Decision support for mastitis on farms with an automatic milking system

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Decision support for mastitis on farms with an automatic milking system

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(met een samenvatting in het Nederlands)

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CONTENTS

Chapter 1

General introduction

A TREND TOWARDS PRECISION DAIRY FARMING

Dairy farming is changing rapidly. Across the world, the trend toward fewer, larger dairy farms continues. These larger farms are more difficult to control and manage. Moreover, there is an increasing need to replace labor. For automation of labor, but also to improve control and detection of diseases, several new technologies were developed. Automatic individual feeding of dairy cows (Ipema and Rossing, 1987) and detection of diseases and estrus (e.g., Firk et al., 2002; Viguier et al., 2009; Brandt et al., 2010) are examples of technologies where individual cows are monitored continuously. These cow monitoring technologies result in a trend towards individual cow decisions instead of group management (Schulze et al., 2007). Taking individual cow decisions is one of the main characteristics of precision dairy farming. This is a relatively new concept of dairy farming, and several definitions exist. Bewley (2010) defined it as the use of new technologies to measure physiological, behavioral, and production indicators on individual cows to improve management strategies and farm performance. It is believed that precision dairy farming technologies allow dairy farmers to make more timely and informed decisions, resulting is a better productivity and profitability (Van Asseldonk et al., 1999).

Already in the 1990's, it was believed that precision dairy farming technologies were useful. However, adoption of these technologies on dairy farms has been relatively slow (Bewley, 2010). Currently, sensors are showing a better performance, and decision support systems can be attached to the sensor outcomes. Moreover, current dairy farmers are more used to working with management systems. These developments have led to an increase in precision dairy farming technologies nowadays. For example, a pedometer, measuring the number of steps of the dairy cow, is a well-known tool for improving estrus detection and prediction of ovulation (Firk et al., 2002; Roelofs et al., 2005). Recently, a new sensor called SensOor® (Agis Automatisering B.V., Harmelen, the Netherlands), attached in the cow's ear, was developed to measure body temperature and movement continuously. It is expected that by using this new sensor it will be possible to improve estrus detection, time of insemination and detection of diseased cows (Mollenhorst et al., 2010). But most precision dairy farming technologies include daily milk yield recording and milk component monitoring. The Herd Navigator® (Delaval, Tumba, Sweden) is able to take milk samples automatically from specific cows and analyses parameters that help farmers to monitor reproduction, mastitis, and energy and protein balance of the cows (Mazeris, 2010). The automatic milking system (AMS) is also an example of a precision dairy farming technology, and is the topic of this thesis.

AUTOMATIC MILKING OF DAIRY COWS

The first AMS was introduced on commercial dairy farms in the Netherlands in 1992. Since that time, the number of farms with an AMS increased considerably (Svennersten-Sjaunja and Pettersson, 2008). At the end of 2009, worldwide over 8,000 commercial farms used one or more AMS. In the Netherlands, almost 2,000 farms currently have an AMS (De Koning, 2010). Reasons for investing in an AMS can be divided into social and economic reasons. The most important social reasons for investing in an AMS are that they allow more free time, provide more flexibility, and require less heavy work (Mathijs, 2004). The economic benefits of an AMS are mainly the savings in labor and increases in milk production per cow. But also high fixed costs are reported, especially for gas, water and electricity (Bijl et al., 2007).

An essential difference between milking dairy cows with an AMS and milking with a conventional milking system is the absence of a milker during the milking process when milking automatically. With an AMS, milk information from individual cows is measured continuously by using sensors. Daily cow management is different from farms milking with a conventional milking system. Dairy farmers milking conventionally observe their cows two times a day very closely, this results in enough opportunities for checking cows for udder health problems. Dairy farmers using AMS have to rely on so-called mastitis alert lists generated by the AMS for information on the udder health status of their cows. These mastitis alert lists are based on sensor measurements (Hogeveen and Ouweltjes, 2003). The sensors continuously measure indicators for clinical mastitis (CM) during the milking process (e.g., Brandt et al., 2010; Viguier et al., 2009). Cows from the alert lists need to be checked visually for CM. Checking only alerted cows instead of checking all cows in the herd at a routinely basis results in “management by exception”. This means that only those cows are checked that might have problems, while the remaining cows in the herd are left unchecked. To make management by exception possible, detection models are needed that alert as many cows with CM as possible (requires a high sensitivity (SE)) and the number of falsely attended cows needs to be reduced to a minimum (requires a high specificity (SP)).

CM DETECTION WITH AN AMS

Recently, Dohmen et al. (2010) concluded that udder health is at risk on farms with an AMS. One of the reasons can be that mastitis detection models of the AMS are unable to detect CM sufficiently. Based on the mastitis detection model, a mastitis alert list is provided to the farmer reporting all cows suspected of having CM. The mastitis alert lists are based on deviated sensor measurements, and the list provides information on milk

yield, time between milkings, electrical conductivity and color of the milk for each mastitis alert (Hogeveen and Ouweltjes, 2003). So far, there is no national or international agreement on the height of the sensitivity a CM detection model should have when implemented in practice. An SE of 70% and an SP of >99% are proposed by Mein and Rasmussen (2008), and an SE of >80% in combination with a specificity of >99% is described by Annex C of the ISO/FDIS 20966 (Automatic milking installations – requirements and testing) of the International Standard Organization (ISO/FDIS 20966, 2007). If a model has to perform similarly as human observation of CM, as stated by the EU legislation regarding milk production (Regulation (EC) No. 853/2004), an SE of 80% should be the target level (Hillerton, 2000). Recently, an SE of 36.8% combined with an SP of 97.9% is reported as test characteristic for detection of CM with a current AMS (Mollenhorst and Hogeveen, 2008). As a consequence of the suboptimal detection performance, a good management by exception is impossible, especially because of the high number of falsely attended cows for CM. These high number of false-positive mastitis alerts are a general complaint of dairy farmers working with an AMS. Several detection models were developed with the aim to reduce the number of false-positive mastitis alerts generated by the AMS (e.g., De Mol and Ouweltjes, 2001; Cavero et al., 2008). The test characteristics of these models, however, did not reach the desired SE and SP levels.

CURRENT SITUATION ON CM DECISIONS ON FARMS WITH AN AMS

With current CM detection models, all mastitis alerts presented have an equal probability of being a true-positive alert, there is no indication which alerts have the highest priority for visual checking. In essence, all mastitis alerts given by an AMS have to be checked visually. Because of lack of time, and the annoyance about the large number of visual checks that turn out to be unnecessary, however, in practice farmers do not check all mastitis alerts (Claycomb et al., 2009). In figure 1 it is presented that currently only the mastitis alert list is provided by the AMS to decide which cows should be checked visually for CM. Results of a large recent survey on Dutch dairy farms using AMS showed, however, that the vast majority of farmers do not use any explicit rules for deciding which cows to check visually for CM (Neijenhuis et al., 2009). These farmers thus take their inspection decisions based on intuition.

Besides the decision on which mastitis alerts to check visually for CM, also for detected CM cases a decision must be made (Figure 1). Deciding on the best treatment regime for detected CM cases on farms with an AMS is not different from farms with a conventional milking system. Although different antimicrobial treatments are available for CM on Dutch dairy farms, differing in antimicrobial compound, route of application, duration,

probability of cure and costs (e.g., Barkema et al., 2006), most CM cases receive a standard 3 day intramammary treatment. Additional information sources are hardly used to differentiate in choice of treatment regime for different cows with CM (Figure 1).

On an increasing number of dairy farms, information on milk production, inseminations, identification and registration is provided automatically via computerized management programs. In addition, on farms with an AMS the mastitis alert information is provided via the management program of the AMS. So, information about individual cows is provided to the farmer via different computerized programs. The alert information is provided without showing which cows from the mastitis alert list have the highest priority for visual checking for CM. Also, no decision support is provided on the choice of treatment regime for a detected CM case. Without decision support, the farmer himself has to take into account all alert and non-AMS cow information, provided via different computerized programs, when making CM decisions. It is known, however, that humans can only use a maximum number of information sources to take the optimal decision (Miller, 1956). As a consequence of the overload of information and the lack of structural support, non-informed CM decisions are made.

IMPROVING CM DECISIONS ON FARMS WITH AN AMS

CM decisions on farms with an AMS can be improved by providing decision support. First, decisions support is needed on which mastitis alerts have the highest priority for visual checking for CM. Subsequently, for detected CM cases decision support is needed to take informed decisions on the choice of treatment regime. It is expected that improved CM decisions can be made by combining all automatically available information sources (Figure 1).

Supporting CM detection decisions

By presenting a probability that a mastitis alert is a true-positive alert for CM, a farmer can determine which alerts have the highest priority for visual checking. The probability that a mastitis alert is true-positive can be based on the alert information only. It is, however, known that also non-AMS cow factors influence the probability of having CM. For instance, cows in early lactation have a higher probability of having CM than cows in late lactation (Barkema et al., 1998). It is expected that using a combination of all available information sources (alert information and non-AMS cow information) is the best basis for deciding which alerted cows have the highest priority for visual checking for CM (Figure 1). Moreover, it is expected that by only presenting mastitis alerts above a certain probability, based on the combination of alert and non-AMS cow information, the number of false-positive mastitis alerts can be reduced.

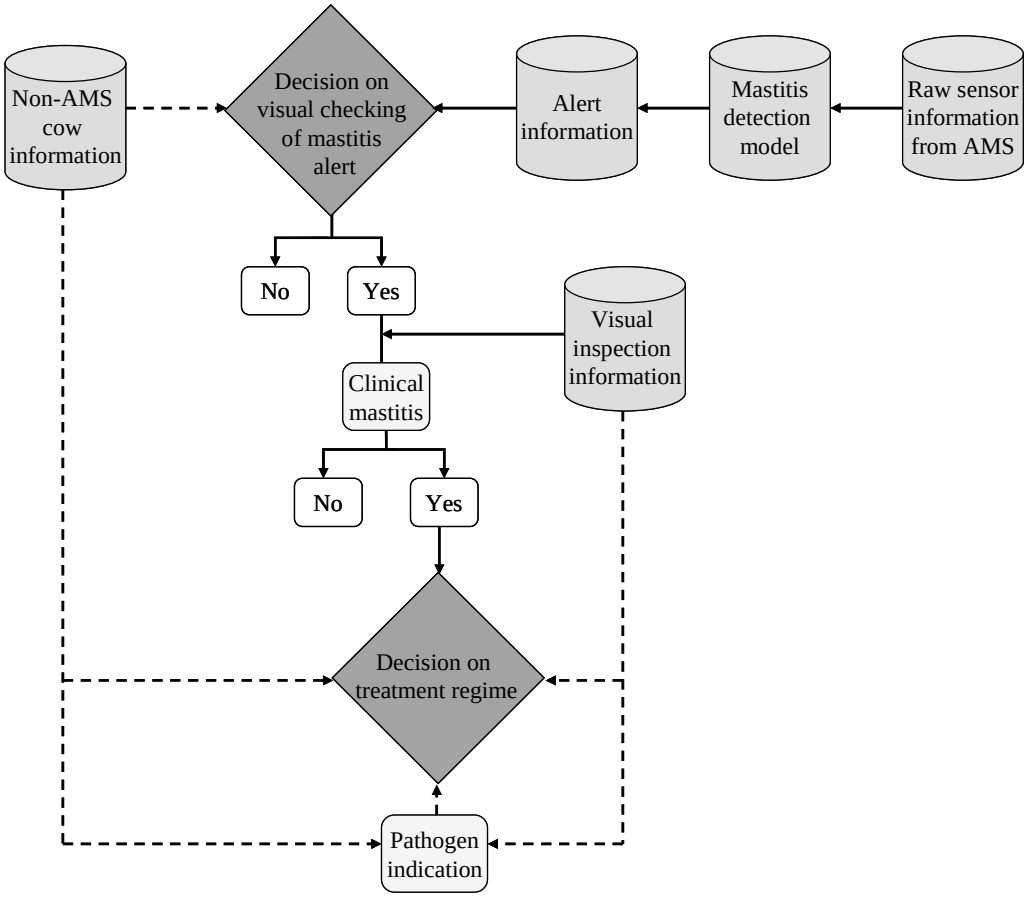

Figure 1. Schematic overview of clinical mastitis decisions to be made on farms with an automatic milking system (AMS) in the current situation (solid line) and the suggested improved situation (dashed line).

By presenting a probability, risk-seeking farmers have the opportunity to check alerts with high probabilities only, and thus taking the risk of missing or detecting later CM cases. Risk-averse farmers have the opportunity to check also alerts with low probabilities, and thus decreasing the risk of missing CM cases at the cost of increasing the number of unnecessary checks. By presenting a probability, it is also possible to adapt the checking behavior of the farmer to the current farm circumstances. For instance, a farmer can decide to check alerts with relatively low probabilities as well if the bulk milk somatic cell count is trending upwards or when clots are found on the filter sock.

Including alert and non-AMS cow information, and subsequently presenting a probability of being a true-positive alert as an outcome can be performed with Bayesian networks. These networks are powerful tools for the representation of probability distributions and for probabilistic reasoning (Jensen, 2001). The advantages of Bayesian networks are that they are flexible in terms of handling missing values, and they allow the computation of prior or posterior probabilities of modelled variables. Bayesian networks have been studied extensively and are being widely applied in human medicine (e.g., Chapman et al., 2005). Applications are not yet common in veterinary science, but they are gaining popularity. For instance, Jensen et al. (2009) estimated the probabilities for different possible causes of leg disorders in finisher pigs.

Supporting CM treatment decisions

The causal pathogen highly influences the success of treatment. Therefore, knowing the causal pathogen of CM, and subsequently using appropriate treatment, will increase the probability of cure (Barkema et al., 2006; McDougall et al., 2007). Information from the visual inspection such as the appearance of the milk and the demeanor of the cow can be used to aid indicating the causal pathogen (Jones and Ward, 1990; Milne et al., 2003). Also cow-specific factors such as parity, stage of lactation, somatic cell count history and CM history can be used to aid indicating the causal pathogen (Zadoks et al., 2001; De Haas et al., 2004).

To aid indicating the causal pathogen, a probability distribution on the causal pathogen based on non-AMS cow information and information from the visual inspection of a cow with CM can be provided. The probability distributions can be provided by using Bayesian networks. By presenting a probability distribution on the causal pathogen of a CM case, a treatment decision will become easier. For instance, for a CM case with a high probability for one particular pathogen, a more specific treatment can thus be decided upon. While for CM cases with almost equal probabilities for different causal pathogens, a broad spectrum use of antibiotics is more appropriate.

By having an indication about the causal pathogen of a CM case, the next step is to provide decision support on the choice of treatment regime. Besides the causal pathogen, also several cow factors (parity, stage of lactation, somatic cell count history and CM history) influence the probability of cure (Sol et al., 2000; Barkema et al., 2006; Bradley and Green, 2009). By using the probability distribution on the causal pathogen and the information on the non-AMS cow factors, the expected probability of cure of different treatment regimes can be presented to the dairy farmer. Based on that information a farmer can decide upon the treatment regime. Because dairy farming is an economic enterprise, the real measure of cure should be the net-benefit of treatment (Barkema et al., 2006). It is expected that for different cows, different treatment regimes have the lowest total costs. In

addition, therefore also the expected net-benefits of different treatment regimes can be used to decide upon the optimal treatment regime for CM.

Presenting the expected probabilities of cure and the expected net-benefit of different treatment regimes with their associated variation, based on information from visual inspection and information from non-AMS cow factors, can be performed by stochastic simulation (Dijkhuizen et al., 1991). By simulating the consequences of different treatment regimes for different cows, it is also possible to determine whether making a differentiation in choice of CM treatment regimes is economically beneficial.

OBJECTIVES

The objectives of this thesis are:

- To improve CM detection with an AMS by combining sensor and non-AMS cow information.
- To improve CM treatment decisions.

To reach these objectives, several studies were conducted:

- Determination of which combination of non-AMS cow factors influence the probability of having CM (Chapter 2).
- Exploration and illustration of combining a probability of having CM (based on non-AMS cow information) and the test characteristics of the CM detection system of the AMS (Chapter 3).
- Validation of using alert and non-AMS cow information to discriminate between true-positive and false-positive mastitis alerts from an AMS (Chapter 4a).
- Validation of a CM detection model using sensor data and non-AMS cow information (Chapter 4b).
- Development of a probability distribution on the causal pathogen of CM, based on non-AMS cow information and information from visual inspection (Chapter 5).
- Determination whether making a differentiation in choice of CM treatment regimes is economically beneficial (Chapter 6).

Finally, in Chapter 7 critical points from the earlier studies as well as concerns that have not been mentioned throughout the earlier chapters are brought up and discussed. That chapter finishes with conclusions based on the work presented in this thesis.

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Chapter 2

The influence of cow factors on the incidence of clinical mastitis in dairy cows

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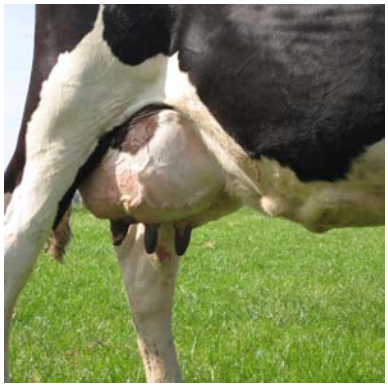

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ABSTRACT

Many cow-specific risk factors for clinical mastitis (CM) are known. Other studies analysed these risk factors separately or only analysed a limited number of risk factors simultaneously. The goal of this study was to determine the influence of cow factors on the incidence rate of CM (IRCM) with all cow factors in one multivariate model. Also, using a similar approach, the probability whether a CM case is caused by Gram-positive or Gram-negative pathogens was calculated. Data were used from 274 Dutch dairy herds that recorded CM over an 18-month period. The final dataset contained information on 28,137 lactations of 22,860 cows of different parities. In total 5,363 CM cases were recorded, while only 2,525 CM cases could be classified as Gram-positive or Gram-negative. The cow factors parity, lactation stage, season of the year, information on SCC from monthly test-day records and CM history were included in the logistic regression analysis. Separate analyses were performed for heifers and multiparous cows in both, the first month of lactation, and from the second month of lactation onwards. For investigating whether CM was caused by Gram-positive or Gram-negative pathogens also quarter position was included in the logistic regression analysis. The IRCM differed considerably among cows, ranging between 0.0002 and 0.0074 per cow-day at risk for specific cows depending on cow factors. In particular, previous CM cases, SCC in the previous month and mean SCC in the previous lactation increased the IRCM in the current month of lactation. Results indicate that it is difficult to distinguish between Gram-positive and Gram-negative CM cases based on cow factors alone.

Keywords: clinical mastitis, incidence rate, dairy cow

INTRODUCTION

Mastitis is one of the most frequent and costly diseases (e.g., Halasa et al., 2007). Many factors influence the incidence of clinical mastitis. These factors may be, amongst others, herd (e.g., Schukken et al., 1991; Barkema et al., 1999; Nyman et al., 2007) and cow factors (e.g., Barkema et al., 1998; Suriyasathaporn et al., 2000; Olde Riekerink et al., 2007). In an individual herd, cow factors are responsible for the difference among cows in having clinical mastitis (CM). It may be worthwhile to know which specific cows have the highest risk to obtain CM. With this information, the farmer may give these cows more attention. This would be particularly useful on farms with an automatic milking system (AMS) because no human being is present during the milking process who would check the milk visually for abnormalities (Hogeveen and Ouweltjes, 2003). When milking with an AMS, cows to be checked for CM are currently selected based on results of sensor measurements only.

