THREE APPROACHES TO EVALUATE THREE DIAGNOSTIC TESTS FOR PARATUBERCULOSIS

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Diagnosis of paratuberculosis is based on detection of immune responses or detection of the aetiologic agent itself. However, neither of the tests has proven accurate enough for both providing a sensitive and a specific diagnosis of paratuberculosis in cattle. The accuracy of the diagnostic tests is often compared to faecal culture as the 'gold standard' (GS), even though the culture method is not very sensitive[1]. The sensitivity of the faecal culture is therefore difficult to obtain in the absence of a true 'GS'.

The purpose of this study was to estimate the diagnostic accuracy of three diagnostic tests for their ability to detect infection with *M. avium* subsp. *paratuberculosis* in cattle using three methods: A) Maximum likelihood (ML); B) Comparison to faecal culturing as 'GS'; C) Comparison to known faecal culture positive samples and defined negative samples from a "paratuberculosis-free area".

Materials & Methods

Serum, milk and faecal samples were collected from all 1844 lactating cows in 29 Danish dairy herds. This is referred to as the Danish material. For maximum likelihood estimations, grouping into populations with different prevalence of paratuberculosis was necessary. Breed[2] and age[3] are both potential risk factors for clinical paratuberculosis and were therefore used for the sub groupings, based on data from the Danish Cattle Database (Table 1).

Parity was used as a substitute for age, and only two levels i.e. parity = 1 and parity > 1, was used. Breed and parity information was not available for 30 cows. The Danish material was

Table 1. Composition of the Danish material

	Breed*						Fungi	Total
	RDM	SDM	Jersey	DRK	Cross	No info		
Parity = 1	30	421	78	4	24	0	85	642
Parity > 1	30	737	226	9	46	0	124	1172
No info	0	0	0	0	0	30	-	30
Fungi	18	162	13	0	16	-	-	-
Total	78	1320	317	13	86	30	-	1844

*RDM=Danish Red; SDM=Danish Holsteins; Jersey=Danish Jersey; DRK=Danish Red-and White; Cross=crossbreed

supplemented with paired milk and serum samples from 68 Norwegian cows assumed to be non-infected, as Norway was considered free of paratuberculosis in cattle at the time of sampling[4].

The samples were tested using three tests: two antibody detecting ELISAs, one on milk (M-ELISA) and one on serum (S-ELISA), and one agent detecting test, i.e. culturing of the mycobacteria from faecal samples[5] (faecal culturing, FC). Only cows where no fungal growth (fungi) was detected in the faecal culturing procedure was used for further analysis (Table 1). The M-ELISA is described elsewhere[6] and the S-ELISA was performed as the M-ELISA except for the following modifications: antigen was used in concentration 1.00 mg/l; serum was diluted 1:5 in phosphate-buffered saline (PBS) subsequent to dilution 1:2 in *M. phlei*; and the peroxidase labelled goat-anti-bovine IgG conjugate was diluted 1:4,000.

Estimation of sensitivity (Se) and specificity (Sp) was done with three different approaches: Method A) in the absence of a 'Gold Standard' (GS) using maximum likelihood (ML)[7]; Method B) using faecal culture as 'GS'; and Method C) using the faecal culture positive samples from the Danish material as 'GS' for positive samples and the Norwegian samples, i.e. samples from an area assumed to be free of the mycobacteria, as the 'GS' for negative samples. The Se and Sp of the faecal culturing were estimated by method A only. Data from method C was selected to determine the optimal cut-point for the ELISAs[8] and this was done using differential positive rate (DPR, (DPR=Se+Sp-1)) analysis. This cut-point was used to dichotomise ELISA results for the ML-procedure. The Danish material was used for Method A and Method B, but in Method A, the 30 cows with no breed and parity information was omitted. For Method C, the defined positive material consisted of 104 samples from faecal culture positive cows and the defined negative material consisted of 68 Norwegian samples.

Results

Determination by DPR analysis gave the optimal cut-off for the S-ELISA at corrected optical densities[6] (OD_C) = 0.22 and the cut-off for the M-ELISA were at OD_C = 0.04 (Table 2), and the dichotomised ELISA data were used for Method A.

Table 2. Sensitivities (Se) and specificities (Sp) of ELISAs at cut-points determined by DPR and Se of each test at a preselected Sp of 0.98.

