

qPCR kits for fecal samples to identify the shedders of Para Tb or Salmonella dublin among highly ELISA-positive cows.

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Background.

The use of ELISA tests in milk and blood is a common way to find problem cows with paratuberculosis (MAP), and in Denmark, Salmonella Dublin as well. Because of the need for repeated samples for MAP (low specificity of ELISA test) the cost is relatively high. It is common knowledge among farmers that even high antibody-positive cows for MAP are not going to show symptoms right away. Saxmose et al. (2016) found that 29% of cows listed as highly positive cows in Denmark, calved after being listed as high ELISA positives and stayed in the farm for an average of 1.4 years (max. 6.9 years). The results are a decreasing participation in the Danish MAP control program.

Among herds positive for Salmonella dublin in bulk tank milk, it is common to look for highly ELISA-positive cows, but often many cows are antibody positive, which makes it difficult to locate cows shedding the Salmonella dublin. Therefore, in both disease complexes, qPCR is a tool to find the right cows to cull, in order to reduce the presence of the pathogen in the herds.

Methods

DNA Diagnostic A/S, Voldbjergvej 14, Risskov, Denmark, has developed two new qPCR test, 'ParaTB', and 'Salmonella 4 cows', both of which can be coupled to the same fecal extraction protocol, also developed by DNA Diagnostic A/S.

Fecal samples from 46 cows listed as high ELISA-positive Red in the Danish control program and 58 control cows from the same herds, were tested by the ParaTB qPCR test. Also 169 cows from an assumingly negative herd were tested.

Fecal samples from 55 high ELISA positive (OD>100) cows, were tested by the Salmonella 4 Cow qPCR. Also 402 cows with lower ELISA-positive (OD<100) measurements Salmonella dublin antibodies in milk or blood were tested.

Results

Figure 1 Fecal qPCR and ELISA results from 46 positive cow and 58 control cows in the Danish MAP control program
Also 169 cows from expected negative herds tested negative in both test.

Para Tb test	ELISA Test	
	Pos*	Neg*
qPCR Fecal		
Pos <33	26	5
Neg >33	20	53

Figure 2 Fecal qPCR and ELISA milk and blod. Results from 55 high positive ELISA cows and 402 ELISA low positive or negative cows Salmonella dublin antibodies.

Salmonella 4 Cow	ELISA Test	
	Pos OD> 100	Neg OD < 100
qPCR Fecal		
Pos ≤ 37	7	1
Neg ≤ 37	48	401

Conclusion

The newly developed qPCRs for MAP, 'ParaTB', and the qPCR for Salmonella dublin, 'Salmonella 4 Cows', have shown to be highly effective in finding cows shedding bacteria for these two infections in fecal samples, and thereby motivating the farmers to effectively reduce the shedding of bacteria by culling these cows immediately.

Follow up test in the Salmonella positive farm in the three month after the last PCR positive cows were culled, all newly introduced heifers where checked by milk ELISA at first test day. None of the animals showed seroconversion. This indicates that new infections seem to have stopped. This is the first period in two years, that newly introduced heifers have not seroconverted for Salmonella dublin in the ELISA test before first test day, on this farm.

Reference: Saxmose S. and C. Kirkeby, 2016. Dairy farmers' compliance with culling recommendation in paratuberculosis programme. <https://www.svepm.org.uk/posters.html>



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