The association between cow factors and CM has been studied frequently. SCC is widely considered to be one of the most important risk factors for CM, with both a high SCC (e.g., Beaudeau et al., 1998; Suriyasathaporn et al., 2000; Green et al., 2004), and a very low SCC associated with an increased risk on subsequent CM (Suriyasathaporn et al., 2000; Peeler et al., 2003; Green et al., 2004). Existing research has established a number of facts about CM. For example, CM most often occurs early in lactation (e.g., Miltenburg et al., 1996; Barkema et al., 1998; Svensson et al., 2006), heifers have the lowest incidence rate of CM (IRCM), except in the first week of lactation (Barkema et al., 1998), and cows that have had CM once have a higher risk for CM later during lactation (Houben et al., 1993; Lam et al., 1997; Zadoks et al., 2001).

Most studies investigating associations between possible cow factors and CM, studied the effect of one or a limited number of cow factors, e.g. the effect of parity, lactation stage and SCC on subsequent CM (e.g., Beaudeau et al., 1998; Suriyasathaporn et al., 2000). In one study, the association between IRCM and several cow factors, including also CM history, was assessed (Houben et al., 1993). No study, however, has analysed IRCM based on all known cow factors together in one multivariate model.

Knowing the pathogen involved in a CM case would be very useful. Particularly, a distinction between Gram-positive and Gram-negative pathogens would be informative, because antimicrobial treatment is necessary when Gram-positive pathogens are involved, while for Gram-negative CM cases supportive treatment is more appropriate (Morin et al., 1998; Pyörälä and Pyörälä, 1998). Inclusion of cow factors may assist in the detection of specific pathogens, but the association between cow factors and the risk for pathogen-specific CM has not been studied frequently (Zadoks et al., 2001; De Haas et al., 2004).

De Haas et al. (2002) recommended that other sources such as parity and lactation stage, besides SCC test-day records, would be used for a more accurate prediction of the pathogen that is involved.

Novel in the current study is that several cow-specific risk factors for CM, that are readily available on most dairy farms, are analysed together using a unique dataset comprising 22,860 cows. The first objective was to investigate if there are differences in the IRCM among cows based on combined information of cow factors. The second objective was to investigate, based on combined cow factors, whether a CM case was caused by Gram-positive or Gram-negative pathogens.

MATERIAL AND METHODS

Herds and data collection

The data used in the present study were described in detail elsewhere (Barkema et al., 1998). Records on CM were collected from 300 dairy herds entering the study between December 1992 and June 1994. Each herd participated in the study for approximately 1.5 years. Eight herds did not complete the study because farming activities ceased. All herds had an annual milk production quota between 300,000 and 900,000 kg, and had cows of the Holstein-Friesian or Dutch Friesian breeds. Lactating cows were housed in a free-stall barn during winter and milked in a double-herringbone or two-sided tandem. During the study, farmers were asked and instructed to collect milk samples from every quarter that had visible signs of CM. Farms were visited monthly to collect milk samples, to motivate for a correct milk sampling procedure and to provide feedback to the farmer. The samples were taken before treatment, stored in a freezer at the farm (at approximately -20°C) and collected every 6 to 8 weeks for bacteriological culture. Bacteriological culturing of milk samples was performed according to the standards of the National Mastitis Council (Harmon et al., 1990). In short, of all milk samples, 0.01 ml was cultured. In each of the cultures, the number of colony-forming units of the bacterial species was counted. The contagious pathogens (*Staphylococcus aureus* and *Streptococcus agalactiae*) were considered to cause IMI if one colony (100 cfu/ml) was isolated. Isolation of ≥ 200 cfu/ml of environmental mastitis pathogens (*Escherichia coli*, streptococci other than *Streptococcus agalactiae*, *Klebsiella* spp., and *Pseudomonas* spp.) or ≥ 1000 cfu/ml of *Corynebacterium bovis* or coagulase-negative staphylococci were considered significant. Collected data contained information on cow identification, date of occurrence, infected quarter, and the outcome of the bacteriological culturing of the milk samples. At the end of the data collection period, farmers were asked to estimate how many cases of CM were not sampled and not recorded. If this number exceeded 10 and was >25% compared with the number of cases sampled, the herd was excluded from analysis of the CM data. In total,

eighteen herds were excluded from the analyses for this reason (Barkema et al., 1998). The Dutch national milk recording system (Nederlands Rundvee Syndicaat, Arnhem, The Netherlands) provided information from the three or four weekly milk production system, including cow identification, date of milk recording, date of calving, date of drying off, test-day milk yields (kg of milk, fat and protein) and SCC (cells/mL) for all cows.

Data preparation

Originally, the dataset consisted of 120,398 lactations from 39,764 cows with a total of 8,571 CM cases. Only lactations that had been recorded from calving onward were included in the dataset, to ensure that no previous cases of CM had occurred within the lactation. For this reason in total 88,220 lactations were excluded. Lactations were included till dry-off or culling. Subsequently, lactations with no milk production information (in total 3,450 lactations) or a calving interval $\leq$ 320 days or $\geq$ 600 days (in total 591 lactations) were excluded from the dataset. Therefore the final dataset consisted of 28,137 lactations from 22,860 cows with 201,708 test-day records and 5,363 CM cases. All cases of CM during dry-off were excluded. Intervals between pathogen specific cases of CM in the same quarter had to be $\geq$14 days for a case to be included in the final dataset.

For this study, all CM cases were classified according to their Gram-status and divided in three groups, 1) Gram-positive CM: *Streptococcus dysgalactiae*, *Streptococcus agalactiae*, *Streptococcus uberis*, other streptococci, *Staphylococcus aureus*, coagulase-negative staphylococci, *Corynebacterium bovis and Arcanobacterium pyogenes*, 2) Gram-negative CM: *Escherichia coli*, *Pseudomonas* and *Klebsiella*, and 3) missing: no growth, contaminants, mould or fungi's, yeast and no samples taken. Mixed cultures containing two Gram-positive pathogens were classified as Gram-positive and those containing two Gram-negative pathogens as Gram-negative. Mixed cultures containing a Gram-positive and a Gram-negative pathogen were classified as missing. In total, 2,491 Gram-positive and 1,007 Gram-negative CM cases were identified. For 1,865 CM cases, the Gram status was missing.

Using information from literature and expertise of the authors, cow-specific risk factors for IRCM were defined (Table 1). Only cow-specific risk factors, for which information is usually available on a farm, were included in this study. Of these, parity (Barkema et al., 1998) was known for each cow in the dataset and lactation (Barkema et al., 1998; Green et al., 2004; Svensson et al., 2006) was divided into 30-day intervals. For every month in lactation the binary trait having CM or not (1/0) was determined. From previous studies it was known that the IRCM is different in the first part of lactation (Houben et al., 1993; Barkema et al., 1998), therefore in this study the first 30 days of lactation (MONTH1) were subdivided into 6 equal periods of each 5 days. Season of the year (Olde Riekerink et al., 2007) was determined for each month in lactation. The SCC from monthly test day

records (e.g., Beaudeau et al., 1998; Suriyasathaporn et al., 2000; Green et al., 2004) was also included. To determine the association between previous SCC and IRCM in the current month of lactation, SCC in the previous month in lactation (SCC1) was determined. Also, SCC of the previous lactation was included (Whist and Østerås, 2006; Green et al., 2007). This variable was defined as the geometric mean SCC from all available test-day records from the previous lactation (SCC2). The natural logarithm of both SCC1 and SCC2 was used for analysis. CM history at the cow level (Houben et al., 1993) was defined with two variables: the accumulated number of CM cases in the previous month in lactation (MAST1) and the accumulated number of CM cases in the month in lactation before the previous month in lactation (MAST2). Because almost no cows with CM information from previous lactation were present in the dataset, no information on CM history from previous lactations was taken into account.

Table 1. Description of study variables with their abbreviation and different levels used for analysis

Variable	Abbreviation	Levels used in analysis
Dependent variables		
Clinical mastitis	CM	0 = no 1 = yes
Gram-status of clinical mastitis case	GRAM	0 = Gram-negative pathogen 1 = Gram-positive pathogen
Independent variables		
Parity		1, 2, 3, ≥4
Month of lactation		1, 2, 3, ..., ≥8
First month of lactation subdivided per 5 days	MONTH1	1, 2, 3, …, 6
Season		January-March April-June July-September October-December
SCC in previous month of the lactation[1]	SCC1	
Geometric mean SCC of all test-days in previous lactation[1]	SCC2	
Accumulated number of CM cases in the previous month of the lactation	MAST1	0, 1, 2
Accumulated number of CM cases in the month of lactation before the previous month of lactation	MAST2	0, 1, 2
Quarter position		1 = right front 2 = left front 3 = right rear 4 = left rear

[1]The natural logarithm was used for these continuous variables.

To prepare for the statistical analyses, a total of six datasets were created (Table 2), four to determine the IRCM (datasets 1 – 4). Datasets 1 and 2 were created for heifers and multiparous cows in the first 30 days of lactation. These datasets contained no information on SCC1, MAST1 and MAST2, and for heifers no information was available on SCC2. In these datasets, having CM was determined per 5 days. Datasets 3 and 4 were created for heifers and multiparous cows from the second month of lactation onwards. In these datasets, having CM was determined per 30 days. Datasets 5 and 6 were created to predict the Gram-status of the CM cases. Dataset 5 included all heifers and dataset 6 all multiparous cows. In these datasets, all records with missing values for SCC1, SCC2 and quarter position were excluded (in total 973 cases). Dataset 5 contained 262 Gram-positive and 102 Gram-negative CM cases. Dataset 6 contained 1,526 Gram-positive and 635 Gram-negative CM cases. In these datasets, information on quarter position was also available.

Table 2. Description of the six created datasets with the total number of lactations and clinical mastitis cases in each dataset

Dataset	Description	# lactations	# CM cases	Variables in dataset[1]
1	All heifers in first 30 days of lactation	8,388	472	CM, MONTH1, season
2	All multiparous cows in first 30 days of lactation	19,749	1,145	CM, parity, MONTH1, season, SCC2
3	All heifers from the second month of lactation onwards	8,121	526	CM, month in lactation, season, SCC1, MAST1, MAST2
4	All multiparous cows from the second month of lactation onwards	19,109	3,220	CM, parity, month in lactation, season, SCC1, SCC2, MAST1, MAST2
5	All CM cases in heifers with known Gram status		364	GRAM, month in lactation, season, SCC1, quarter position
6	All CM cases in multiparous cows with known Gram status		2,161	GRAM, parity, month in lactation, season, SCC1, SCC2, quarter position

[1]MONTH1 = First month of lactation subdivided in 6 equal 5-day periods, SCC1 = SCC in previous month of lactation, SCC2 = Geometric mean SCC of all test-days in the previous lactation, MAST1 = Accumulated number of CM cases in the previous month of the lactation, MAST2 = Accumulated number of CM cases in the month of the lactation before the previous month of the lactation.

Statistical analysis

Clinical mastitis detection in first month of lactation. Statistical analyses were carried out to determine the relation between the independent variables and the IRCM in the first 30

days of lactation for heifers and multiparous cows (dataset 1 and 2), using SAS (PROC GENMOD) version 9.1 (SAS Institute Inc., Cary, NC). Because of the repeated measurements in the data, all datasets were analysed using generalised estimating equations (GEE) (Dohoo et al., 2003) with an exchangeable correlation matrix within herd, a binomial variance and a logit link. All cow factors were analysed using a backward stepwise procedure. For a categorical variable, all dummy variables were entered. Only variables at $P \leq 0.05$ in the likelihood ratio test were retained in the model. Goodness of fit of the model was assessed by judging the residuals. The residuals were plotted against the fitted values and judged for peculiarities (Dohoo et al., 2003).

To determine IRCM in a specific period during MONTH1 for multiparous cows, equation (1) was used:

$$\text{IRCM} = \left(\frac{e^{\beta_0 + \beta_1 * parity\ + \beta_2 * MONTH\ \ 1 + \beta_3 * season\ + \beta_4 * SCC\ \ 2}}{1 + e^{\beta_0 + \beta_1 * parity\ + \beta_2 * MONTH\ \ 1 + \beta_3 * season\ + \beta_4 * SCC\ \ 2}} \right) / 5 \qquad (1)$$

where the outcome is the IRCM per cow-day at risk in a specific period during MONTH1. β_0 is the estimated intercept and the regression coefficients (log odds ratios) were estimated for parity (β_1), MONTH1 (β_2), season (β_3) and SCC2 (β_4). Because the data was ordered per 5 days, the IRCM was divided by 5 to calculate an IRCM per cow-day at risk. For heifers, the same model was used, except that the terms parity and SCC2 were omitted because they were not applicable.

Clinical mastitis detection from second month of lactation onwards. The statistical analysis to determine the relation between the independent variables and IRCM from the second month of lactation onwards for heifers and multiparous cows (datasets 3 and 4) was performed identical to the one described above for datasets 1 and 2. To determine IRCM in a specific month in lactation for multiparous cows, equation (2) was used:

$$\text{IRCM} = \left(\frac{e^{\beta_0 + \beta_1 * parity\ + \beta_2 * month\ + \beta_3 * season\ + \beta_4 * MAST\ \ 1 + \beta_5 * MAST\ \ 2 + \beta_6 * SCC\ 1 + \beta_7 * SCC\ \ 2}}{1 + e^{\beta_0 + \beta_1 * parity\ + \beta_2 * month\ + \beta_3 * season\ + \beta_4 * MAST\ \ 1 + \beta_5 * MAST\ \ 2 + \beta_6 * SCC\ 1 + \beta_7 * SCC\ \ 2}} \right) /30 \qquad (2)$$

where the outcome is the IRCM per cow-day at risk in a specific month of lactation. β_0 is the estimated intercept and the regression coefficients (log odds ratios) were estimated for parity (β_1), month in lactation (β_2), season (β_3), MAST1 (β_4), MAST2 (β_5), SCC1 (β_6) and

SCC2 (β_7). In these analyses, biologically plausible two-way interactions were also tested (parity by SCC1, season by SCC1, parity by month in lactation, month in lactation by SCC1). Because the data was ordered per 30 days, the IRCM was divided by 30 to calculate an IRCM per cow-day at risk. For heifers, the same model was used, except that the terms for parity and SCC2 were omitted, because they were not applicable.

Gram-status of the clinical mastitis cases. The analysis to determine the probability whether CM cases were caused by Gram-positive or Gram-negative pathogens was performed in a similar way, using GEE (Dohoo et al., 2003) with a binomial variance and a logit link. In these analyses, biologically plausible interactions were also tested (parity by SCC1, season by SCC1, parity by month in lactation, month in lactation by SCC1). Analyses were performed identically to the ones described above, using datasets 5 and 6.

To determine the probability that a CM case was caused by Gram-positive pathogens for multiparous cows equation (3) was used:

$$\text{GRAM} = \left(\frac{e^{\beta_0 + \beta_1 * parity + \beta_2 * month + \beta_3 * season + \beta_4 * SCC1 + \beta_5 * SCC2 + \beta_6 * quarter}}{1 + e^{\beta_0 + \beta_1 * parity + \beta_2 * month + \beta_3 * season + \beta_4 * SCC1 + \beta_5 * SCC2 + \beta_6 * quarter}} \right) \quad (3)$$

where the outcome is the probability that a CM case was caused by Gram-positive pathogens. β_0 is the estimated intercept and the regression coefficients (log odds ratios) were estimated for parity (β_1), month in lactation (β_2), season (β_3), SCC1 (β_4), SCC2 (β_5) and quarter position (β_6). For heifers, the same model was used, except that the terms for parity and SCC2 were omitted, because they were not applicable.

RESULTS

Clinical mastitis detection in first month of lactation

Results of the multivariate analysis of cow-specific risk factors in the first month of lactation for heifers and multiparous cows are given in Table 3. All cow-specific risk factors in the first month of lactation, described in Table 2, significantly contributed to the fit of the model. Heifers had a much higher IRCM in the first 5 days of lactation compared with other intervals (OR = 12.2). The values for OR after day 5 of lactation decreased to 1 rapidly. Multiparous cows also had a high OR during the first 5 days of lactation (OR = 4.0), but not as high as for heifers. For both heifers and multiparous cows, season of the year was significantly associated with IRCM. Multiparous cows with a higher SCC2 had an increased IRCM in the first month of lactation (OR = 1.25).

Clinical mastitis detection from second month of lactation onwards

Results of the multivariate analysis for the second month of lactation onwards for heifers are given in Table 4 and for multiparous cows in Table 5. All the cow-specific risk factors

from the second month of lactation onwards described in Table 2 significantly contributed to the fit of the model. Two interaction terms (parity by SCC1 and season by SCC1) were also associated with multiparous cow IRCM. Heifers and multiparous cows in the first months of lactation (but after the first month), during winter periods, with a CM history and with higher SCC1 had the highest IRCM. IRCM was highest for cows in higher parities and that had a higher SCC2 (Tables 4 and 5).

In Figure 1, the IRCM during the first 8 months of lactation is presented for heifers and multiparous cows. Additionally, the IRCM in the first 30 days of lactation is presented separately. The IRCM in the first 10 days of lactation was higher for heifers than for multiparous cows. From day 10 of lactation onwards, the IRCM for multiparous cows was higher than for heifers. For heifers and multiparous cows, the IRCM from the second month of lactation onwards varied between 0.0002 and 0.0012 per cow-day at risk (Figure 1).

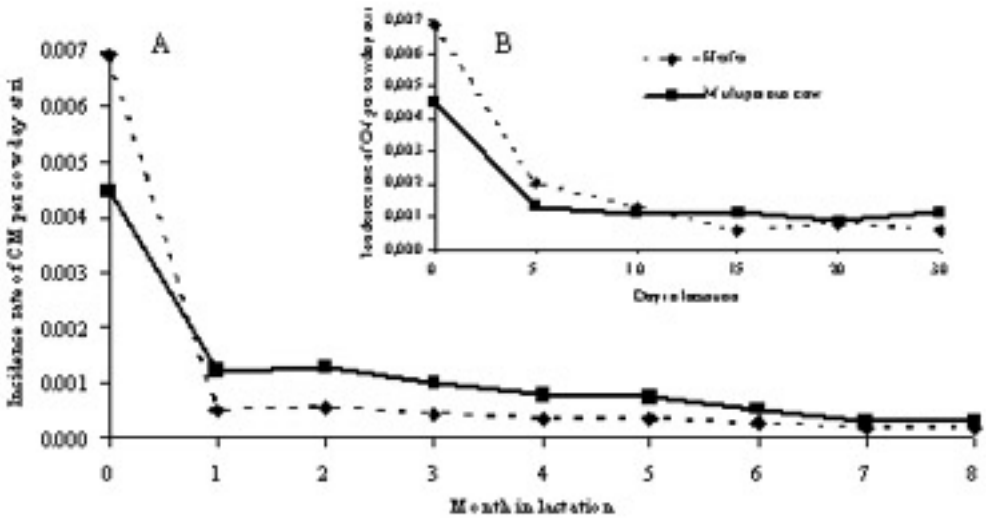

Figure 1A. Incidence rate of clinical mastitis (CM) per cow-day at risk for a specific heifer (during last three months of the year, no CM history and SCC of 100,000 cells/mL in the previous month) and a specific multiparous cow (in third parity, during last three months of the year, with no CM history, SCC of 100,000 cells/mL in the previous month and geometric mean SCC of 100,000 cells/mL in the previous lactation) in different months of lactation. Also, the incidence rate for CM, for the same cows, in the first month of lactation is presented (Figure 1B).

