly indicate a positive attitude towards initiating increased information and advisory services on paratuberculosis, eventually leading to control of the disease.

The fact that cross-reactions to avian tuberculosis is a possibility may also influence the farmers opinion on their own infection status, as some believe that positive results are false positive results due to cross-reactions.

References

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