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Use of online measures of L-lactate dehydrogenase for classification of posttreatment mammary *Staphylococcus aureus* infection status in dairy cows

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ABSTRACT

An automated method for determining whether dairy cows with subclinical mammary infections recover after antibiotic treatment would be a useful tool in dairy production. For that purpose, online L-lactate dehydrogenase (LDH) measurements was modeled using a dynamic linear model; the variance parameters were estimated using the expectation-maximization algorithm. The method used to classify cows as infected or uninfected was based on a multiprocess Kalman filter. Two learning data sets were created: infected and uninfected. The infected data set consisted of records from 48 cows with subclinical *Staphylococcus aureus* infection from 4 herds collected in 2010. The uninfected data set came from 35 uninfected cows collected during 2013 from 2 herds. Bacteriological culturing was used as gold standard. To test the model, we collected data from the 48 infected cows 50 d after antibiotic treatment. As a result of the treatment, this test data set consisted of 25 cows that still had a subclinical infection and 23 cows that were recovered. Model sensitivity was 36.0% and specificity was 82.6%. To a large extent, L-lactate dehydrogenase reflected the cow's immune response to the presence of pathogens in the udder. However, cows that were classified correctly before treatment had a better chance of correct classification after treatment. This indicated a variation between cows in immune response to subclinical mammary infection that may complicate the detection of subclinically infected cows and determination of recovery.

Key words: dairy cow, mastitis, L-lactate dehydrogenase, multiprocess model

INTRODUCTION

Intramammary infections in dairy cows are of great concern to the dairy industry (Bradley, 2002). *Staphylococcus aureus* is a common cause of such infections. It is highly contagious, and the majority of infections are subclinical (Barkema et al., 2006). For this reason, it is important for farmers to know which of their cows are infected. To keep transmission low, infected cows should be treated with antibiotics or separated from healthy cows (Lundberg, 2015), but this is possible only if infections can be detected with measures of sufficient quality. Conventional methods for determination of whether or not bacteria are present are bacteriological culturing or PCR analysis of milk samples. These methods are very time consuming because milk samples from individual cows are needed and cows' infection status should be updated regularly. The aim of the present study was to develop an automated method for detecting pathogens in dairy cow udders based on inline L-lactate dehydrogenase (**LDH**) measurements.

Traditionally, monitoring in animal production has been based on select static key figures isolated in time, such as milk production per cow or average daily gain in slaughter pigs. Observed key figures are interpreted as a true underlying value plus random variation and compared with a predefined target. The fundamental assumption behind this approach is that the true underlying mean is constant over the period of interest. However, in animal production, the underlying mean will often show some kind of variation. This variation can be accounted for when a time series of measurements is available (Kristensen et al., 2010). Therefore, time series are also very well suited to measuring milk quality and pathogens in milk.

Dynamic monitoring of time series has become an important tool in different areas such as medicine and finance, and this technique has found use in animal production. For instance, Thysen (1993) monitored milk quality in dairy cows, Roush et al. (1992) detected changes in feed consumption in broilers, and de Mol et

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al. (1999) and Cornou and Lundbye-Christensen (2008) detected estrus and disease in dairy cattle and sows, respectively. With online monitoring, level shifts can be detected in real time as new observations are made. However, to use the continual stream of measurements, a framework that allows knowledge to accumulate is needed. Mathematical and statistical modeling of time series processes can be based on classes of state space models, also called dynamic models.

Technological development has made it possible to conduct automatic online sampling and measurement of components in milk. The enzyme LDH is found in the cytoplasm of all cells in the body, and during an inflammatory process involving cell damage and breakdown, as observed during IMI, it is released from the cell into the milk (Zank and Schlatterer, 1998). In dairy milk, LDH is correlated with SCC (Chagunda et al., 2006a; Akerstedt et al., 2011; Nyman et al., 2016), and it is used as a mastitis indicator in commercial herd management (Chagunda et al., 2006b; Friggens et al., 2007). The aim of the present study was to develop a model that could classify cows correctly according to their true infection status after antibiotic treatment.

MATERIALS AND METHODS

Herds and Data Collection

In this study, we included 2 data sets. The first included only cows infected with *Staph. aureus*, and the second included only uninfected cows.

We collected data set 1 during the autumn of 2010; it contained data from 4 dairy herds that were chosen because they used the Herd Navigator management program (DeLaval, Tumba, Sweden). All cows were housed in freestall barns with cubicles. Two herds used automatic milking systems and 2 herds used milking parlors. The predominant breed was Holstein. Herd characteristics are presented in Table 1.

The aim of the data collection was to evaluate milk bacteriology and LDH concentration in milk before and after antibiotic treatment in cows infected with *Staph. aureus*. Cows that were likely to have chronic IMI were selected for screening of milk samples (209 cows). Selected cows had a SCC >100,000 cells/mL in the latest monthly test recording or a SCC >150,000 cells/mL in at least 1 of 2 consecutive milk recordings in the present lactation. Cows were not eligible for the screening if they (1) had received antimicrobial treatment in the 11 d before enrollment; (2) were expected to be culled, sold, or dried off during the trial; (3) had fewer than 3 lactating quarters; or (4) was over 250 DIM. The 3 latter groups were not expected to stay in the herd for the 56 d required for the trial. After the above-described selection of cows, data set 1 included 48 cows.

Quarter milk samples were collected on 2 or 3 occasions from each cow for bacteriological culture before initiation of treatment on d 0. The time between sampling was at least 1 week, and the time between last sampling and therapy was 14.2±6.75 d. If only 1 of the first 2 samplings was *Staph. aureus* positive, an additional sample was collected. Cows were considered infected when at least 1 of the quarter samples was *Staph. aureus* positive. All samples were collected either by veterinary students or veterinarians employed by the Faculty of Agricultural Science, Aarhus University (Tjele, Denmark).

Aseptic samples for bacteriology were collected after disinfection of the teat ends with cotton swabs soaked in 70% alcohol and the first few mL of milk were discarded; samples were collected in sterile plastic tubes. Latex gloves were worn during sample collection. Samples were stored in a freezer at −18°C until they were thawed for culturing.

Laboratory examinations were performed as follows: an inoculum of 0.02 mL milk was cultured on blood agar plates supplemented with esculin, and on ChromID *Staph. aureus* medium (bioMérieux, Marcy l'Etoile, France). Plates were incubated at 37°C for 18 to 24 h. Identification was based on colony morphology and biochemical tests. Strains producing double hemolysis were presumed to be *Staph. aureus*, and the

Table 1. Description of the 5 participating herds

Herd no.	Herd size	No. of cows		Cow breed	Milk yield (kg of ECM/305 d)	Milking system[1]	Bulk tank SCC ($\times 10^3$ cells/mL)
		Data set 1	Data set 2				
1a	240	5	—	Holstein	9,600	Voluntary milking system	167
1b	240	—	17	Holstein	10,700	Voluntary milking system	136
2	120	31	—	Holstein	9,100	Milking parlor	216
3	140	3	—	Holstein	9,300	Milking parlor	199
4	100	—	18	Holstein	10,300	Milking parlor	119
5	180	9	—	8 Holstein/ 1 Jersey	8,900	Voluntary milking system	137

[1]Both the voluntary milking system and the milk parlor were from Herd Navigator (DeLaval, Tumba, Sweden).