Table 3. Results of the analysis of cow-specific risk factors for the incidence of clinical mastitis in first month of lactation for heifers (dataset 1) and multiparous cows (dataset 2). Estimated coefficients (ß), standard error (SE) for the coefficient, odds ratio (OR), 95% confidence interval (CI) for OR, and significance level are given for each cow-specific risk factor

	Heifer					Multiparous cow				
Variable	ß	SE	OR[1]	95% CI for OR	P-value	ß	SE	OR[1]	95% CI for OR	P-value
Intercept	-6.141	0.250				-6.380	0.221			
Parity					NA[4]					0.0417
2						Ref.	–	1.00		
3						-0.046	0.090	0.96	0.80 –1.14	
≥4						0.146	0.073	1.16	1.00 – 1.34	
MONTH1					<.0001					<.0001
0-5 days	2.504	0.244	12.23	7.57 – 19.74		1.385	0.113	3.99	3.20 – 4.98	
6-10 days	1.244	0.264	3.47	2.07 – 5.82		0.162	0.142	1.18	0.89 – 1.55	
11-15 days	0.791	0.278	2.21	1.28 – 3.80		-0.034	0.118	0.97	0.77 – 1.22	
16-20 days	-0.003	0.325	0.99	0.53 – 1.89		-0.017	0.126	0.98	0.77 – 1.26	
21-25 days	0.337	0.295	1.40	0.79 – 2.50		-0.278	0.138	0.76	0.58 – 0.99	
26-30 days	Ref.[2]	–	1.00			Ref.	–	1.00		
Season					0.0026					0.0030
January-March	Ref.	–	1.00			Ref.	–	1.00		
April-June	-0.318	0.175	0.73	0.52 – 1.03		0.011	0.103	1.01	0.83 – 1.24	
July-September	0.143	0.171	1.15	0.83 – 1.61		0.277	0.098	1.32	1.09 – 1.60	
October-December	0.304	0.140	1.36	1.03 – 1.78		0.242	0.093	1.27	1.06 – 1.53	
SCC2[3]					NA[4]	0.222	0.039	1.25 [5]	1.16 – 1.35	<.0001

[1]Odds ratio for having clinical mastitis in a specific period in the first month of lactation versus not having clinical mastitis. [2]Ref. = Reference category. [3]SCC2 = Geometric mean SCC of all available test-day records of the previous lactation. [4]NA = not applicable. [5]For an increase in 1 unit of natural logarithm of SCC.

Table 4. Results of analysis of cow-specific risk factors for the incidence of clinical mastitis from second month of lactation onwards for heifers (dataset 3). Estimated coefficients (ß), standard error (SE) for the coefficient, odds ratio (OR), 95% confidence interval (CI) for OR, and significance level are given for each cow-specific risk factor

Variable	ß	SE	OR[1]	95% CI for OR	P-value
Intercept	-7.167	0.267			
Month of lactation					<.0001
2	1.008	0.161	2.74	2.00 – 3.76	
3	1.160	0.159	3.19	2.34 – 4.36	
4	0.919	0.169	2.51	1.80 – 3.49	
5	0.632	0.187	1.88	1.30 – 2.72	
6	0.672	0.191	1.96	1.35 – 2.84	
7	0.397	0.205	1.49	1.00 – 2.22	
≥8	Ref.[5]	–	1.00		
Season					0.0210
January-March	Ref.	–	1.00		
April-June	-0.313	0.126	0.73	0.57 – 0.94	
July-September	-0.211	0.135	0.81	0.62 – 1.05	
October-December	0.053	0.134	1.05	0.81 – 1.37	
MAST1[2]					0.0081
0	Ref.	–	1.00		
1	0.910	0.262	2.49	1.49 – 4.16	
≥2	1.237	0.479	3.45	1.35 – 8.81	
MAST2[3]					0.0020
0	Ref.	–	1.00		
1	0.712	0.182	2.04	1.43 – 2.91	
≥2	1.056	0.289	2.88	1.63 – 5.07	
SCC1[4]	0.412	0.049	1.51[6]	1.37 – 1.66	<.0001

[1]Odds ratio for having clinical mastitis in a specific month of lactation versus not having clinical mastitis. [2]MAST1 = Accumulated number of CM cases in the previous month of the lactation. [3]MAST2 = Accumulated number of CM cases in the month of lactation before the previous month of the lactation. [4]SCC1 = SCC in the previous month of the lactation. [5]Ref. = Reference category. [6]For an increase in 1 unit of natural logarithm of SCC.

Table 5. Results of analysis of cow-specific risk factors for the incidence of clinical mastitis from the second month of lactation onwards for multiparous cows (dataset 4). Estimated coefficients (ß), standard error (SE) for the coefficient, odds ratio (OR), 95% confidence interval (CI) for OR, and significance level are given for each cow-specific risk factor

Variable	ß	SE	OR[1]	95% CI for OR	P-value
Intercept	-6.864	0.272			
Parity					
2	Ref.[6]	–	1.00		0.0014
3	0.476	0.231	1.61	1.02 – 2.53	
≥4	0.766	0.209	2.15	1.43 – 3.24	
Month of lactation					<.0001
2	1.405	0.071	4.07	3.54 – 4.68	
3	1.449	0.071	4.26	3.71 – 4.90	
4	1.209	0.075	3.35	2.90 – 3.88	
5	0.960	0.082	2.61	2.22 – 3.07	
6	0.889	0.080	2.43	2.08 – 2.85	
7	0.540	0.090	1.72	1.44 – 2.05	
≥8	Ref.	–	1.00		
Season					0.0007
January-March	Ref.	–	1.00		
April-June	-0.700	0.239	0.50	0.31 – 0.79	
July-September	-0.737	0.245	0.48	0.30 – 0.77	
October-December	0.030	0.210	1.03	0.68 – 1.55	
MAST1[2]					<.0001
0	Ref.	–	1.00		
1	0.800	0.093	2.23	1.85 -2.67	
≥2	1.138	0.258	3.12	1.88 – 5.17	
MAST2[3]					<.0001
0	Ref.	–	1.00		
1	0.366	0.069	1.44	1.26 – 1.65	
≥2	0.600	0.130	1.82	1.41 – 2.35	
SCC1[4]	0.288	0.048	1.33[7]	1.21 – 1.47	<.0001
SCC2[5]	0.142	0.030	1.15[7]	1.09 – 1.22	<.0001
Interaction 1					0.0159
Parity 2 * SCC1	Ref.	–	1.00[7]		
Parity 3 * SCC1	-0.068	0.047	0.93[7]	0.85 – 1.02	
Parity 4 * SCC1	-0.118	0.041	0.89[7]	0.82 – 0.96	
Interaction 2					0.1005
Jan-March * SCC1	Ref.	–	1.00[7]		
April-June * SCC1	0.090	0.047	1.09[7]	1.00 – 1.20	
July-Sept * SCC1	0.082	0.045	1.09[7]	1.00 – 1.19	
Oct-Dec * SCC1	0.002	0.041	1.00[7]	0.93 – 1.09	

[1]Odds ratio for having clinical mastitis in a specific month of lactation versus not having clinical mastitis. [2]MAST1 = Accumulated number of CM cases in the previous month in lactation. [3]MAST2 = Accumulated number of CM cases in the month in lactation before the previous month in lactation. [4]SCC1 = SCC in the previous month of the lactation. [5]SCC2 = Geometric mean SCC from all available test-day records of the previous lactation. [6]Ref. = Reference category. [7]For an increase in 1 unit of natural logarithm of SCC.

The IRCM increased with increasing values for SCC1 and SCC2 (Figure 2). For instance, while a specific multiparous cow with values for SCC1 and SCC2 of 100,000 cells/mL had an IRCM of 0.0013 per cow-day at risk, this becomes 0.0034 per cow-day at risk for a multiparous cow with values of 1,000,000 cells/mL for both SCC1 and SCC2. The IRCM was lower for heifers than for multiparous cows: a specific heifer with a SCC1of 1,000,000 cells/mL had an IRCM of 0.0011 per cow-day at risk (Figure 2).

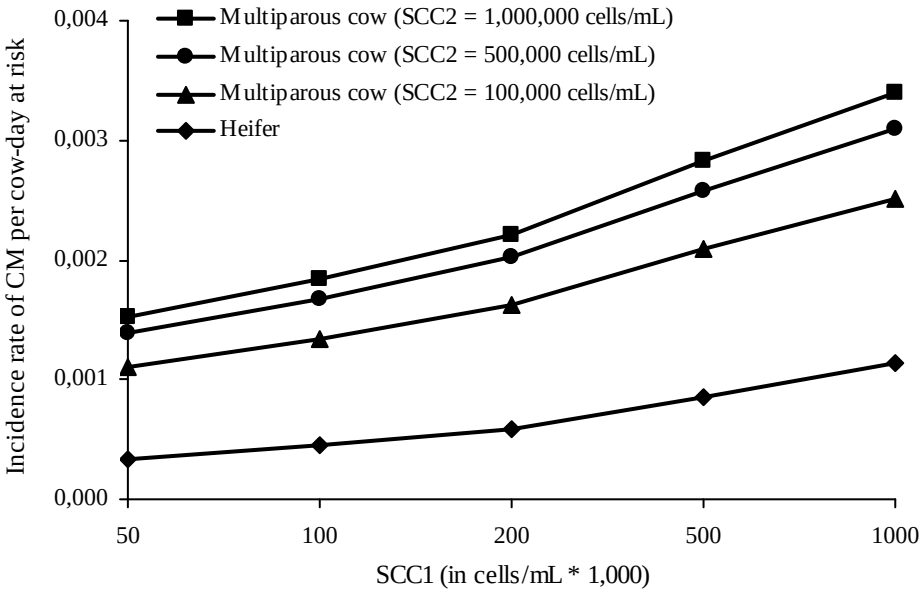

Figure 2. The association between different values for SCC in the previous month (SCC1), geometric mean SCC in the previous lactation (SCC2), and the incidence rate of clinical mastitis (CM) per cow-day at risk for a specific cow (parity 3, fourth month of lactation, during last three months of the year and no CM history). Also, the association between different values of SCC1 and the incidence rate of CM for a specific heifer (fourth month of lactation, during last three months of the year and no CM history) is presented.

The IRCM increased with increasing values for MAST1 and MAST2 (Figure 3). A specific multiparous cow with no CM history (MAST1 = 0, MAST2 = 0) had an IRCM of 0.0016 per cow-day at risk, whereas the same cow with 2 CM cases in the previous month of lactation (MAST1=2 and MAST2=0) would have an IRCM of 0.0045 per cow-day at risk. In a worst case scenario (MAST1 = 2 and MAST2 = 2), the IRCM increased to 0.0074 per cow-day at risk (Figure 3).

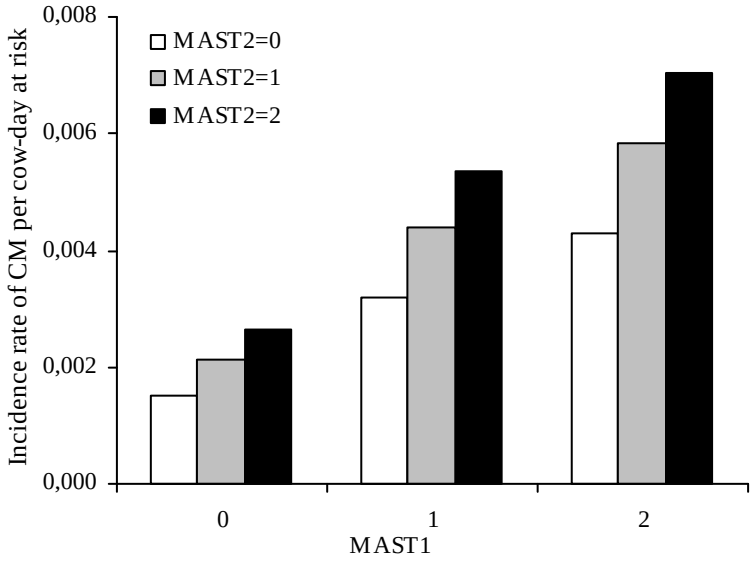

Figure 3. The incidence rate of clinical mastitis (CM) per cow-day at risk for a specific multiparous cow (parity 3, fourth month of lactation, during last three months of the year, SCC of 150,000 cells/mL in the previous month in lactation and a geometric mean SCC of 150,000 cells/mL in the previous lactation) in relation to different values for the accumulated number of CM cases in the previous month in lactation (MAST1) and the accumulated number of CM cases in the month in lactation before the previous month in lactation (MAST2).

Gram-status of the clinical mastitis cases

In total, 2,525 CM cases (in heifers and multiparous cows) with known Gram status were analysed. The causing pathogens were *Streptococcus dysgalactiae* (10%), *Streptococcus agalactiae* (0.6%), *Streptococcus uberis* (7%), other streptococci (8%), *Staphylococcus aureus* (21.9%), coagulase-negative staphylococci (5.4%), *Escherichia coli* (26.7%), *Arcanobacterium pyogenes* (0.7%), *Corynebacterium bovis* (2.7%), *Pseudomonas* (0.6%) and *Klebsiella* (1.5%). The remaining 14.9% of all CM cases were caused by mixed cultures containing two Gram-positive or two Gram-negative pathogens.

Results of the analysis to determine the Gram status of the CM cases for heifers and multiparous cows are given in Table 6. From all cow-specific risk factors for heifers, described in Table 2, only season and SCC1 significantly contributed to the Gram status of the CM cases. For multiparous cows only month in lactation, SCC1 and quarter position significantly contributed to the Gram status of the CM cases (Table 6). During the first six months of the year, the probability that a CM case in a heifer was caused by Gram-positive pathogens was higher than in the last six months of the year. Using the coefficients (Table

6), a CM case in a heifer (with a corresponding value of 100,000 cell/mL for SCC1) had a probability of being caused by Gram-positive pathogens of 0.76 in the first, 0.80 in the second, 0.61 in the third, and 0.54 in the last three months of the year.

Location in the udder also matters. In multiparous cows, CM cases in front quarters had a higher probability of being caused by Gram-positive pathogens (OR = 1.43) than those in rear quarters. Using the coefficients (Table 6), the probability that a CM case in a front quarter in a multiparous cow (in the second month of lactation with a corresponding value of 100,000 cells/mL for SCC1) was caused by Gram-positive pathogens was 0.68. The probability that an identical CM case in a rear quarter was caused by Gram-positive pathogens was 0.60. In addition, higher values for SCC1 increased the probability that a CM cases was caused by Gram-positive pathogens.

Table 6. Results of the analysis of cow-specific risk factors for the prediction of the Gram status of the clinical mastitis case in heifers (dataset 5) and multiparous cows (dataset 6). Estimated coefficients (ß), standard error (SE) for the coefficient, odds ratio (OR), 95% confidence interval (CI) for OR, and significance level are given for significant (P≤0.05) cow-specific risk factors

	Heifer					Multiparous cow				
Variable	ß	SE	OR[1]	95% CI for OR	P-value	ß	SE	OR[1]	95% CI for OR	P-value
Intercept	-2.544	0.519				-0.874	0.249			
Month of lactation					NS[4]					0.0032
1						-0.726	0.207	0.48	0.32 – 0.73	
2						-0.482	0.181	0.62	0.43 – 0.88	
3						-0.112	0.176	0.89	0.63 – 1.26	
4						-0.206	0.194	0.81	0.56 –1.19	
5						-0.359	0.199	0.70	0.47 – 1.03	
6						-0.470	0.197	0.63	0.42 – 0.92	
7						0.082	0.259	1.09	0.65 – 1.80	
≥8						Ref.	–	1.00		
Season					0.0059					NS[4]
January-March	Ref.[5]	–	1.00							
April-June	0.215	0.435	1.24	0.58 – 2.91						
July-September	-0.717	0.406	0.49	0.22 – 1.08						
October-December	-0.987	0.381	0.37	0.18 – 0.79						
SCC1[2]	0.804	0.103	2.23[3]	1.82 – 2.74	<.0001	0.377	0.037	1.46[3]	1.36 –1.57	<.0001
Quarter position					NS[4]					0.0150
Front						0.356	0.112	1.43	1.15 – 1.78	
Rear						Ref.	–	1.00		

[1]Odds ratio for being infected with a Gram-positive pathogen versus a Gram-negative pathogen. [2]SCC1 = SCC in the previous month of lactation. [3]For an increase of 1 unit in natural logarithm of SCC. [4]NS = Not significant variable for the prediction of the Gram status of the CM case. [5]Ref. = Reference category.

DISCUSSION

The current study presents a cow-level risk study simultaneously analyzing all cow factors in one model to study their association with IRCM. While most other studies investigated only some cow-specific risk factors for CM (e.g., Beaudeau et al., 1998; Suriyasathaporn et al., 2000; Olde Riekerink et al., 2007), our study is novel, since it combined the significant cow-specific risk factors mentioned in previous studies in a single model to determine the IRCM for specific cows. Our results indicate that a combination of all these cow-specific risk factors can give an indication for the risk of individual cows of having CM.

There were large differences in IRCM among cows; the IRCM ranged between 0.0002 and 0.0074 per cow-day at risk (Figures 1, 2 and 3). The results of our study correspond with the results of studies on some individual cow factors: cows in higher parities, in beginning of lactation and with higher values for SCC have the highest IRCM (e.g., Barkema et al., 1998; Beaudeau et al., 1998; Suriyasathaporn et al., 2000). Results from our study are also comparable with results from a previous study (Houben et al., 1993) that combined four cow-specific risk factors to detect CM.

Danish researchers reported that treatment of a CM case leading to a record of CM is determined by a series of cow and herd factors; some farmers treat cases with any signs of CM, while others will only record severe cases (Vaarst et al., 2002). In the current study, however, all farmers were in great detail informed at the start of the study about recording CM; a clear case definition was provided (Barkema et al., 1998). Of course, in a study with 300 participating herds it is impossible to state that each farm collected the samples in exactly the same way. Lam et al. (1993), however, determined that samples collected by farmers are a useful tool in epidemiologic studies on CM. Because of the information provided we are comfortable that recording of CM cases was as good as possible in a study of this size. A severity score was not present in the dataset. This information will be especially useful in detection systems for CM, when it is very serious when severe cases of CM are missed.

SCC is widely known as an important risk factor for CM (e.g., Beaudeau et al., 1998; Suriyasathaporn et al., 2000; Green et al., 2007). Results from our study indicate that both SCC in the previous month (SCC1) and SCC in the previous lactation (SCC2) are significant cow-specific risk factors for an increased IRCM. The IRCM for a multiparous cow, with different corresponding values for SCC1 and SCC2, ranged between 0.0011 and 0.0034 per cow-day at risk (Figure 2). From this it seems that using SCC from monthly test-day records in AMS would improve the detection of CM. Our results also emphasize the importance of the cow-specific risk factor CM history for the risk of having CM later

in lactation. With different values for MAST1 and MAST2 the IRCM ranged between 0.0016 and 0.0074 per cow-day at risk (Figure 3). Previous studies have also found that previous CM cases and recovery from infections was a risk factor for reinfection (Houben et al., 1993; Zadoks et al., 2001). Therefore, recording CM and using this historical information in CM detection systems could be very useful.

In literature, other cow factors, which are usually available on a farm, were mentioned that increase the IRCM. Diseases, such as milk fever, were associated with an increased IRCM (Østergaard et al., 2003), but this information was not available in our dataset. Also milking speed (Waage et al., 1998; Klaas et al., 2005) and udder depth (Slettbakk et al., 1995; Klaas et al., 2004) are risk factors associated with a higher risk of CM. This information was available for the cows in our study. Information on udder depth, however, was available for only 70% of the cows in the dataset, while milking speed was only recorded for 40% of the cows. To prevent problems with removed records because of missing values, it was decided not to include these risk factors in the analyses.

