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## **The use of new qPCR kits for fecal samples to find the shedders of paratuberculosis or salmonella dublin among highly elISA-positive cows**

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The use of ELISA tests in milk and blood is a common way to find problem cows with Paratuberculosis, and in Denmark, Salmonella Dublin as well. Because of the need for repeated samples for Paratuberculosis the cost is relatively high. It is common knowledge among farmers that even high antibody-positive cows for Paratuberculosis 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 paratuberculosis 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, there is a need for tests that find the right cows to cull, in order to reduce the presence of the pathogen in the herds.

DNA Diagnostic A/S, Voldbjergvej 14, Risskov, Denmark, has developed a 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.

For the fecal test for paratuberculosis, in total 46 Cows listed as high ELISA-positives in the Danish control program and 58 control cows from the same herds, were tested by the ParaTB qPCR test. Only 26 (57%) was found to be high shedders of paratuberculosis bacteria in the fecal samples (Ct values <33). Of the 58 control cows 5 (9%) were shedding high numbers of Paratuberculosis bacteria. Also 169 cows from an assumingly negative herd were tested and all tested negative. Finally, fecal samples from 5 cows with diarrhea and clinical signs of paratuberculosis were tested, of which all tested positive (Ct values from 21.4 to 32.6).

For the fecal test for Salmonella dublin, a total of 55 high ELISA positive (OD>100) cows, were tested, and only 7 (13%) were positive shedding Salmonella dublin bacteria. Among 402 cows with lower ELISA-positive measurements in milk or blood, one extra shedder was found. All shedders were culled immediately. During the two 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.

The newly developed qPCRs for paratuberculosis, 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.

**Keywords:** paratuberculosis, salmonella dublin, elisa, qpcr, culling strategy