An In-house ELISA for Treponema Antibodies In Bulk Milk is useful for Monitoring Claw Health

Bovine digital dermatitis (DD) is a painful, infectious claw disorder that causes ulcerative lesions mainly at the coronary band of the hind legs of dairy cows and lameness. DD is not only a serious issue in terms of animal welfare, but also has significant economic consequences due to milk loss, decreased fertility and treatment costs. Prevalence of DD in the Netherlands is estimated to be 20% to 30%. DD was first described in 1974, in Italy. Common bacteria associated with DD are multiple phylotypes from the genus Treponema, of which *T. medium/T. vincentii*-like, *T. phagedenis*-like, and *T. pedis* are most representative. Lesions can be classified using the M-scoring system, developed by Döpfer *et al.* Evaluation of lesions by lifting the cow's feet for visual inspection is the most accurate DD identification system but is expensive, time consuming, labor-intensive, and stressful for cattle. Regular claw trimming and inspection of feet in the West-European countries is mostly performed twice a year. In the meantime, it is useful for dairy farmers to obtain information about the prevalence of *Treponema spp.* in the herd.

Current laboratory tests for DD diagnostics in individual cows are based on histology, cultivation, PCR techniques and also ELISA for antibody detection. The advantage of the use of ELISA in milk is the availability of samples and the limited costs.

Aim

The objective of this study was to develop, validate and implement a Treponema antibody ELISA in bulk milk to monitor and assess DD prevalence at the herd level.

Materials and Methods

The farms used in this study were chosen by convenience sampling based on the willingness of the farmers to participate. In 2017 and 2018, seven herds were visited by trained GD employees three times in 2017-2018, with six months between each visit at the moment of regular preventive claw trimming. In these seven herds, all claws were scored according to the M-score system for presence and severity of DD. This M-score classification comprises six classes (M0, M1, M2, M3, M4, and M4.1). Class M0 is coding for healthy skin without lesions, M1 for an active granulomatous area of 0–2 cm, M2 for an ulcerative lesion of >2 cm, M3 for an ulcerative lesion covered by a scab, class M4 for alteration of the skin with hyperkeratotic lesions and class M4.1 codes for scar tissue with a new small lesion. In general, M2 stage is seen as most painful, but chronic lesions might be more infectious. After scoring, claws were trimmed and treated topically by the claw trimmer when necessary. Four weeks after each trimming, milk from all lactating cows was sampled. From another 110 herds, DD scores from one regular claw trimming were obtained, and one bulk milk sample was taken. Milk samples were tested for antibodies against Treponema spp. using an indirect ELISA based on a mixture of whole cell antigens from *T. medium/T. vincentii*-like, *T. phagedenis*-like, and two strains of T. pedis of which one was formerly known as *T. denticola.* The optical densities (OD) were measured and S/P values were calculated.

Main Results

ELISA results in milk samples of all individual cows and the bulk milk samples of the seven herds obtained during three visits showed a good association (n=20, r2= 0.82, p<0.001) between the average S/P ratio of the individual samples and the S/P ratio of the bulk milk. When the average S/P ratios of the individual milk samples in ELISA were set off against the average M-scores or the M2-prevalence in these herds, in both cases a weak, but statistical significant association between the severity of the lesions and the S/P ratio was found (Figure 1 and 2).

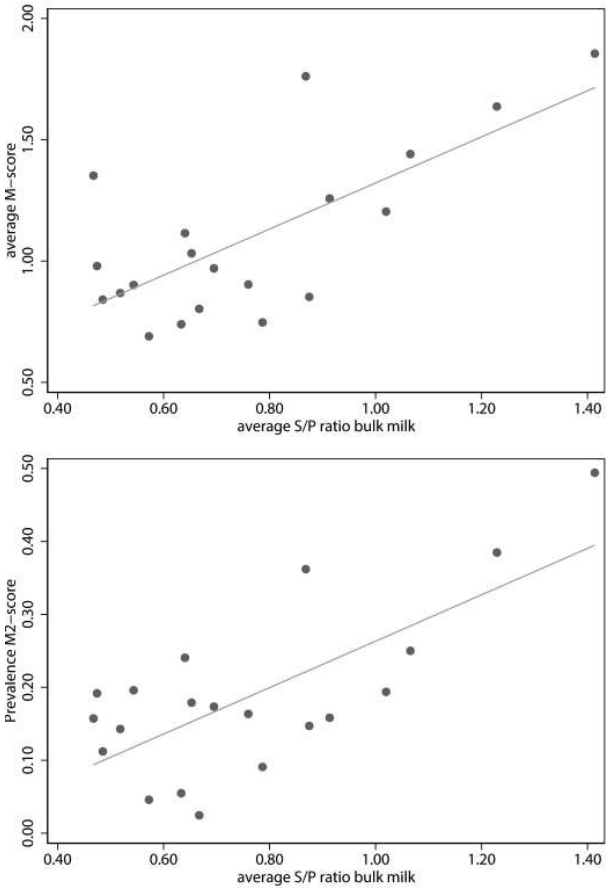

Figure 1. Scatterplots of trimming scores and bulk milk ELISA S/P-ratio's on 7 herds sampled thrice; average M-score and S/P-ratio (n=20, r2=0.504, P); and M2-prevalence and S/P-ratio (n=20, r2=0.513, p<0.001).

To be able to distinguish herds with high and low level of Treponema antibodies in bulk milk, two cut-off values (0,87 and 1,24) were determined based on the 25 and 75 percentile of the S/P-ratio obtained for all bulk milk samples from the seven herds. These ELISA cutoff's were evaluated in the 110 herds. Comparing the M2 prevalence with the bulk milk results, a good association was found. Only one of the herds had a high ELISA S/P-ratio in bulk milk, but a low M2 prevalence, while eleven herds were found with a high M2 prevalence and a low ELISA S/P-ratio.

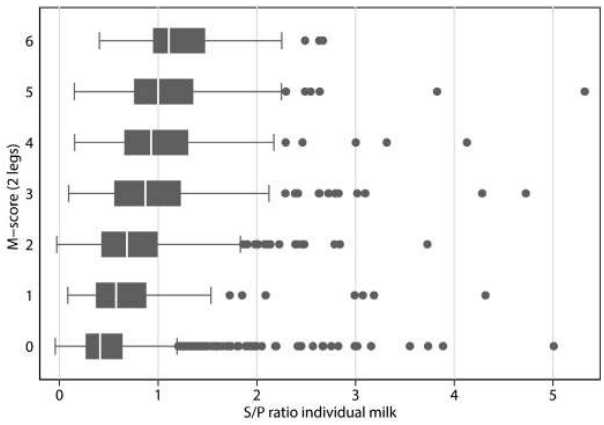

Figure 2. Comparison of M-scores and bulk milk ELISA S/P-ratio's on 95 herds with information on both M2-scores and the bulk milk S/P ratio (r2=0.23, p<0.001).

From 2019 on, the Treponema ELISA was added to the Royal GD Claw Health program, using quarterly bulk milk testing. Other parameters tested in this programme are feed associated parameters biotin, zinc and manganese. Results of these tests help farmers to improve claw health.

Conclusions

A Treponema bulk-milk ELISA was developed to get insight in the DD status at herd level, and the ELISA is suitable to be used in a claw-health monitoring programme for dairy cattle in the Netherlands. Periodically bulk milk testing for antibodies against Treponema spp. is valuable to monitor the DD prevalence at herd level and may support dairy farmers in applying the correct curative and preventive measures.

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