Many studies have been carried out to find risk factors for CM at the herd level (e.g., Schukken et al., 1991; Barkema et al., 1999; Nyman et al., 2007). We did not analyse these types of risk factors. In the model, the repeated herd term accounted for these factors. On a single farm, circumstances are equal for all cows and therefore herd level risk factors cannot distinguish in the IRCM among cows. Probably, when there is no information at all about the individual cows, the herd level incidence of CM might serve as a base value in detection systems. In a similar way, also for pathogen or Gram-status detection it may be worthwhile to include the pathogen prevalence of a herd in detection systems as a base value. These base values will reflect the herd factors for CM or specific pathogens.

Because it is useful for treatment decisions to know the Gram status of the CM case (Morin et al., 1998; Pyörälä and Pyörälä, 1998), we created with logistic regression a simple predictive model with a binary (positive or negative) outcome. It might still be useful, however, to predict the exact involved pathogen. Because of low incidence rates for most pathogens and also a lot of missing values for SCC1, it was decided to predict the Gram-status of the CM cases. Knowing the Gram-status of previous CM cases could be an important risk factor for the Gram status of the current CM case. Because of statistical problems, however, it was not possible to include this risk factor. The most probable reason would be that there were almost no differences between the dependent (Gram-status of the current CM case) and the independent variable (Gram-status of the previous CM case).

SCC1 is a significant variable in predicting the Gram-status of the CM cases for both heifers and older cows. Higher values for SCC1 increased the probability that CM cases were caused by Gram-positive pathogens, which was in accordance with another study (De Haas et al., 2002). Other significant variables were different for heifers and multiparous cows (Table 6). One explanation of that difference could be that the dataset for heifers is relatively small (only 364 CM cases), while for multiparous cows 2,161 cases were included. We found that for specific heifers, the probability that a CM case is caused by a Gram-positive pathogen ranged from 0.54 to 0.80 depending on the season of the year. This seasonal variation has also been identified in other studies (Morin et al., 1998; Makovec and Ruegg, 2003; Olde Riekerink et al., 2007). For multiparous cows, rear quarters were more susceptible to Gram-negative pathogens, possibly because rear quarters are more soiled and more susceptible to the environmental (and Gram-negative) pathogens such as *E. coli* and *Klebsiella*. Results indicate that it is difficult to distinguish between Gram-positive and Gram-negative CM cases based on cow factors alone.

The current study indicates that a differentiation can be made among cows in the risk of having CM based on a combination of cow factors. These differences among cows can be useful to aid automatic detection of CM. On farms with an AMS, where the farmer is not present during the milking process, this will be particularly worthwhile. The number of false-positive warnings for CM, based on sensor measurements, of an AMS needs improvement (Hogeveen and Ouweltjes, 2003). For instance, in a recent study using electrical conductivity measurements of an AMS, a sensitivity of 56% and a specificity of 82% to detect CM were found (Mottram et al., 2007). Combining sensor information with the individual risk of having CM based on additional cow factors may possibly reduce the number of these false-positive CM warnings generated by the AMS and may improve the interpretation of the sensor outputs. Also, in previous studies a combination of sensor measurements and cow factors was presented for a better interpretation of the sensor outputs (De Mol and Woldt, 2001; Chagunda et al., 2006). A study should be conducted to quantify the possible decrease in the number of false-positive warnings for CM by combining sensor measurements with the individual risk on having CM based on cow factors. That study will have some special requirements, a dataset including both sensor information and information on cow factors is needed. Also, a validation of the developed model is needed to assess the usefulness of combining the two information sources.

CONCLUSIONS

This study contains an integrated analysis with several cow factors to determine the IRCM of dairy cows. All cow factors together (parity, month in lactation, season of the year, SCC in previous month, geometric mean SCC in previous lactation and CM history) significantly influenced the risk of having CM. There was a large difference in IRCM among dairy cows. The IRCM ranged between 0.0002 and 0.0074 per cow-day at risk. CM history was an important factor in determining the IRCM. Therefore, registering CM cases and using this historical information would be very useful in detecting CM. Results indicate that additional cow factors should not be the sole criteria for differentiating between Gram-positive and Gram-negative CM cases.

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Chapter 3

Simplify the interpretation of alert lists for clinical mastitis in automatic milking systems

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ABSTRACT

Based on sensor measurements, an automatic milking system (AMS) generates mastitis alert lists indicating cows which are likely to have clinical mastitis (CM). Because of the general assumption of equal probabilities of developing CM for all cows, all alerts on the list have the same success rate. As a consequence, it is not possible to rank-order the alerts in terms of their likelihood of CM. In practice, the performance of a CM detection system is not only based on the sensitivity (SN) and specificity (SP) of the system, but is also influenced by the prior probability of a cow having CM. This study illustrates the idea of using cow-specific prior probabilities of CM, based on non-AMS information, to provide a rank-order on the alerts from an AMS. A tree-augmented naive Bayesian network was trained from available data to determine these cow-specific prior probabilities for CM. The graphical structure of the network and the probability tables for its variables in the network were based on data from 274 Dutch dairy herds that recorded each case of CM over an 18-month period. The final data set contained information on a total of 5,363 CM cases derived from 28,137 lactations and 22,860 cows. The available prior cow information (parity, days in milk, season of the year, somatic cell count history and CM history) were included as variables in the network. By combining the cow-specific prior probabilities of CM with the SN and SP of the detection system of the AMS, the computed success rates can be used to discriminate between CM alerts. Our illustrations indicate that the success rate might range from 3 to 84%, while assuming an equal overall probability would result in a success rate of 21%. Using the computed success rates, the CM alerts on an alert list can be ranked-ordered, thereby providing the dairy farmer information about which cows have the highest priority for visual inspection for CM.

Keywords: clinical mastitis, automatic milking systems, detection

INTRODUCTION

The number of farms with an automatic milking system (AMS) continues to increase considerably (Svennersten-Sjaunja and Pettersson, 2008). Approximately 5% of Dutch dairy farms and approximately 5,500 dairy farms worldwide now work with an AMS. Regardless of whether or not a farm has an AMS, clinical mastitis (CM) remains one of the most frequent and costly diseases of dairy cows (e.g., Halasa et al., 2007). Farmers milking with conventional systems rely on visual inspection of the milk to detect abnormalities during the milking process, while farmers with an AMS rely on mastitis alert lists from their AMSs for information on the udder health status of their cows (Hogeveen and Ouweltjes, 2003). These alert lists report the cows likely to have CM. A general complaint of dairy farmers working with an AMS is the relatively large number of false-positive alerts on the mastitis alert lists. Moreover, current AMSs do not rank-order the alerts on their lists in terms of likelihood of CM.

Expressed per milking, the prevalence of CM is very low. To substantially decrease the number of false-positive alerts on the mastitis alert lists, therefore, an extremely high specificity (SP) is required of the AMS. A sensitivity (SN) of 70% and an SP of at least 99% have been mentioned as minimum requirements for a reliable mastitis detection system (Mein and Rasmussen, 2008). Previous studies have examined ways of improving the SN and SP of CM detection models in AMSs (De Mol and Ouweltjes, 2001; De Mol and Woldt, 2001; Kamphuis et al., 2008). However, the resulting detection models were unable to improve detection performance to the extent that the detection of CM cases remained satisfactory and the number of false-positive alerts was reduced to a reasonable level.

In practice, the performance of a CM detection system is influenced by the prior probability of a cow having CM as well as by the SN and SP of the system. This prior probability is usually assumed to be the same for all cows on a farm. Cows, however, are different and will have different prior probabilities of CM. For instance, cows in early lactation have a higher probability of CM than cows in late lactation (Steeneveld et al., 2008). Using a cow-specific prior probability of CM to determine the success rate for an alert, instead of assuming an equal probability for all cows, is likely to improve interpretation of the CM alert lists from an AMS. For instance, by taking cow-specific prior probabilities into consideration, it should be possible to rank-order the alerts on a CM alert list according to their success rate. This cow-specific prior probability of CM can be based on non-AMS information from a cow, such as parity, days in milk (DIM), monthly somatic cell count (SCC) records, and CM history.

To examine the idea of using cow-specific prior probabilities to rank-order CM alerts, a probabilistic model is required to calculate such probabilities based upon non-AMS information from a cow. A logistic regression model has recently been described to determine the cow-specific risk of CM based upon a combination of cow factors (parity, month in lactation, season, SCC history and CM history) (Steeneveld et al., 2008). This model can be exploited to compute the prior probabilities to be used with the sensor-driven detection models of an AMS. Logistic regression models, however, have several limitations for this purpose. Firstly, they cannot handle missing values, and secondly, including intricate dependencies among variables is difficult. To address these limitations Bayesian networks (BN) were used in our study. BNs are powerful tools for the representation of probability distributions and for probabilistic inference (Jensen, 2001). The advantages of BNs are that they are flexible in terms of handling missing values, they can capture complicated dependencies among their variables, and they allow the computation of prior or posterior probabilities of modelled variables. In addition, the simpler types of BN provides better results with smaller data sets than logistic regression (Van der Gaag et al., 2009). BNs have been studied extensively and are being widely applied in human medicine (e.g., Chapman et al., 2005). Applications are not yet common in veterinary science, but they are gaining popularity (e.g., McKendrick et al., 2000; Otto and Kristensen, 2004; Jensen et al., 2009).

The overall objective of the current study was to explore and illustrate the idea of combining prior non-AMS cow information with the test characteristics of the CM detection system, with the aim of providing a rank-order on alerts. This rank-order would allow the farmer to more adequately detect cows with CM.

MATERIAL AND METHODS

Herds and data collection

The data used in the present study were described in detail elsewhere (Barkema et al., 1998). In short, records of CM were collected from 274 dairy herds entering the study between December 1992 and June 1994. Each herd participated in the study for approximately 1.5 years. All herds had an annual milk production quota between 300,000 and 900,000 kg, and had cows of the Holstein-Friesian or Dutch Friesian breeds. Lactating cows were housed in a free-stall barn during winter and milked in a double-herringbone or two-sided tandem milking parlor. During the study, the farmers were asked to collect milk samples from cows with signs of CM. The samples were taken before treatment, stored in a freezer at the farm (at approximately -20°C) and collected every 6 to 8 weeks for bacteriological culturing. The collected data contained cow identification, the date of occurrence of CM, the infected quarter, clinical signs, and the outcome of the

bacteriological culturing of the milk samples (Barkema et al., 1998). In addition, the Dutch national milk recording system (Nederlands Rundvee Syndicaat, Arnhem, the Netherlands) provided information from the 3- or 4-weekly milk production system, including cow identification, the date of the milk recording, the date of calving, the date of drying off, test-day milk yields (kg of milk, fat and protein), and SCC (cells/mL) measurements for all cows.

Data preparation

The originally available dataset consisted of 120,398 lactations from 39,764 cows, and included a total of 8,571 CM cases. Only lactations that had been recorded from calving onwards were included in the datasets for our study, to ensure that no previous cases of CM had occurred within the same lactation. Lactations without any information on milk production or with a calving interval ≤ 320 days or ≥ 600 days were further excluded from our datasets. Also, the dry period was excluded from our dataset. Intervals between pathogen-specific repeated CM cases in the same quarter had to be at least 14 days for a case to be included in our final datasets.

From previous studies it was known that the incidence of CM can vary considerably within the first month of lactation (Barkema et al., 1998; Steeneveld et al., 2008). Therefore, for the current study two datasets were created. Dataset1 included information from the first 30 DIM. Information from the remaining DIM were included in dataset2. For both datasets, cow-specific risk factors for CM were defined. These factors were defined based upon information from the literature and based upon the expertise of the authors. A further criterion was that information for a risk factor had to be readily available on a farm.

Dataset1 contained information from the first 30 DIM of each cow. These first 30 DIM were subdivided into 6 periods of 5 days each. For each period for each cow, it was determined whether or not the cow had CM in that period. Dataset1 contained 168,822 records with a total of 1,617 CM cases. In addition to the period variable, four other variables were defined as risk factors for CM (Table 1). For all cows, parity (Barkema et al., 1998) was known. For each period, the season of the year (Olde Riekerink et al., 2007) was determined. For multiparous cows, the geometric mean SCC from all available test-day records of the previous lactation was defined (Whist and Østerås, 2006; Green et al., 2007) (5.1% missing values). From the second period of 5 days onwards, it was determined whether or not a cow had CM in the previous period of 5 days (Houben et al., 1993), including the dry period.

In dataset2, DIM (Barkema et al., 1998; Green et al., 2004; Svensson et al., 2006) from 31 DIM onwards were divided into 30-day intervals. For every 30 DIM for each cow, it was

determined whether or not the cow had CM in these 30 days. The dataset contained 195,454 records with a total of 3,746 CM cases. In addition to the 30 DIM variable, seven other variables were defined as risk factors for CM (Table 1).

Table 1. Description of the study variables with their different levels used for learning tree-augmented naive Bayesian networks to determine cow-specific prior probabilities of clinical mastitis (CM).

Description	# classes	Levels
Clinical mastitis[1, 2]	2	no, yes
Parity[1, 2]	4	1, 2, 3, ≥4
First 30 days in milk divided in periods of 5 days[1]	6	1-5, 6-10, 11-15, 16-20, 21-25, 26-30
30-day intervals from 31 days in milk onwards[2]	7	31-60, 61-90, 91-120, 121-150, 151-180, 181-210, ≥211
Season of the year[1, 2]	4	January-March, April-June, July-September, October-December
Somatic cell count in the previous 30 days in milk[2]	2	<200,000 cells/mL, ≥200,000 cells/mL
Somatic cell count in the 30 days before the previous 30 days in milk[2]	2	<200,000 cells/mL, ≥200,000 cells/mL
Geometric mean somatic cell count of all test-days in the previous lactation[1,2]	2	<200,000 cells/mL, ≥200,000 cells/mL
Having CM in the previous 5-day period[1]	2	no, yes
Accumulated number of CM cases in the previous 30 days in milk[2]	3	0, 1, 2
Accumulated number of CM cases before the previous 30 days in milk[2]	3	0, 1, 2

[1]Included in dataset1,

[2]Included in dataset2

Parity (Barkema et al., 1998) was known for each cow in the dataset. Season of the year (Olde Riekerink et al., 2007) was determined for each period of 30 DIM. In addition, three variables on SCC were constructed (e.g., Beaudeau et al., 1998; Suriyasathaporn et al., 2000; Green et al., 2004): the SCC in the previous 30 DIM (9.9% missing values), the SCC in the 30 days before the previous 30 DIM (8.3% missing values) and, for multiparous cows, the geometric mean SCC from all available test-day records from the previous lactation was established (5.1% missing values). Because continuous variables cannot be handled in standard BNs, the values of all SCC variables were classified into < and ≥ 200,000 cells/mL (Dohoo and Leslie, 1991; Schukken et al., 2003). In addition, two variables for the CM history of a cow were constructed (Houben et al., 1993): 1) the

accumulated number of CM cases in the previous 30 DIM and 2) the accumulated number of CM cases in the DIM before the previous 30 DIM. Also, CM cases in the dry period were taken into account to determine the accumulated number of previous CM cases. Because hardly any CM information from earlier lactations was present in the originally available dataset, no further history information was taken into account in our study. Data preparation was performed using SAS version 9.1 (SAS Institute Inc., Cary, NC).

Learning of Bayesian networks

BNs allow different graphical structures, of varying complexity. Naive Bayesian networks (NBN) are the simplest type of BN and consist of a single class variable that represents the possible classes for the dependent variable of a study, and a set of feature variables modelling the relevant levels of the study's independent variables. Additionally, an NBN includes arrows from the class variable to each feature variable to describe the dependence of the latter on the class variable. NBNs build upon the assumption that all feature variables are mutually independent given the class variable. Dependencies among the feature variables, therefore, cannot be modelled in an NBN (Friedman et al., 1997). Because previous studies reported significant dependencies among the different risk factors for CM (Olde Riekerink et al., 2007; Steeneveld et al., 2008), the somewhat more complex tree-augmented naive Bayesian network (TAN) was used for the present study. A TAN, just like an NBN, consists of a single class variable and multiple feature variables, with arrows pointing from the class variable to each of the feature variables. A TAN, in addition, has a tree-like dependency structure over its feature variables, in which each feature variable has at most one arrow from another feature variable pointing to it (Friedman et al., 1997).

In the current study, the variable whether or not a cow had CM in a particular period, was taken for the class variable. All other variables, representing cow-specific risk factors for CM, were taken as feature variables. Two TANs were trained, one for establishing the probability of CM in the first 30 DIM and one for computing the probability of CM from 31 DIM onwards. Learning the two TANs, which includes establishing the dependencies between the feature variables and estimating the prior and conditional probabilities required, was done by using the Bayesian-network editing package Dazzle (Schrage et al., 2005). The strengths of the dependencies among the feature variables were captured using the information-theoretic criterion of conditional mutual information given the class variable. Based upon the computed mutual-information terms, an undirected tree structure of maximum likelihood was created over the feature variables; from this tree structure, a single variable was chosen randomly for the root variable and all links were subsequently directed from this variable onwards (Friedman et al., 1997). It should be noted that any choice of root variable for the tree will result in a TAN which is equivalent to the actually constructed TAN in terms of performance, yet may include different directions for the

links between the feature variables. The directions of the links can thus not be interpreted as modelling a direction of causality. These directions should rather be interpreted as capturing joint effects of the feature variables on the class variable. For the two TANs, prior probabilities for the class variable had to be estimated, capturing the overall probability of CM per 5 days and capturing the overall probability of CM per 30 days, respectively. For each feature variable moreover, conditional probabilities had to be estimated, dependent on its incoming arrows. All probabilities were estimated based upon frequency counts in the datasets.

After learning the dependency structure and the required prior and conditional probabilities, the two TANs were used to compute cow-specific prior probabilities of CM based upon the cow's risk factors. The following formula can be used for computing this probability Pr ($c_1 \mid f_1, \ldots, f_n$) given levels $f_1,\ldots,f_n$ for the n feature variables:

$$\Pr(c_1 \mid f_1,\ldots, f_n) = \frac{\Pr(c_1) * \Pr(f_1,\ldots, f_n \mid c_1)}{\Pr(f_1,\ldots, f_n)}$$

$$= \frac{\Pr(c_1) * \prod_{i=1}^{n} \Pr(f_i \mid pa(f_i), c_1)}{\sum_{j=1}^{2} \Pr(c_j) * \prod_{i=1}^{n} \Pr(f_i \mid pa(f_i), c_j)} \qquad (1)$$

where Pr(c_1) is the overall prior probability of CM and Pr(c_2) is the prior probability of not having CM. The term Pr($f_i \mid pa(f_i), c_j$) in the formula denotes the conditional probability of level f_i for the ith feature variable given the level pa(f_i) for its parent feature variable and given the $_j$th level of the class variable.