Test and method (met)	Se and Sp at optimal cut-points, OD_C			Se at Sp = 0.98	
	OD_C	Se	Sp	OD_C	Se
S-ELISA, Met B	0.34	0.56	0.69	0.96	0.06
S-ELISA, Met C	0.22	0.65	0.94	0.29	0.60
M-ELISA, Met B	0.32	0.44	0.90	1.13	0.25
M-ELISA, Met C	0.04	0.56	0.91	0.30	0.44

Attempts to use breed for sub grouping failed, as the estimated true prevalence was not different between breeds. This was a prerequisite for the ML-procedure. Only sub grouping with parity provided a proper difference.

Sensitivity (Se) and specificity (Sp) of each of the tests using each method is given in Table 3. For comparative purposes Sp of the ELISAs are given at the Se given by method A, i.e. $Se_{S\text{-}ELISA}$= 0.64 and $Se_{M\text{-}ELISA}$=0.87.

Discussion

Which of the estimates for accuracy is the better is difficult to judge, but method B is not the method of choice as the Se of faecal culture is quite low. Method A gives higher estimates of sensitivity for ELISA than is often seen in literature. However, many of these estimates are still based partly on animals shedding bacteria, and this population might not be representative of the infected population in general. Surprisingly, faecal culture is not 100% specific, as it is usually assumed[1]. One reason may be that some cows probably ingest and shed bacteria without ever being infected. The Se estimate of only 0.09 for faecal culture is low compared to the 0.45 previously published[1], and the estimate could be underestimated. However, the previously published data were done in populations with high numbers of cows with clinical paratuberculosis and therefore the sensitivity there could be overestimated. The true Se of faecal culture in either case is even lower because a high number of cows will have fungal growth on their culture. Method C is not optimal because of potential differences in the mycobacterial background flora in Norway and Denmark. Method A takes into account both immunological status and shedding status of a cow and combines these into an estimate for infection. The other methods do not consider the aspect of shedding from animals that are not infected. The estimates for method A are therefore more likely to be true. The true sensitivity and specificity are probably somewhere in between the estimates provided by Method A and Method C.

Table 3. Sensitivities (Se) and specificities (Sp) of three tests using different methods for evaluation. Sp of the ELISAs are given at the Se given by method A

Evaluation method	A: ML		B: Culture+ & Culture -		C: Culture+ & Area -	
Test	Se	Sp	Se	Sp	Se	Sp
Faecal culture	0.09	0.98	-	-	-	-
S-ELISA	0.64	1.00	0.64	0.58	0.64	0.94
M-ELISA	0.87	1.00	0.87	0.36	0.87	0.06

References

[1] Sockett DC, Carr DJ, Collins MT. Evaluation of conventional and radiometric fecal culture and a commercial DNA probe for diagnosis of *Mycobacterium paratuberculosis* infections in cattle. Can J Vet Res, 1992; 56: 148-153

[2] McNab WB, Meek AH, Duncan JR, Martin SW, van Dreumel AA. An epidemiological study of paratuberculosis in dairy cattle in Ontario: Study design and prevalence estimates. Can J Vet Res, 1991; 55: 246-251.

[3] Kalis CHJ, Hesselink JW, Russchen EW, Barkema HW, Collins MT, Visser IJR. Factors influencing the isolation of *Mycobacterium avium* subsp. *paratuberculosis* from bovine fecal samples. J Vet diagn Invest, 11: 345-351.

[4] Mørk T, Sund A. Paratuberkuose hos storfe i Norge (Paratuberculosis in cattle in Norway). Norsk Veterinærtidsskrift, 1997; 109: 558.

[5] Giese SB, Huda A, Wedderkopp A, Klausen J, Ahrens P. Cultivation of Mycobacterium paratuberculosis in Dubos broth combined with PCR. Proc 5th International Colloquium on Paratuberculosis, 1996: 5: 302-306.

[6] Nielsen SS, Agger JF. Prevalence of paratuberculosis in Danish dairy herds. Proc 9th ISVEE, Breckenridge, Colorado, USA, 2000.

[7] Hui SL, Walter SD. Estimation of error rates of diagnostic tests. Biometrics, 1980; 36: 167-171.

[8] Jensen AL, Poulsen JS. Evaluation of diagnostic tests using relative operating characteristic (ROC) curves and the differential positive rate. J Vet Med A, 1992; 39: 656-668.