Illustrating the use of cow-specific prior probabilities

Based on sensor measurements of the milk, an AMS generates a mastitis alert list of cows likely to have CM. The numbers of alerts on these lists have their origin in the test characteristics of the detection system of the AMS. Results from a recent Dutch study, for example, found an SN of 43% and an SP of 97% (Mollenhorst and Hogeveen, 2008). The percentage of true-positive alerts (the success rate as defined by Sherlock et al. (2008)) on the list is based on the SN and SP of the detection system and on the overall prior probability (Pr(c)) of CM (equation 2).

$$\text{Success rate} = \frac{\Pr(c) * SN}{(\Pr(c) * SN) + (1 - \Pr(c)) * (1 - SP)} * 100\% \qquad (2)$$

Because the overall prior probability of CM is assumed to be the same for all cows on a farm, all alerts on the list have the same likelihood of being a true-positive alert. To discriminate between the cows on the alert list in terms of their likelihood of having CM, in this study cow-specific prior probabilities of CM (Pr ($c \mid f_1, \ldots, f_n$)), computed from the TAN given the non-AMS risk factors, were used to determine a cow-specific success rate (equation 3).

$$\text{Success rate } (f_1,\ldots,f_n) = \frac{\Pr(c \mid f_1,\ldots, f_n) * SN}{(\Pr(c \mid f_1,\ldots, f_n) * SN) + (1 - \Pr(c \mid f_1,\ldots, f_n)) * (1 - SP)} * 100\% \quad (3)$$

The ability to discriminate between cows on a mastitis alert list was illustrated with two detection scenarios. In the first scenario an SN of 43% and an SP of 97% were used, as recently found in a Dutch study (Mollenhorst and Hogeveen, 2008); the second scenario used an SN of 70% and an SP of 99% which were recently mentioned as minimum requirements for mastitis detection systems (Mein and Rasmussen, 2008). The illustrations contained CM alerts for different cows, differing in prior information, in the first 30 DIM and from 31 DIM onwards.

RESULTS

Trained tree-augmented naive Bayesian networks

Figure 1 presents the trained TAN for computing prior probabilities of CM in the first 30 DIM, while Figure 2 presents the trained TAN for computing prior probabilities for CM from 31 DIM onwards. The prior probability of CM in the first 30 DIM computed from the available data was 0.0087 per 5 days, while the prior probability of CM after 30 DIM was 0.0178 per 30 days. The conditional probability tables established from the data for the feature variables of the two TANs are available upon request from the authors. In Figure 1, the arrow from the class variable “CM” to the feature variable “Season”, for example, describes the correlation of the season of the year with the occurrence of CM. The arrow from the feature variable “Mean SCC in previous lactation” to the feature variable “Parity” implies that the influence of a cow’s parity on its probability of having CM depends on its mean SCC in the previous lactation. In Figure 2, the arrow from the feature variable “SCC in previous 30 DIM” to the feature variable “SCC before previous 30 DIM” implies that the SCC more than 30 days ago and the SCC in the previous 30 days have a combined influence on the probability of CM.

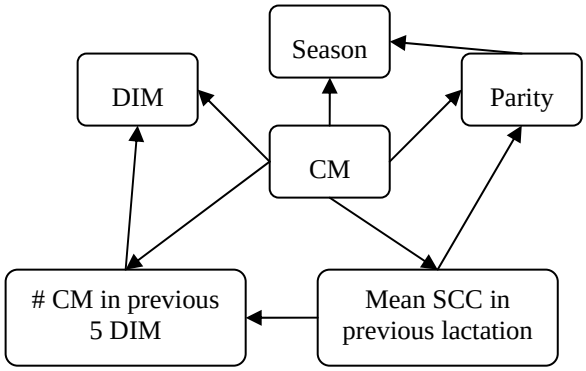

Figure 1. Trained graphical structure of the tree-augmented naive Bayesian network for computing a cow-specific prior probability of clinical mastitis (CM) in the first 30 days in milk; DIM = days in milk, SCC = somatic cell count.

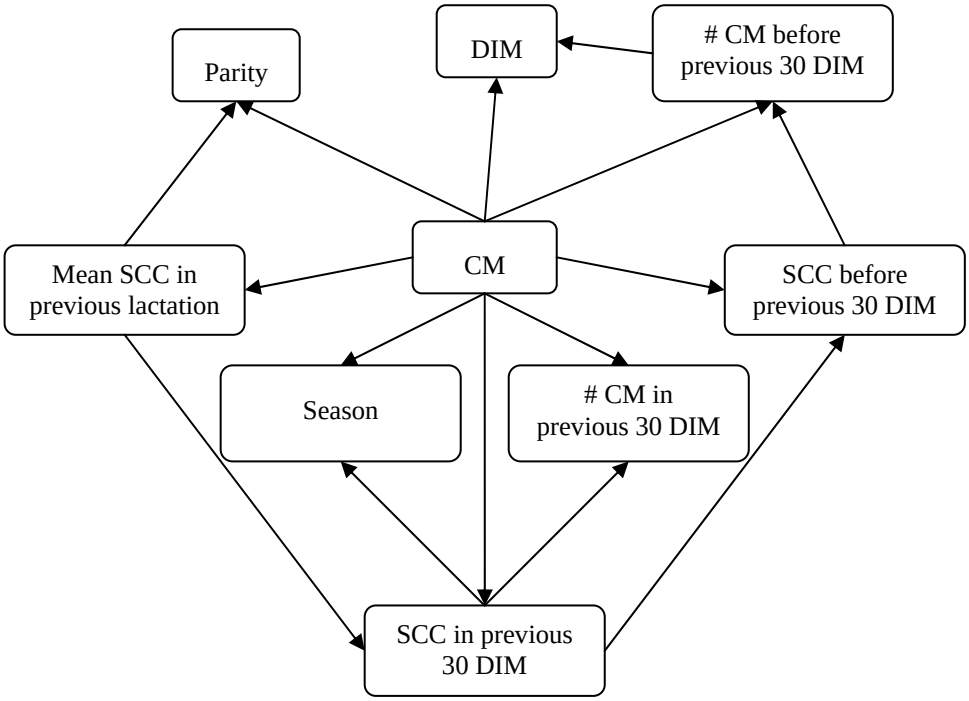

Figure 2. Trained graphical structure of the tree-augmented naive Bayesian network for computing a cow-specific prior probability of clinical mastitis (CM) from 31 days in milk onwards; SCC = somatic cell count, DIM = days in milk.

Success rate for clinical mastitis alerts before 30 days in milk

With the trained TAN, prior probabilities of CM were computed for 6 example cows early in lactation. The prior probabilities established for these example cows ranged from 0.0033 to 0.0348 per 5 days. Using these cow-specific prior probabilities, detection scenario 1 resulted in a success rate ranging from 5 to 34% (Table 2). A CM alert for a cow which is in third parity, between 20 and 25 DIM (period 5) in January, with a mean SCC in the previous lactation of < 200,000 cells/mL and no previous CM in the current lactation, for example, had a success rate of 5%. This particular CM alert thus had a probability of 0.05 to be a true-positive alert. Alerts for other cows on the list have higher success rates, i.e., these alerts have higher probabilities of being true-positive alerts. For instance, a CM alert for a cow which is in first parity, between 1 and 5 DIM (period 1) in December, had a success rate of 34%.

Using the computed cow-specific prior probabilities of CM, detection scenario 2 resulted in a success rate ranging from 19 to 72% (Table 2). All success rates calculated with detection scenario 2 were higher than those calculated with detection scenario 1.

Table 2. Illustration of the success rate for clinical mastitis alerts based on a cow-specific prior probability of clinical mastitis (CM), determined with different detection scenarios; illustrations are given for CM alerts for 6 cows before 30 days in milk, differing in prior information.

Prior knowledge of the cow					Cow-specific prior probability of CM[2]	Success rate of alert[3] (%)	Success rate of alert[4] (%)
Parity	Days in milk	Season	Mean SCC[1] in previous lactation (cells/mL)	# CM cases in previous 5 days in milk			
3	20-25	January	<200,000	0	0.0033	5	19
2	25-30	April	≥200,000	0	0.0045	6	24
3	5-10	December	<200,000	0	0.0067	9	32
3	5-10	December	<200,000	1	0.0091	12	39
2	1-5	December	≥200,000	NA[5]	0.0289	30	68
1	1-5	December	NA[5]	NA[5]	0.0348	34	72

[1]SCC = somatic cell count,
[2]calculated with the trained tree-augmented naive Bayesian network, expressed per 5 days,
[3]computed using a detection scenario with a sensitivity of 43% and a specificity of 97%,
[4]computed using a detection scenario with a sensitivity of 70% and a specificity of 99%,
[5]information is not available.

Success rate for clinical mastitis from 31 days in milk onwards

With the trained TAN, cow-specific prior probabilities of CM were computed for 8 example cows later in lactation. The computed probabilities ranged from 0.0018 to 0.2697 per 30 days. Using these cow-specific prior probabilities, detection scenario 1 resulted in a success rate ranging from 3 to 84% (Table 3). A CM alert for a cow with a low prior probability (third parity, from 121 to 150 DIM, in January, with previous SCC records of < 200,000 cells/mL and no previous CM cases in the current lactation) had a success rate of 19%. A CM alert for this particular cow thus had a probability of 0.19 to be a true-positive alert. An alert for the same cow, but now with an SCC in the previous 30 DIM of ≥ 200,000 cells/mL and two previous CM cases in the current lactation, had a success rate of 78%, thus had a higher probability of being truly positive.

Using the computed cow-specific prior probabilities, detection scenario 2 resulted in a success rate ranging from 11 to 96% (Table 3).

Table 3. Illustration of the success rate for clinical mastitis (CM) alerts based on a cow-specific prior probability of clinical mastitis, determined with different detection scenarios; illustrations are given for CM alerts for 8 cows after 31 days in milk, differing in prior information.

Prior knowledge of the cow										
Parity	DIM[1]	Season	SCC[2] in previous 30 DIM[1] (cells/mL)	SCC[2] before the previous 30 DIM[1] (cells/mL)	Mean SCC[2] in previous lactation (cells/mL)	# CM cases in previous 30 DIM[1]	# CM cases before previous 30 DIM[1]	Cow-specific prior probability of CM[3]	Success rate of alert[4] (%)	Success rate of alert[5] (%)
1	≥211	April	<200,000	<200,000	NA[6]	0	0	0.0018	3	11
2	≥211	August	<200,000	<200,000	<200,000	0	0	0.0034	5	19
1	31-60	August	<200,000	NA[6]	NA[6]	0	NA[6]	0.0080	10	36
3	121-150	January	<200,000	<200,000	<200,000	0	0	0.0166	19	54
1	31-60	August	≥200,000	NA[6]	NA[6]	0	NA[6]	0.0285	30	67
3	121-150	January	≥200,000	<200,000	<200,000	1	0	0.0828	56	86
3	121-150	January	≥200,000	<200,000	<200,000	1	1	0.1938	78	94
≥4	121-150	January	≥200,000	≥200,000	≥200,000	2	2	0.2697	84	96

[1]DIM = days in milk,
[2]SCC = somatic cell count,
[3]calculated with the trained tree-augmented naive Bayesian network, expressed per 30 days,
[4]computed using a detection scenario with a sensitivity of 43% and a specificity of 97%,
[5]computed using a detection scenario with a sensitivity of 70% and a specificity of 99%,
[6]information is not available.

DISCUSSION

The number of false-positive alerts for CM is considerable at commercial Dutch dairy farms with an AMS (Mollenhorst and Hogeveen, 2008). Although new CM detection models for AMS were developed (De Mol and Ouweltjes, 2001; De Mol and Woldt, 2001) or are under construction (Kamphuis et al., 2008), their SN and SP remain too low to substantially reduce the number of false-positive alerts in practice. Moreover, the alerts on the CM alert list are not ranked-ordered. For instance, it is not known which cows on the list have a higher probability of actually having CM than other cows. The farmer thus has to visually inspect all cows on the list, without any indication of priority.

The performance of a CM detection system in practice is not just based on the test characteristics of the system, but also on the prior probability of a cow having CM. Usually, this probability is assumed to be the same for all cows on a farm. Consequently, all CM alerts generated by an AMS have equal success rates. For instance, using the overall prior probability of having CM from our dataset (0.0178 per 30 days) in combination with an SN of 43% and an SP of 97% will result in a success rate of 21% for all alerts. A previous study reported, however, that cows are different and will have different prior probabilities of CM (Steeneveld et al., 2008). The present study aimed at establishing a cow-specific prior probability of CM, based on prior non-AMS cow information. These cow-specific prior probabilities were subsequently combined with the test characteristics of an AMS to illustrate the possible range in success rates for CM alerts. Illustrations of these success rates were presented in Tables 2 and 3. The results showed that combining test characteristics of an AMS with a cow-specific prior probability of CM based on non-AMS information of a cow, has potential to discriminate between CM alerts based upon their likelihood of being truly positive. For instance, alerts for cows with high values on SCC and/or previous CM cases had high success rates, while alerts for cows in late lactation, with low values on SCC and no previous CM cases before, had low success rates. Currently, most Dutch dairy farmers know the history of most cows on their farm by heart. Based on that knowledge, the decision which CM alert to inspect visually can be made. With increasing herd sizes, however, it will be more difficult for a farmer to know the history of each individual cow. For those farmers, a rank-ordering of CM alerts based on their success rates will be particularly worthwhile.

The illustrations in the present study were based on two different detection scenarios. For the first scenario, a detection system was assumed with an SN of 43% and an SP of 97%. In our opinion, this scenario matches the CM detection characteristics of current AMSs. These characteristics were in fact found from visual inspections of all milkings during three days on three Dutch commercial dairy farms (Mollenhorst and Hogeveen, 2008). An improvement in sensors or in the detection models themselves will lead to higher values

for SN and SP, and consequently will result in higher values for the success rate. In the second detection scenario, therefore, higher values for SN (70 %) and SP (99%) were used; these values were mentioned in a previous study as minimum requirements for a mastitis detection system (Mein and Rasmussen, 2008). Although mastitis detection models were presented meeting these requirements (e.g., De Mol and Ouweltjes, 2001; De Mol and Woldt, 2001), they were not implemented on commercial dairy farms. Moreover, most of these detection models have high values for SN and SP as a result of using long time-windows for CM detection (Sherlock et al., 2008).

The present study built on the observation that the success rate for CM alerts is based not just on the test characteristics of the CM detection system, but also on the prior probability of CM. Figure 3 presents the effects of varying the prior probability of CM on the success rate for the two detection scenarios under study. Figure 3A presents the entire range of prior probabilities of CM; Figure 3B zooms in on the range of smaller prior probabilities. For very small prior probabilities, Figure 3 shows that even a slight increase results in a considerable increase in the success rate. For instance, with detection scenario 1, an increase in the prior probability of CM from 0.01 to 0.03 results in an increase in the success rate from 0.13 to 0.31. Such variations in success rate were seen also in Tables 2 and 3. For larger prior probabilities, no major increase in the success rate is expected (Figure 3A). Based on prior non-AMS cow information (e.g., parity, DIM, SCC measurements), however, it is not very likely that such high probabilities of CM are reached. For instance, a cow with values $\geq$ 200,000 cells/mL for all three SCC variables and with two previous CM cases in the current lactation, had a cow-specific prior probability of CM of 0.27. Higher values will be quite exceptional. Low cow-specific prior probabilities are more likely and especially these low values are useful for discriminating between CM alerts. Figure 3 thus supports the idea that cow-specific prior probabilities can be used to discriminate between CM alerts in terms of their success rate.

The dataset used in our study was not collected on farms with an AMS. Although the use of data from farms with an AMS would have been preferred, such data with comparable completeness, size and variety were not available. In our opinion, however, it was reasonable to exploit the current dataset because it was used for exploratory purposes only. For application in the field, clearly the TAN needs to be retrained with current AMS data.

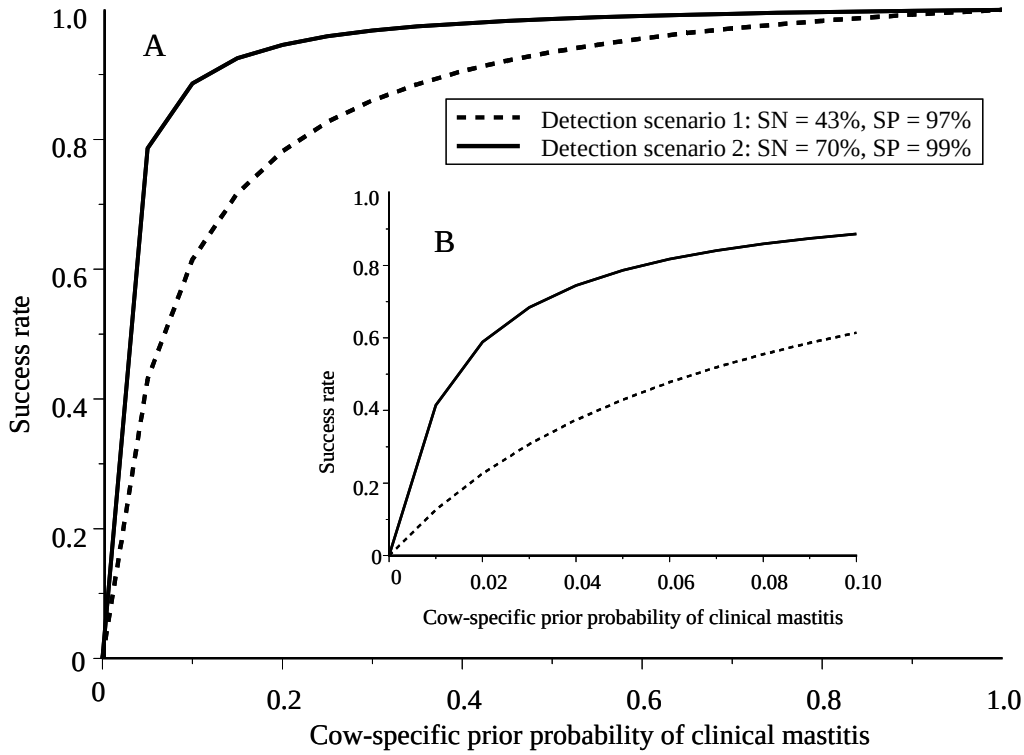

Figure 3. Association between a cow-specific prior probability of clinical mastitis and the success rate of a mastitis alert for the whole range of cow-specific prior probabilities (A) and for cow-specific prior probabilities below 0.10 only (B). SN = sensitivity, SP = specificity

In the current study, a TAN was trained from the available data for the purpose of determining cow-specific prior probabilities of CM. The dependencies in the trained graphical structures (Figures 1 and 2) seem plausible. The main advantage of using a TAN over a simpler NBN is that dependencies between the independent variables are taken into account (Friedman et al., 1997), such as the dependencies between successive SCC measurements. Yet, the restriction imposed by the TAN on the numbers of incoming arrows for the feature variables prevents an increasing complexity of the dependency structure and thereby forestalls having to estimate conditional probabilities from unrealistically small subsets of the data. For the present study, moreover, BNs were used instead of the more commonly used logistic regression. One of the advantages of using BNs is their adaptability. The prevalence of CM on a farm is a measure of the herd circumstances. A farm-specific prevalence can be easily included in the trained TAN model by simply adapting the overall prior probability of CM, without the need to have to

train the model anew. Also, including other information sources at the individual cow level as variables in the developed TAN is easy. Additional information sources can help to establish better cow-specific prior probabilities of CM and, consequently, a better discrimination between CM alerts based on their success rates. In our study, SCC measurements were simply divided into < and ≥ 200,000 cells/mL. In recent studies, alternative traits for SCC as CM indicators were developed (De Haas et al., 2005; De Haas et al., 2008; Ten Napel et al., 2009). Also genetic differences between dairy sires in their daughters' susceptibility to mastitis (Zwald et al., 2004) and udder skin temperature (Hovinen et al., 2008) can probably serve as new information sources. The presented method can also be applied in other countries as well as on farms milking conventionally, since also CM alert systems are incorporated in conventional milking systems. Implementation in other countries and in different milking environments, however, will need retraining from representative data. When training a model for a different country or milking system, there might be other information sources than the ones used in the current study. These other information sources can also be included in such a country-specific model.

Chagunda et al. (2006) also combined prior knowledge of a cow and sensor measurements to improve detection of CM. They integrated the two information sources into a very complicated model. Adding extra non-AMS cow information or implementing newly developed detection sensors into this model will be difficult. The advantage of our method is that the TAN for computing cow-specific prior probabilities of CM and the detection model of the AMS were kept separate. Consequently, the TAN can be extended with other non-AMS cow information independent of the detection model and can also be combined with any new model for the detection of CM based on sensor measurements. For improved oestrus detection cow information was also successfully added (De Mol and Woldt, 2001; Firk et al., 2003).

With our method, it has become possible to determine a cut-off value on the success rate and to present only alerts with a success rate over this cut-off value to the farmer. By using a higher cut-off value, the alert list will become shorter, minimising the additional labour of checking false-positive alerts. However, as a side effect, some true clinical cases might be missed. It is expected that presenting shorter alert lists would increase the confidence in the system and the farmers' compliance to the system. Ideally, the choice of the cut-off value will be farmer dependent, taking the risk attitude of the farmer on missing true cases into account. To study the practicability of rank-ordering the CM alerts from an AMS according to their corresponding success rates and to investigate the effect of setting a cut-off value on the success rate, a field validation has to be performed. For this field validation, data will have to be collected on farms with AMSs.

CONCLUSIONS

Currently, all CM alerts generated by an AMS are assumed to have an equal success rate, i.e., each alert has the same likelihood of being a true-positive alert. By using cow-specific prior probabilities of CM (based on non-AMS cow information) in combination with the SN and SP of the detection system of the AMS, cow-specific success rates can be established for each alert separately. In the current study, a TAN model was developed to determine these cow-specific prior probabilities of CM. Subsequently, these cow-specific probabilities were combined with the test characteristics of an AMS to illustrate the possible range in success rates for CM alerts generated by an AMS. The illustrations indicate that using cow-specific prior probabilities allows discriminating between CM alerts by their success rates and has potential for improving the interpretation of CM alert lists. Using the success rates, the alerts on a CM alert list can be ranked-ordered, thereby providing the dairy farmer information about which cows have the highest priority for visual inspection for CM.

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Chapter *4a*

Discriminating between true-positive and false-positive clinical mastitis alerts from automatic milking systems

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ABSTRACT

Automatic milking systems (AMS) generate alert lists reporting cows likely to have clinical mastitis (CM). Dutch farmers indicated that they use non-AMS cow information or the detailed alert information from the AMS to decide whether to check an alerted cow for CM. It is, however, not yet known to what extent such information can be used to discriminate between the true-positive and false-positive alerts. The overall objective was to investigate whether a selection of the alerted cows can be made, that need further investigation for CM. For this purpose, non-AMS cow information and detailed alert information were used. During a 2-year study period, a total of 11,156 alerts for CM, including 159 true-positive alerts, were collected at one farm in the Netherlands. Non-AMS cow information on parity, days in milk, season of the year, somatic cell count history and CM history was added to each alert. In addition, six alert information variables were defined. These are the height of electrical conductivity, the alert origin (electrical conductivity, color, or both), whether or not a color alert for mastitic milk was given, whether or not a color alert for abnormal milk was given, the deviation from the expected milk yield and the number of alerts of the cow in the preceding 12-96 hours. Subsequently, naive Bayesian networks (NBN) were constructed to compute the posterior probability of an alert being truly positive based on only non-AMS cow information, based on only alert information, and based on both types of information. The NBN including both types of information had the highest area under the receiver operating characteristic curve (AUC) (0.78), followed by the NBN including only alert information (AUC=0.75) and the NBN including only non-AMS cow information (AUC=0.62). By combining the two types of information and by setting a threshold on the computed probabilities, the number of false-positive alerts on a mastitis alert list was reduced by 35%, while then 10% of the true-positive alerts will not be identified. For detecting CM cases at a farm with an AMS, checking all alerts is still the best option but will result in a high work load. Checking alerts based on a single alert information variable will result in missing too many true cases. Using a combination of alert information variables, however, is the best way to make a selection of cows that need further investigation. The effect of adding non-AMS cow information on making a distinction between true-positive and false-positive alerts will be minor.

Keywords: clinical mastitis, detection, automatic milking, dairy cow

INTRODUCTION

Mastitis is one of the most frequent and costly diseases in dairy cows (e.g., Halasa et al., 2007). Detection of clinical mastitis (CM) is important to maintain a high standard of milk quality. While with conventional milking systems, detection of CM is based on visual inspection of the milk during milking, farmers with an automatic milking system (AMS) have to rely on mastitis alert lists from the AMS for information on the udder health status of their cows (Hogeveen and Ouweltjes, 2003). These alert lists are generated from sensor measurements during milking (e.g., electrical conductivity (EC) and color measurements) and report the cows suspected of having CM. A general complaint of dairy farmers working with an AMS is the relatively large number of false-positive alerts on the mastitis alert lists. Several detection models were developed with the aim to reduce the number of false-positive alerts (e.g., De Mol and Ouweltjes, 2001; Cavero et al., 2008; Kamphuis et al., 2010). The sensitivity (SE) and specificity (SP) of these models, however, remain too low to substantially reduce the number of false-positive alerts and at the same time retain a sufficient detection of true cases.

Deciding on which alerts have the highest priority to be visually checked is difficult. In essence, all mastitis alerts given by an AMS have to be visually checked. Because of lack of time, and the annoyance about the large number of fruitless visual checks, however, in practice farmers do not check all alerts (Claycomb et al., 2009; Neijenhuis et al., 2009). Results of a large recent survey on Dutch dairy farms using AMS showed that the vast majority of farmers (65%) does not use any explicit rules for deciding upon which cows to check visually for CM (Neijenhuis et al., 2009). These farmers thus take their inspection decisions based on intuition. Furthermore, 12% of the farmers used cow information, such as a cow's SCC and CM history, and 23% of the farmers used alert information, such as the height of EC, to decide whether or not to check a particular cow (Neijenhuis et al., 2009). To which extent non-AMS cow information, alert information or both can be used to discriminate between the true-positive and false-positive alerts of a mastitis alert list is unknown.

In two recent Danish studies, non-AMS cow information was taken into account for the detection of CM in addition to sensor measurements (Chagunda et al., 2006; Friggens et al., 2007). Steeneveld et al. (2010) presented a method in which a prior probability of CM (based on parity, DIM, season, SCC history and CM history) was combined with the test characteristics (SE and SP) of the AMS detection system to discriminate between alerts in their likelihood of being a true-positive alert. In these studies, however, the additional value of non-AMS cow information to discriminate between true-positive alerts and false-positive alerts was not investigated. It was also not yet investigated whether the detailed

alert information itself can be used to discriminate between true-positive and false-positive alerts.

The overall objective of the current study was to investigate whether alerted cows can be selected that need further investigation for CM. For this purpose, Bayesian networks were used. Bayesian networks are readily constructed and allow easy computation of posterior probabilities (Jensen, 2001). For our study, several Bayesian networks were constructed for computing the posterior probability of an alert being truly positive based on non-AMS cow information only, based on just alert information, and based on both types of information.

MATERIALS AND METHODS

Data collection and herd description

From October 1, 2007, to October 1, 2009, data were collected at the Dutch research farm Waiboerhoeve (Lelystad, the Netherlands). During this period, the average herd size was approximately 500 cows. From the herd, 250 cows were milked with four AMS (Lely Industries N.V., Maassluis, the Netherlands). The other cows were milked in another barn with a conventional milking system. Due to different experiments on the farm, cows frequently changed between the two barns. All cows were housed in a free-stall barn and were of the Holstein Friesian breed. The average 305-day milk yield was 9,500 kg/cow and the average bulk milk SCC during the study period was 185,000 cells/mL.

In the 2-year study period, data were collected from all milkings of all cows milked with an AMS. These data included quarter-based EC and milk color reflection measurements (red, green and blue) measured with MQC® (Lely Industries N.V., Maassluis, the Netherlands). Information on milk yield was available at cow level. The EC measurements represented the mean value of the 20 highest measurements of a quarter milking and were available as index values. The color measurements represented the mean value of the milking. In addition to these sensor measurements, information was collected for each milking about whether the quarter milking was alerted for mastitis or not based on EC and/or color measurements. An EC alert was given for a quarter if the EC of the milk was 20% higher than that from the lowest quarter (inter-quarter ratio of 1.20) in two consecutive milkings. In addition, the inter-quarter ratio of the running average over the last three milkings had to exceed 1.20. Based on milk color measurements by the color sensor (Espada and Vijverberg, 2002), a quarter could receive the following color alerts: abnormal milk, mastitic milk, colostrum and milk with blood. During the study period no color alerts for colostrum and milk with blood were given, therefore only the alerts for abnormal milk and mastitic milk were used in the current study.

For all cows at the farm, information was collected about the occurrence of CM within the study period and in the preceding year. For the cows milked with the AMS, herd employees were instructed to check alerts two times a day. Cows appearing on the alert list were eligible for inspection. There was, however, no explicit protocol to guide employees in their decisions which alerts to visually check. The employees also used other triggers than the mastitis alert list to check cows for CM. These included clots on the filter sock and long milking intervals. These other triggers occasionally resulted in the detection of CM in cows for which no alert was given by the AMS detection system. A total of 8 employees were involved in visually checking alerts. If CM was confirmed, by obvious signs of wateriness or clots in any quarter or by other clear signs of CM such as swelling or redness, the infected quarter was treated with antibiotics. Immediately after detecting CM, the infected cow was removed from the AMS-barn to the sick cow pen and were milked twice daily with the conventional milking system for an initially unknown period of time.

For all cows at the farm, the Dutch national milk production recording system (CRV, Arnhem, the Netherlands) provided information from the four-weekly milk recordings, which included date of milk recording, test-day milk yields (kg of milk, fat and protein) and SCC measurements (cells/mL). Data from the milk production recording system were available from the study period and from the preceding year.

Data preparation

During the study period, information from 511,744 milkings of 602 different cows was collected. Milkings without any recorded milk yield (n = 2,842) were excluded from the data. In total, 227 CM cases were recorded for 148 different cows milked with the AMS.

For each milking, it was determined if any quarter of the cow had an alert based on EC and/or color. For 11,314 milkings, at least one quarter milking received an alert based on EC or color measurements (abnormal milk or mastitic milk). The average number of alerts per day was 15, with a minimum of 1 and a maximum of 48. For each of these alerts, it was determined whether it was a true-positive, a false-positive or an inconclusive alert for CM. If a cow had a single alert on the day at which CM was recorded, then that alert was assigned to be a true-positive alert. If a cow had multiple alerts on the day at which CM was recorded, then only the last alert of that day was assigned to be a true-positive alert. Because not all alerts are visually checked, it was not possible to know whether the other alerts during the 24 hours preceding the true-positive alert were true-positive or false-positive alerts. Therefore, these alerts were considered inconclusive and were excluded from the final dataset (n = 110). Some cows with CM were detected in the early morning without having been milked yet on that day. If such a cow had an alert on the previous

day, the last alert of that previous day was assigned to be a true-positive alert and the remaining alerts of that previous day were considered inconclusive and were again excluded from the final dataset (n = 48). All other alerts from the dataset were defined to be false-positive alerts.

In the resulting dataset, information from 508,744 milkings was available. A total of 11,156 milkings were alerted for CM, of which 159 were true-positive alerts and 10,997 were false-positive alerts. For 68 CM cases, no alert was given by the AMS. The CM detection performance of the AMS can thus be summarized in an SE of 70%, an SP of 97.8% and a predictive value positive of 1.4% (Table 1). Because not all milkings are checked, it must be noted that the SE and SP values were not the exact values.

Table 1. Clinical mastitis detection performance of the automatic milking system

		Clinical mastitis		
		Yes	No	
Alert	Yes	159	10,997	11,156
	No	68	497,520	497,588
		227	508,517	508,744

Six variables were defined to describe the detailed alert information. Only variables were defined that were readily available and directly usable from the alert lists currently presented to dairy farmers using AMS. Firstly, for each alert, the highest EC of the four quarters was determined and classified into the intervals ≤80, 81-90, 90-100 and >100. Secondly, for each alert it was determined whether it originated from an increased EC, from a deviated color measurement or from both. If, for instance, one quarter of a cow was alerted based on EC and another quarter was alerted based on color, then that alert was defined to be based on both EC and color. Thirdly, for each alert, it was determined whether or not a color alert for mastitic milk (Lely Industries N.V., Maassluis, the Netherlands) was given. The fourth variable describes whether or not a color alert for abnormal milk (Lely Industries N.V., Maassluis, the Netherlands) was given. Fifthly, for each alert, the deviation from the expected milk yield was determined. To determine the expected milk yield, the milk yield per hour was calculated by dividing the milk yield during a milking by the time since the last milking of a cow. The deviation from the expected milk yield was determined by comparing the milk yield per hour of the current milking with the mean milk yield per hour of the last 5 days. The deviation from the expected milk yield was classified into the intervals >40%, 30-40, 20-30, 10-20, <10% less milk than expected. Finally, for each alert, the number of alerts (0, 1, 2, 3, ≥4) for that

cow in the preceding 12-96 hours was determined. A time-window starting with 12 hours was chosen because herd employees were instructed to check alerts two times a day. Therefore, it was assumed that alerts in the 12 hours before the current alert were checked at the same time as the current alert.

Non-AMS cow information was added to each alert in the dataset, as suggested by Steeneveld et al. (2010). The parity of the cow, the DIM, the season of the year, the SCC in the previous 30 days, the SCC in the 30 days before the previous 30 days and, for multiparous cows, the geometric mean SCC from all available test-day records from the previous lactation were added to each alert. In addition, to each alert the accumulated number of CM cases of the cow in the previous 30 days and the accumulated number of CM cases of the cow in the days before the previous 30 days were added.

Logistic regression was used to determine whether true-positive and false-positive alerts differ in their non-AMS cow information and in their alert information. Each variable was investigated individually using a random cow effect to account for the repeated measurements on cows. Data preparation and logistic regression were performed using SAS version 9.1 (SAS Institute Inc., Cary, NC).

Construction of a naive Bayesian network

Bayesian networks allow different graphical structures, of varying complexity. Naive Bayesian networks (NBN) are the simplest type of Bayesian network. In veterinary research, they were described for example by Steeneveld et al. (2009). NBN consist of a single output variable that represents the possible classes for the dependent variable of a study, and a set of feature variables modeling the levels of the study's independent variables. An NBN further includes arrows from the output variable to each feature variable to describe the dependence of the latter on the output variable (Friedman et al., 1997). In the current study, the variable capturing whether a CM alert is a true-positive or a false-positive alert was the output variable. The variables describing detailed alert information and the variables capturing non-AMS cow information were feature variables.

NBN are typically constructed from data, which consists of determining prior probabilities for the output variable and of estimating conditional probabilities for the feature variables given the possible classes of the dependent variable. In this study, the prior probabilities for the output variable reflect the probability of an alert being a true-positive alert. Like these prior probabilities, the conditional probabilities for the feature variables are based on frequency counts in the data. For instance, the conditional probability of EC over 100 given that the alert is truly positive, that is, the probability Pr (EC $\geq$ 100 | true-positive alert), was computed as the proportion of alerts with an EC over 100 among the true-positive alerts.

In essence, all available feature variables can be included in an NBN. Methods exist, however, for selecting only those feature variables that best discriminate between the different classes of a dependent variable, thereby forestalling overfitting of the data (Langley and Sage, 1994). In the present study, wrapper-based backward elimination was used for selecting appropriate feature variables from all defined variables (Kohavi and John, 1997). With this method, feature variables were selected to optimize the area under the receiver operating characteristic (ROC)-curve (AUC) (these will be described in more detail in the next section) of the NBN under construction. The method started with an NBN including the output variable and all feature variables. In each subsequent step, a single feature variable was removed. The variable chosen to this end was the one which served to improve the AUC of the NBN the most, if any. The removal of feature variables was continued until the AUC of the NBN no longer improved.

To determine which information (non-AMS cow information, alert information or both) was the most valuable for discriminating between true-positive and false-positive alerts, different NBN were constructed. These were an NBN with only feature variables with non-AMS cow information, an NBN with only feature variables with alert information, and an NBN with feature variables capturing both types of information.

For the purpose of constructing and subsequently validating the NBN, the available dataset was split into a construction set and a validation set. From the dataset, the alerts of 2/3 of the cows were selected randomly for construction; the alerts of the remaining cows were included in the validation dataset. Constructing the different NBN, which included performing backward elimination and estimating prior and conditional probabilities, was done by using the Bayesian-network editing package Dazzle (Schrage et al., 2005).

Validation

For each alert from the validation dataset, the constructed NBN was used to calculate the posterior probability of the alert being truly positive, given the available non-AMS cow information and/or the alert information. For computing posterior probabilities, an NBN builds upon Bayes' rule together with the assumption that all feature variables are mutually independent given the output variable (Friedman et al., 1997). More specifically, for computing the posterior probability Pr ($c_1 \mid f_1, \ldots, f_n$) of the output c_1 given levels $f_1,\ldots,f_n$ for its n feature variables, the model uses

$$\Pr(c_1 \mid f_1,\ldots, f_n) = \frac{\prod_{i=1}^{n} \Pr(f_i \mid c_1) * \Pr(c_1)}{\sum_{j=1}^{2} \prod_{i=1}^{n} \Pr(f_i \mid c_j) * \Pr(c_j)} \quad (1)$$

where $Pr(c_1)$ is the overall prior probability of an alert being truly positive and $Pr(c_2)$ is the prior probability of an alert being a false-positive alert. The probabilities $Pr(f_i \mid c_1)$ are the conditional probabilities of finding the level f_i for the i^{th} selected feature variable given that the alert is a true-positive alert. Note that the prior probabilities $Pr(c_j)$ and the conditional probabilities $Pr(f_i \mid c_j)$ for all levels, have already been estimated from the data upon constructing the NBN and therefore are readily available in the NBN for the computation of the posterior probabilities using formula (1). Computing the posterior probabilities was done using Dazzle (Schrage et al., 2005).

To evaluate the performance of the different constructed NBN, the posterior probabilities obtained for the validation dataset were used to calculate the SE and SP of the NBN over the whole range of possible threshold probabilities for classification. ROC-curves were constructed to visualize the performance. An ROC-curve is a graphic representation of the SE versus 1-SP over the whole range of classification thresholds. To summarize the ROC-curves into a single quantity, the AUC was computed (e.g., Detilleux et al., 1999; Dohoo et al., 2003). In the current study, the AUC can be interpreted as the probability that a randomly selected true-positive alert has a higher posterior probability to be truly positive than a randomly selected false-positive alert. The ROC-curves were visualized using TIBCO S^+ version 8.1 (TIBCO Software Inc) and the AUC using the trapezoidal rule were calculated in SAS (%roc macro: http://support.sas.com/kb/25/017.html).

Subsequently, using the validation dataset with the computed posterior probabilities, different threshold values on these probabilities were set such that 95%, 90% and 80% of all true-positive alerts ended up being alerted. The effect of setting these thresholds on the number of false-positive alerts was investigated.

RESULTS

The descriptive statistics of the true-positive alerts and of the false-positive alerts are given in Tables 2 and 3. Most alerts are given for older cows and for cows later in lactation. From the non-AMS cow information, only the distribution of DIM was found to be significantly different between true-positive and false-positive alerts ($P = 0.002$). The SCC information captured in the three SCC variables did not significantly differ between true-positive and false-positive alerts. For instance, while 30% of the true-positive alerts were from cows with an SCC in the last 30 days above 500,000 cells/mL, the same information was found in 24% of the false-positive alerts ($P = 0.135$) (Table 2).

The distributions of five of the 6 variables capturing the detailed alert information were significantly ($P<0.05$) different between true-positive and false-positive alerts. The

variable whether or not a color alert for abnormal milk was given, was the single exception (Table 3). For instance, while 45% of the true-positive alerts had an EC-value over 100, this information was found in just 20% of the false-positive alerts. While 40% of the true-positive alerts had a decreased milk production of more than 30%, just 9% of the false-positive alerts had such a large decrease in milk production in comparison with the expected milk yield.

Upon constructing the three NBN, backward elimination resulted in the removal of feature variables. From the NBN with just non-AMS cow information, only the variable capturing the season of the year was removed. From the NBN containing just alert information, the variable capturing the number of alerts for the cow in the preceding 12-96 hours and the variable describing whether or not a color alert for abnormal milk was given were removed. From the NBN containing both types of information, two variables were removed by backward elimination. These were the variable capturing the mean SCC in the previous lactation and the variable whether or not a color alert for abnormal milk was given.

The ROC-curves of the three constructed NBN are presented in Figure 1. The AUC of the three NBN are reported in Table 4. The combination of non-AMS cow information and detailed alert information resulted in the highest AUC. The difference in AUC between the NBN with alert information only (AUC = 0.7499) and the NBN containing both information sources (AUC = 0.7792) was not significant (P = 0.210), but both differed significantly from the AUC of the NBN containing cow information only (AUC = 0.6175, P = 0.014 and < 0.0001 respectively).

Table 2. Number of true-positive (tp) and false-positive (fp) alerts for different levels of cow information

Cow information	tp alerts (n = 159)	fp alerts (n = 10,997)	P-value[1]
Parity			0.121
1	8 (5%)	941 (9%)	
2	58 (37%)	3,541 (32%)	
3	45 (28%)	3,325 (30%)	
≥ 4	48 (30%)	3,190 (29%)	
DIM			0.002
1-30	9 (6%)	273 (3%)	
31-60	18 (11%)	477 (4%)	
61-90	18 (11%)	726 (7%)	
91-120	13 (8%)	664 (6%)	
121-150	12 (8%)	733 (7%)	
151-180	12 (8%)	883 (8%)	
181-210	24 (15%)	822 (7%)	
≥211	53 (33%)	6,419 (58%)	
Season			0.518
January – March	48 (30%)	2,818 (26%)	
April – June	31 (20%)	2,374 (21%)	
July – September	37 (23%)	2,650 (24%)	
October – December	43 (27%)	3,155 (29%)	
SCC in last 30 days			0.135
<500,000 cells/mL	79 (70%)	6,911 (76%)	
≥500,000 cells/mL	34 (30%)	2,183 (24%)	
SCC before last 30 days			0.864
<500,000 cells/mL	94 (73%)	7,049 (77%)	
≥500,000 cells/mL	35 (27%)	2,091 (23%)	
Mean SCC previous lactation			0.501
<500,000 cells/mL	132 (89%)	9,045 (93%)	
≥500,000 cells/mL	17 (11%)	708 (7%)	
# CM[2] cases in last 30 days			0.054
0	148 (93%)	10,271 (93%)	
1	10 (6%)	654 (6%)	
2	1 (1%)	72 (1%)	
# CM[2] cases before last 30 days			0.158
0	84 (53%)	5,026 (46%)	
1	39 (24%)	3,413 (31%)	
2	36 (23%)	2,558 (23%)	

[1]Indicates whether the distribution over the levels for a cow information variable is different between true-positive and false-positive alerts, [2]CM = clinical mastitis.

Table 3. Number of true-positive (tp) and false-positive (fp) alerts for different levels of alert information

Alert information	tp alerts (n = 159)	fp alerts (n = 10,997)	P-value[1]
Electrical conductivity			<0.0001
≤80	17 (11%)	3,586 (33%)	
81-90	34 (21%)	2,270 (21%)	
90-100	36 (23%)	2,893 (26%)	
>100	72 (45%)	2,248 (20%)	
Alert origin			<0.0001
Electrical conductivity	25 (16%)	4,618 (42%)	
Color	73 (46%)	5,548 (50%)	
Electrical conductivity + color	61 (38%)	831 (8%)	
Color alert: mastitic milk			<0.0001
Yes	75 (47%)	2,346 (21%)	
No	84 (53%)	8,651 (79%)	
Color alert: abnormal milk			0.685
Yes	63 (40%)	4,256 (39%)	
No	96 (60%)	6,741 (61%)	
Decreased milk production			<0.0001
>40%	46 (29%)	609 (6%)	
30-40%	17 (11%)	303 (3%)	
20-30%	22 (14%)	702 (6%)	
10-20%	24 (15%)	1,770 (16%)	
<10%	50 (31%)	7,613 (69%)	
# alerts of cow in preceding 12-96 hours			0.020
0	80 (50%)	3,856 (35%)	
1	26 (17%)	1,790 (16%)	
2	19 (12%)	1,291 (12%)	
3	16 (10%)	980 (9%)	
≥4	18 (11%)	3,080 (28%)	

[1]Indicates whether the distribution over the levels for an alert information variable differs between true-positive and false-positive alerts.

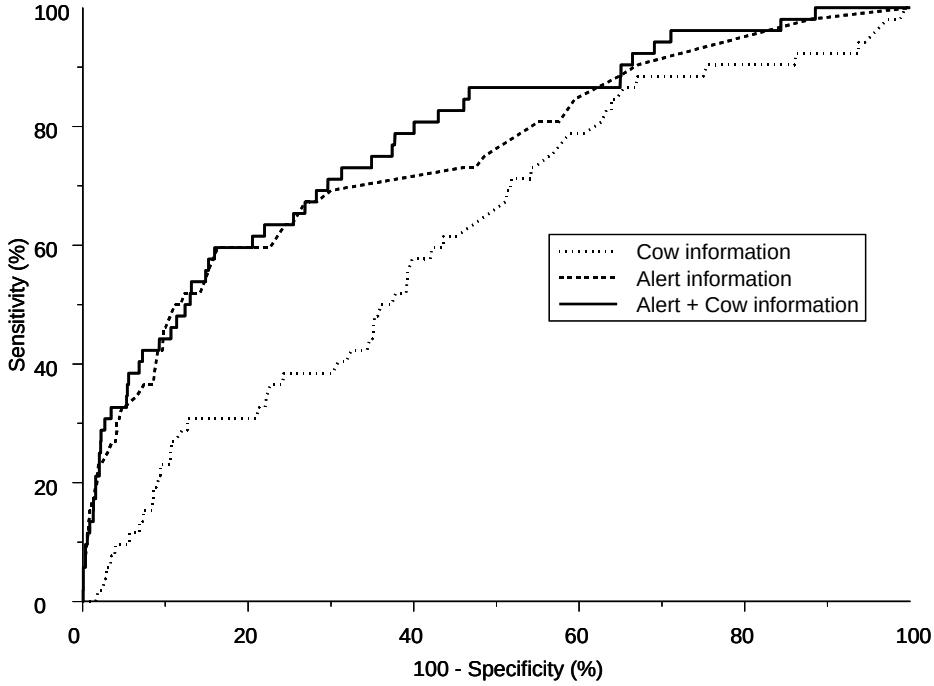

Figure 1. ROC-curves for the three developed naive Bayesian networks including different types of information

Table 4. Area under the ROC-curve (AUC) with associated standard error and confidence interval of naive Bayesian networks including different types of information

Type of information	AUC	Standard error	95% confidence interval
Cow information	0.6175	0.0387	0.5416 - 0.6934
Alert information	0.7499	0.0379	0.6756 - 0.8243
Cow + Alert information	0.7792	0.0342	0.7121 - 0.8463

The validation dataset contained 52 true-positive alerts and 3,636 false-positive alerts. Table 5 summarizes the classification results from the three NBN for these alerts, by using threshold values for which at least 95%, 90% and 80% of all true-positive alerts were identified, respectively. A threshold value on the posterior probability computed from the NBN containing both cow and alert information, for example, for which at least 95% of all true-positive alerts were identified, resulted in 2,527 false-positive alerts. Compared to the original 3,636 false-positive alerts, this was a reduction of 31%. A threshold value set such that at least 95% of all true-positive alerts were identified, but this time using the NBN with non-AMS cow information only, resulted in 3,493 false-positive alerts, this was a reduction of 4%. A threshold value on the posterior probability computed from the NBN containing both types of information for which at least 80% of all true-positive alerts were identified, resulted in 1,568 false-positive alerts, which meant a reduction of 57% when compared to the original 3,636 false-positive alerts.

Table 5. The effect of three different probability threshold values on the number of true-positive (tp) and false-positive (fp) alerts. The reduction in the number of true-positive and false-positive alerts compared to the original numbers is given between brackets

	Cow information		Alert information		Cow + Alert information	
	tp	fp	tp	fp	tp	fp
Original number	52	3,636	52	3,636	52	3,636
Threshold value A[1]	50 (4%)	3,493 (4%)	50 (4%)	2,723 (25%)	50 (4%)	2,527 (31%)
Threshold value B[2]	47 (10%)	2,939 (19%)	47 (10%)	2,439 (33%)	47 (10%)	2,363 (35%)
Threshold value C[3]	42 (19%)	2,186 (40%)	42 (19%)	1,684 (54%)	42 (19%)	1,568 (57%)

[1]Highest value such that at least 95% of all true-positive alerts were identified.
[2]Highest value such that at least 90% of all true-positive alerts were identified.
[3]Highest value such that at least 80% of all true-positive alerts were identified.

DISCUSSION

The test characteristics of the AMS in the current study were an SE of 70% and an SP of 97.8%. Since an SE of at least 70% combined with an SP of at least 99% is desired (Mein and Rasmussen, 2008), the CM detection performance of this AMS is suboptimal. Although in a number of previous studies new detection models were developed (e.g., De Mol and Ouweltjes, 2001; Cavero et al., 2008; Kamphuis et al., 2010), these models were unable to improve detection performance to the extent that the detection of CM cases remained satisfactory and the number of false-positive alerts was reduced to a reasonable level. As a consequence of the suboptimal detection performance, and of the unsatisfactory SP more specifically, the interpretation of the alert lists is difficult. For instance, the current alert lists contain a large number of false-positive alerts and it is not possible to select those cows that have a priority for visual checking. In our study, no new detection model was developed. The aim was to investigate whether from the alert lists cows needing further investigation for CM can be selected based on non-AMS cow information, on alert information or on both.

For farmers milking with an AMS, handling the mastitis alert lists generated by the AMS is daily practice. The majority of Dutch farmers milking with an AMS make their inspection decisions based on intuition. Only a minority of the farmers indicated that they use non-AMS cow information or detailed alert information to decide which cows to check visually (Neijenhuis et al., 2009). Results of our study show that checking alerts based on a single alert variable is not satisfactory. For instance, visual checking of just the alerts with an EC over 100 would result in missing 55% of the true-positive alerts and checking of just the newly alerted cows without any alerts in the preceding 12-96 hours, would result in missing 50% of the true-positive alerts (Table 3). The results of our study show that a combination of variables capturing alert information is necessary to make a meaningful selection of alerts for visual checking. In fact, using a combination of 4 alert variables (height of EC, alert origin, color alert for mastitic milk and expected milk production) proved to be very useful in discriminating between the true-positive and false-positive alerts from a mastitis alert list. This combination resulted in a reduction of the number of false-positive alerts by 33% at the cost of missing or detecting later 10% of the true-positive alerts (Table 5). Combining both non-AMS cow information and detailed alert information served to even further reduce the number of false-positive alerts (Table 5).

Using only non-AMS cow information to discriminate between alerts was not very useful (Tables 4 and 5). The idea of using non-AMS cow information to discriminate between alerts, originated from the observation that cows having CM differ in several aspects from cows not having CM (e.g., Barkema et al., 1998; Steeneveld et al., 2008; Steeneveld et al.,

2010). Based on that finding, better performance from the NBN with only non-AMS cow information was expected than was actually found in the present study. The true-positive alerts indeed differed in their cow information from the true-negative milkings. Especially the SCC history was found to be significantly different between true-positive and true-negative milkings (data not shown). Within the group of alerted cows, however, the SCC histories of the true CM cases and of the false alerts proved to be no longer significantly different (Table 3). Most likely, the AMS had given many alerts for cows with subclinical mastitis. These alerts were not given without reason, but these cows were not (yet) clinically diseased. The lack of significant differences in non-AMS cow information between the true-positive and false-positive CM alerts explains the relatively low AUC of the constructed NBN based on non-AMS cow information only.

Upon constructing the three NBN, backward elimination resulted in the removal of some variables from the NBN. For instance, from the NBN containing only alert information, the variable capturing the number of alerts for the cow in the preceding 12-96 hours and the variable describing whether or not a color alert for abnormal milk was given, were removed. Because these variables were the least significant in the univariate analysis (Table 3), it was not unexpected that they were removed. The presence of all other feature variables in the NBN indicates that each of these variables, in the presence of the previously selected variables served to contribute to the model's discriminative performance. It further indicates that the selected variables do not exhibit a large overlap in the information they contribute.

The method for discriminating between true-positive and false-positive alerts as presented in the current paper is based on the use of a relatively simple Bayesian network. Bayesian networks have been studied extensively and are being widely applied in human medicine (e.g., Chapman et al., 2005). Applications are not yet common in veterinary science, but they are gaining popularity (e.g., Otto and Kristensen, 2004; Jensen et al., 2009; Steeneveld et al., 2009). The NBN used in this study constitute the simplest type of Bayesian network. Despite their simplicity, these models are surprisingly effective showing good classification performance even if the independence assumption for the feature variables does not hold in the data (Friedman et al., 1997). In the current study, Bayesian networks of increasing complexity were developed as well. More specifically, tree-augmented NBN, which include dependencies between the feature variables (Friedman et al., 1997), were constructed and validated. The more sophisticated dependency structures, however, did not result in significantly higher AUC than the ones obtained with the simple NBN (data not shown).

The posterior probabilities computed from the NBN in this study can be combined with the current detection algorithms in several different ways. For instance, it is possible to

add a posterior probability to each of the alerts currently given by the AMS. The farmer then is provided with additional information to decide which alerts have the highest priority for visual checking. Another possibility is to only present the alerts from the current alert lists which have a posterior probability of being truly positive above a particular threshold value. In this way, fewer alerts are presented to the farmer, and fewer false-positive alerts more specifically. It can not be prevented that for some of the true CM cases no alert will be given and consequently, these CM cases will be missed or later detected by the farmer. The risk attitude of the farmer, with respect to missing CM cases versus checking large numbers of false-positive alerts, can be incorporated by allowing the farmer to adjust the threshold value himself. For instance, the threshold values can lowered if the bulk milk SCC is trending upwards (Claycomb et al., 2009). For actual implementation, software is required for combining all available information into a posterior probability and subsequently comparing this probability against a pre-set farmer-specific threshold value.

For a total of 68 CM cases, no alert was given by the AMS (Table 1). The majority of these cases were non-alerted as a consequence of the use of a small time-window. Only the last alert on the day at which CM was recorded, was considered as a true-positive alert. The definition of a proper time-window is difficult and is still under debate (Sherlock et al., 2008). In our study, using a wider time-window resulted in more true-positive alerts and fewer CM cases without an alert. For instance, considering also alerts from the day before the day at which CM was recorded as true-positive alerts resulted in 28 CM cases instead of 68 CM cases without an alert. We decided, however, to use a small time-window because of the systematic way of working on the research farm. Moreover, Sherlock et al. (2008) also argued that using small time-windows for CM detection is more realistic and better for application in practice. The use of other triggers than the alert list for visually checking cows for CM is an explanation for the detection of the non-alerted CM cases.

The herd employees indicated that approximately 40% of the alerts were visually checked. Because not all milkings and not all alerts were checked visually, it was not possible to calculate the exact values for the SE and SP. It is possible that non-alerted cows had CM, and remained undetected. So, in fact some assigned true-negative milkings may have been false-negative. The herd employees did, however, also use other triggers than the alert list to detect CM, thereby minimizing the number of undetected CM cases. Also some true-positive alerts for CM may have been missed and consequently, some alerts may have been incorrectly assigned as false-positive alerts. Although there were some missed CM cases, the effect of these missed CM cases on the conclusions would be limited. Moreover, we believe that the systematic way of working on this research farm, and the serious consideration of the alert lists have minimized the missing of true-positive alerts.

Because our research farm was used for several studies, the proportion of cows at risk for CM may have been somewhat different from that at commercial Dutch farms. For instance, heifers were more frequently housed in the other barn, cows did not enter the AMS barn in the colostrum period and cows detected with CM were removed from the AMS barn. These observations explain the relatively small numbers of heifers and of cows in the first weeks of lactation with alerts (Table 2). Most likely, the cows with chronic mastitis remained in the barn with the conventional milking system. In proportion, therefore, totally new CM cases occurred more frequently in cows milked with the AMS. Because of these specific characteristics of the research farm, it is not possible to generalize the developed model to other farms just like that. For future implementation, it will be necessary to construct farm-specific models or a more generic model based on a variety of farms.

For the detection of CM cases on a farm milking with an AMS, checking all alerts is still the best option. Checking all alerts, however, is often not feasible because of the higher work load caused by the large number of false-positive alerts. Selecting alerts for visual checking based on a single alert information variable, for instance only checking alerts with EC values over 100, resulted in missing too many true-positive alerts. To reduce both the work load and the annoyance of fruitless visual checks, a selection of cows requiring further inspection is best based on a combination of alert information. The effect of using non-AMS cow information on making a distinction between true-positive and false-positive alerts proved to be minor in our study. NBN can very well be used to combine the variables to select cows for visual checking.

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Chapter *4b*

The effect of adding cow information to a clinical mastitis detection model using sensor information from automatic milking systems

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ABSTRACT

Using a probability of having clinical mastitis (CM) based on sensor measurements given by an automatic milking system (AMS) in combination with a probability of having CM based on non-AMS cow information was expected to improve CM detection. Based on data of 9 Dutch commercial dairy farms, a CM detection model was developed. The model provided for each cow milking the probability that the cow milking is a mastitis one, based on sensor measurements of electrical conductivity, color and milk yield. In total, 47,049 cow milkings, including 99 milkings for which CM was reported, were available with a probability of having CM based on the sensor measurements. To each of these cow milkings, non-AMS cow information (parity, day in milk, season, somatic cell count history, and CM history) was added. After adding a probability of having CM based on the non-AMS cow information, a posterior probability of having CM was calculated for each cow milking. To evaluate the CM detection performance by adding non-AMS cow information, the posterior probabilities were used to calculate the sensitivity and specificity over the whole range of possible threshold probabilities for classification. ROC-curves were constructed to visualize the performance. To summarize the ROC-curves into a single quantity, the AUC was computed. The AUC based on the probability of having CM based on sensor measurements only was compared with the AUC based on the posterior probability. The AUC was 83.0 for the CM detection model based on only sensor measurements, while the AUC based on sensor measurements and non-AMS cow information was 80.8. Results of the current study show that adding non-AMS cow information to all milkings did not improve CM detection performance. So, if probabilities based on sensor measurements are available, adding non-AMS cow information showed no additional value for the detection of CM.

INTRODUCTION

Using a probability of having clinical mastitis (CM) based on sensor measurements given by an automatic milking system (AMS) in combination with a probability of having CM based on non-AMS cow information was expected to improve CM detection (Steeneveld et al., 2010a). However, Steeneveld et al. (2010b) reported a minor effect of using non-AMS cow information (parity, day in milk (DIM), somatic cell count (SCC) history, and CM history) on making a distinction between true-positive and false-positive mastitis alerts. This minor effect was explained by the fact that within the group of alerted cows, amongst others the SCC history of the true-positive alerts was not significantly different from the false-positive alerts. In that study, the effect of adding non-AMS cow information to only those cow milkings with mastitis alerts was investigated. It was not investigated if adding non-AMS cow information to all milkings could improve CM detection. Moreover, reported results were based on data from a single research farm, where the proportion of cows at risk for CM differed from commercial Dutch dairy farms (Steeneveld et al., 2010b).

Based on data of 9 commercial dairy farms in the Netherlands, a new CM detection model has been developed by Kamphuis et al. (2010). That new CM detection model was capable of keeping the sensitivity (SE) at about the same level as models currently used by AMS, but at the same time to decrease the number of false-positive alerts by more than 50%. The model provided a probability for having CM at quarter milking level, based on sensor measurements of electrical conductivity, color and milk yield. The CM detection performance of the described model was highly farm dependent, with SE levels ranging between 0.0 and 71.4% at a fixed specificity (SP) of 99% for the 9 farms.

The objective of the current study is to determine whether CM detection can be improved by adding non-AMS cow information to the sensor measurement based CM detection model developed by Kamphuis et al. (2010). CM detection performance will be determined for 9 farms together and for each farm separately. CM detection performance of a CM detection model including only sensor information will be compared with a CM detection model including both sensor information and non-AMS cow information.

MATERIALS AND METHODS

A CM detection model based on sensor measurements

A detailed description of the development and validation of a new CM detection model, using decision-tree induction, is provided by Kamphuis et al. (2010). In short, sensor measurements of electrical conductivity, color and milk yield were collected for each

quarter milking at 9 commercial dairy farms in the Netherlands using a total of twelve Lely Astronaut AMS (version A2 (n = 10) or A3 (n = 2); Lely Industries N.V., Maassluis, the Netherlands). These sensor measurements were used to define descriptive variables (n = 1,065) as described in Kamphuis et al. (2008). These independent variables described characteristics (level, variability and shape) of the sensor measurement patterns from each quarter milking.

A decision-tree was trained using 24,960 quarter milkings from 404 cows. From these quarter milkings, 243 had CM as observed and recorded by the participating farmers. All others had a very low likelihood of having CM: these quarter milkings had SCC information (from the milk production test day system) and belonged to cows that never exceeded a SCC level of 200,000 cells/mL within the lactation, and in addition were never visually checked by the farmers during the whole study period. The training procedure used decision-tree induction as base classifier and combined it with bagging. Bagging is a commonly used data mining technique that is able to improve detection performance of a classification model (Witten and Frank, 2005). Data was validated using data from cows that were not used for training. In addition, the test set also included quarter milkings with a less clear mastitis status: all quarter milkings outside a 2-week range from a CM case were considered as negative for having CM. A random sample of 50,000 of these quarter milkings was then used for testing. The final test set included 50,000 quarter milkings being negative for CM and 105 quarter milkings with CM from 368 cows. The decision-tree model provided probability estimates for having CM for each of the 50,105 quarter mikings in the test set.

The non-AMS cow information is at cow level, so it was necessary to transform the probability for having CM at quarter milking level to a probability estimate at cow milking level. To do so, all quarter milkings of the cow were added to the quarter milking originally in the test dataset. These added quarter milkings also received a probability of having CM determined by the CM detection model developed by Kamphuis et al. (2010). A cow level probability for CM ($P_{cowmilk}$) was calculated using the probabilities for CM of the quarter milkings as follows:

$$P_{cowmilk} = P_{Q1} + (1-P_{Q1})*P_{Q2} + (1-P_{Q1})*(1-P_{Q2})*P_{Q3} + (1-P_{Q1})*(1-P_{Q2})*(1-P_{Q3})*P_{Q4} \quad (1)$$

where P_{Q1}, P_{Q2}, P_{Q3} and P_{Q4} are the probabilities of having CM for each of the four quarter milkings of the cow, respectively. Subsequently, per cow milking, one record remained which resulted in a dataset of 47,111 cow milkings, each with a probability of having CM at cow milking level. To all these cow milkings, the non-AMS cow information on parity, month in lactation, the season of the year, the SCC in the previous 30 days, the SCC in the 30 days before the previous 30 days and, for multiparous cows, the geometric mean SCC

from all available test-day records from the previous lactation were added. In addition, for each cow milking the accumulated number of CM cases of the cow in the previous 30 days and the accumulated number of CM cases of the cow in the days before the previous 30 days were added. For 62 cow milkings, it was not possible to add non-AMS cow information, and therefore these milkings were excluded for further analyses. The final test set, used for analyses in the current study, consisted of 47,049 cow milkings with in total 99 CM cases. The test dataset was constructed for all farms together and for the 9 farms separately. Creating the test dataset was performed using SAS version 9.1 (SAS Institute Inc., Cary, NC).

Adding non-AMS cow information using a naive Bayesian network

To update the probabilities for having CM based on the CM detection model developed by Kamphuis et al. (2010) with non-AMS cow information, a naive Bayesian network (NBN) was constructed. An NBN determines conditional probabilities for the feature variables given the possible classes of the class variable (Friedman et al., 1997). In the current study, the variable capturing whether a cow had CM or not at a particular DIM was used as the class variable. The variables describing non-AMS cow information were feature variables. To construct the NBN, a dataset including each DIM for all cows not present in the test set of Kamphuis et al. (2010) was used. That dataset was established using data provided by the Dutch national milk production recording system (CRV, Arnhem, the Netherlands), including information from the monthly milk recording. For each DIM the following feature variables were defined: parity, month in lactation, the season of the year, the SCC in the previous 30 days, the SCC in the 30 days before the previous 30 days and, for multiparous cows, the geometric mean SCC from all available test-day records from the previous lactation. In addition, for each DIM the accumulated number of CM cases of the cow in the previous 30 days and the accumulated number of CM cases of the cow in the days before the previous 30 days were determined (Steeneveld et al., 2010a). The final set for constructing the NBN consisted of 260,046 DIM, including 261 DIM for which CM was recorded. The descriptive statistics of this dataset used for constructing the NBN are presented in Table 1.

NBN based on the whole dataset and for the 9 farms separately were constructed. Creating the dataset for constructing the NBN was performed using SAS. Constructing the NBN, which included estimating conditional probabilities, was done by using the Bayesian-network editing package Dazzle (Schrage et al., 2005).

Table 1. Number of clinical mastitis (CM) cases for different levels of cow information in the dataset used for constructing the naive Bayesian network. The P-value indicates whether the distribution over the levels for a cow information variable is different between CM and no CM cases.

Cow information	CM = 1 (n = 261)	CM = 0 (n = 259,785)	P-value
Parity			<0.0001
1	34 (13%)	97,242 (37%)	
2	88 (34%)	72,139 (28%)	
3	47 (18%)	36,348 (14%)	
≥ 4	92 (35%)	54,056 (21%)	
Day in milk			0.025
1-30	29 (11%)	23,800 (9%)	
31-60	34 (13%)	23,646 (9%)	
61-90	22 (9%)	23,431 (9%)	
91-120	32 (12%)	23,338 (9%)	
121-150	24 (9%)	22,603 (9%)	
151-180	26 (10%)	21,629 (8%)	
181-210	26 (10%)	20,415 (8%)	
≥211	68 (26%)	100,923 (39%)	
Season			0.012
January – March	69 (26%)	72,618 (28%)	
April – June	54 (21%)	63,983 (25%)	
July – September	82 (31%)	54,904 (21%)	
October – December	56 (22%)	68,280 (26%)	
SCC in last 30 days			<0.0001
<200,000 cells/mL	89 (34%)	162,439 (63%)	
≥200,000 cells/mL	130 (50%)	53,436 (21%)	
SCC before last 30 days			<0.0001
<200,000 cells/mL	102 (39%)	163,399 (63%)	
≥200,000 cells/mL	105 (40%)	50,025 (19%)	
Mean SCC previous lactation			<0.0001
<200,000 cells/mL	88 (34%)	73,607 (28%)	
≥200,000 cells/mL	62 (24%)	30,485 (12%)	
# CM cases in last 30 days			0.169
0	236 (90%)	253,580 (98%)	
1	20 (8%)	5,906 (2%)	
2	5 (2%)	299 (0%)	
# CM cases before last 30 days			0.335
0	179 (69%)	228,676 (88%)	
1	53 (20%)	22,358 (9%)	
2	29 (11%)	8,751 (3%)	

Validation

For each cow milking in the test set, the constructed NBN was used to calculate a posterior probability of the cow milking being positive for CM (P_{cow}), based on the prior probability of a cow having CM ($P_{cowmilk}$), as calculated with formula 1, and the added non-AMS cow information. For computing the posterior probability P_{cow} ($c \mid f_1, \ldots, f_n$) of the output c given levels $f_1,\ldots,f_n$ for its n feature variables, the model uses

$$P_{cow}(c \mid f_1,..., f_n) = \frac{\prod_{i=1}^{n} P(f_i \mid c_1) * P_{cowmilk}(c_1)}{\sum_{j=1}^{2} \prod_{i=1}^{n} P(f_i \mid c_j) * P_{cowmilk}(c_j)} \quad (2)$$

where $P_{cowmilk}(c_1)$ is the prior probability of cow milking being positive for CM and $P_{cowmilk}(c_2)$ is the prior probability of a cow milking being free of CM. The probabilities $P(f_i \mid c_1)$ are the conditional probabilities of finding the level f_i for the i^{th} feature variable given that milking is positive for CM. The conditional probabilities $P(f_i \mid c_j)$ for all levels for the i^{th} selected feature have already been estimated from the training data upon constructing the NBN and therefore are readily available in the NBN for the computation of the posterior probabilities using formula (2). Computing the posterior probabilities for each cow milking was done using Microsoft Excel.

To evaluate the CM detection performance by adding non-AMS cow information, the posterior probabilities (P_{cow}) obtained for the validation dataset were used to calculate the SE and SP over the whole range of possible threshold probabilities for classification. ROC-curves were constructed to visualize the performance. To summarize the ROC-curves into a single quantity, the AUC was computed (e.g., Dohoo et al., 2003). The AUC based on P_{cow} was compared with the AUC based on $P_{cowmilk}$. The difference between SE levels at 2 fixed SP levels was tested for significant difference using McNemar's test in SAS. Also at fixed SE levels the SP levels were compared. Validation was performed for all farms together and for each of the 9 farms separately.

RESULTS AND DISCUSSION

For construction of the NBN, the dataset as described in Table 1 was used. The non-AMS cow information variables on parity, DIM, season and SCC history variables were found to be significantly different between CM cases and non-CM cases. For instance, while 50% of the CM cases were from cows with an SCC in the last 30 days above 200,000 cells/mL, the same information was found in only 21% of the non-CM cases ($P < 0.0001$) (Table 1).

Although the significant difference in having CM and not having CM for different non-AMS cow information variables (Table 1), adding these variables to the sensor measurements given by the AMS did not improve CM detection. Based on data of all 9 herds, the AUC was 83.0 for the CM detection model based on only sensor measurements, while the AUC based on sensor measurements and non-AMS cow information was 80.8 (Table 2). For the farm-specific CM detection models, the AUC was ranging between 63.6 and 89.4 when non-AMS information was added. Except for herd 4, these AUC-values were lower than the AUC-values for the CM detection model based on only sensor measurements (Table 2). Previously it was reported that adding non-AMS cow information to alerted cows did not improve the ability to discriminate between true-positive and false-positive mastitis alerts (Steeneveld et al., 2010b). Results of the current study showed that adding non-AMS cow information to all cow milkings also did not improve CM detection performance. So, if probabilities based on sensor measurements are available, adding non-AMS cow information showed no additional value for the detection of CM.

Table 2. Area under the curve (AUC) based on a clinical mastitis (CM) detection model including sensor measurements ($P_{cowmilk}$), and for a clinical mastitis detection model including sensor measurements and non-AMS cow information (P_{cow}).

Herd[1]	# cow milkings	# CM cases	AUC ($P_{cowmilk}$)	AUC (P_{cow})
all	47,049	99	83.0	80.8
1	6,834	26	92.3	89.0
2	3,104	16	72.8	70.4
3	3,822	5	79.3	69.1
4	3,614	14	87.9	89.4
5	8,284	11	73.4	63.6
6	9,816	9	82.1	81.9
7	6,364	14	79.3	74.6

[1]For 2 farms the AUC was not calculated because they had only 2 CM cases in the test set.

The CM detection model based on only sensor measurements showed an SE of 26.3% at an SP of 99% and an SE of 37.4% at an SP of 97.9% (Table 3). The CM detection model based on sensor measurements and non-AMS cow information showed lower SE-values at both SP-levels. Also for the herd-specific CM detection models, SE-values were lower when non-AMS cow information was added at a fixed SP of 99%. It must be noted that the herd-specific CM detection models were based on a small amount of CM cases, for

instance, for herd 3 only 5 CM cases were present in the test set. At a fixed SP of 99%, the SE of 26.3% is much lower than the SE of 40% found by Kamphuis et al. (2010). This can be explained by the fact that the current study is performed at cow milking level, while the study of Kamphuis et al. (2010) was performed at quarter milking level, which makes comparison of SP and SE values very difficult. For instance, reaching a SP of 99% at cow milking level is more difficult than reaching a SP of 99% at quarter milking level. Subsequently, comparison of the SE at a fixed SP level does not give a good indication.

By adding non-AMS cow information, the SP values at fixed SE-levels did also not improve (Table 4). For instance, based on sensor measurements only, the SP was 95.7% at a fixed SE-level of 50%, while the SP decreased to 94.5% when non-AMS cow information was added (P<0.0001). Also for all herd specific CM detection models, the SP at a fixed SE of 50%, were significantly lower when non-AMS cow information was added. For 2 herds, the SP at a fixed SE of 70% were higher (P<0.0001). The reason for significant differences in SP between CM detection models including only sensor information and CM detection models including both sensor information and non-AMS cow information was the large amount of milkings without CM (n = 46,950).

Table 3. Sensitivity (SE) at a specificity of 99% (SE^{SP99}) and at a specificity of 97.9% ($SE^{SP97.9}$) for the clinical mastitis detection model including sensor measurements ($P_{cowmilk}$) and the clinical mastitis detection model including sensor measurements and non-AMS cow information (P_{cow}).

Herd[1]	SE^{SP99} ($P_{cowmilk}$)	SE^{SP99} (P_{cow})	P-value[2]	$SE^{SP97..9}$ ($P_{cowmilk}$)	$SE^{SP97..9}$ (P_{cow})	P-value[2]
all	26.3	20.2	0.157	37.4	29.3	0.033
1	34.6	23.1	0.257	38.5	30.8	0.414
2	12.5	12.5	1	12.5	12.5	1
3	60	40	0.317	60	40	0.317
4	71.4	28.5	0.014	78.6	50	0.046
5	9.1	0	0.317	9.1	18.2	0.317
6	44.4	33.3	0.564	66.6	55.5	0.317
7	14.3	14.3	1	14.3	21.4	0.317

[1]For 2 farms no SE-values were calculated because they had only 2 clinical mastitis cases in the test set.
[2]Indicates whether the SE is statistically significantly different between the CM detection model including sensor measurements ($P_{cowmilk}$) and the CM detection model including both sensor measurements and non-AMS cow information (P_{cow}).

Table 4. Specificity (SP) at a sensitivity of 50% (SP^{SE50}) and at a sensitivity of 70% (SP^{SE70}) for the clinical mastitis detection model including sensor measurements ($P_{cowmilk}$) and the clinical mastitis detection model including sensor measurements and non-AMS cow information (P_{cow}).

Herd[1]	SP^{SE50} ($P_{cowmilk}$)	SP^{SE50} (P_{cow})	P-value[2]	SP^{SE70} ($P_{cowmilk}$)	SP^{SE70} (P_{cow})	P-value[2]
all	95.7	94.5	<0.0001	91.6	89.6	<0.0001
1	96.2	94.5	<0.0001	93.4	90.0	<0.0001
2	87.2	81.5	<0.0001	69.5	75.6	<0.0001
3	99.2	81.2	<0.0001	91.7	49.0	<0.0001
4	99.8	98.6	<0.0001	99.1	94.9	<0.0001
5	94.5	56.5	<0.0001	89.1	43.7	<0.0001
6	98.7	98.5	0.0196	95.2	94.4	0.0001
7	93.6	91.4	<0.0001	87.5	89.3	<0.0001

[1]For 2 farms no SP-values were calculated because they had only 2 clinical mastitis cases in the test set.
[2]Indicates whether the SP is statistically significantly different between the CM detection model including sensor measurements ($P_{cowmilk}$) and the CM detection model including both sensor measurements and non-AMS cow information (P_{cow}).

Besides the CM detection model based on the described NBN, an NBN was constructed using an SCC threshold of 500,000 cells/mL instead of an SCC threshold of 200,000 cells/mL for the three SCC history variables. In addition, also an NBN was developed using backward elimination of feature variables. This resulted in an NBN without the feature variable “season of the year”. The AUC of the CM detection model based on an SCC threshold of 500,000 cells/mL and the AUC of the CM detection model developed with the backward NBN were lower than the AUC-values presented in Table 2 (data not shown). This indicates that adding non-AMS cow information with different constructed NBN did also not to improve CM detection performance.

To develop an NBN, also forward selection of feature variables was performed. This resulted, however, in an NBN without any feature variable. This indicates that each of the feature variables on non-AMS cow information was not able to increase the AUC of the NBN under construction. So, this denotes that, although the significant differences between CM cases and no CM cases in non-AMS cow information, the variables on non-AMS cow information did not contain enough discriminative power to distinguish between having CM and not having CM.

CONCLUSIONS

The results of the current study show that, in presence of sensor information, detection of CM cannot be improved by adding non-AMS cow information. Test characteristics of the CM detection model including sensor measurements and non-AMS cow information showed lower performance than the CM detection model including only sensor measurements.

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Chapter 5

Providing probability distributions for the causal pathogen of clinical mastitis using naive Bayesian networks

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ABSTRACT

Clinical mastitis (CM) can be caused by a wide variety of pathogens and a farmer will have to start treatment before the actual causal pathogen is known. By providing a probability distribution for the causal pathogen, naive Bayesian networks (NBN) can serve as a management tool for a farmer to decide which treatment to use. The advantage of providing a probability distribution for the causal pathogen, rather than only providing the most likely causal pathogen, is that the uncertainty involved is visible for a farmer and a more informed treatment decision can be made. The objective of this study was to illustrate provision of probability distributions for the Gram-status and for the causal pathogen for CM cases to a farmer. For constructing the NBNs, data were used from 274 Dutch dairy herds in which the occurrence of CM was recorded over an 18-month period. The dataset contained information on 3,833 CM cases. Two-thirds of the dataset were used for the construction process and one-third was retained for validation. One NBN was constructed with the CM cases classified according to their Gram-status, and another was built with the CM cases classified into streptococci, *Staphylococcus aureus* or *Escherichia coli*. Information usually available at a dairy farm was included in both NBNs (parity, month in lactation, season of the year, quarter position, SCC and CM history, being sick or not, and color and texture of the milk). Accuracy was calculated to obtain insight in the quality of the constructed NBNs. The accuracy of classifying CM cases into Gram-positive or Gram-negative pathogens was 73%, while the accuracy of classifying CM cases into streptococci, *Staph. aureus* or *E. coli* was 52%. Since only CM cases with a high probability for a single causal pathogen will be considered for pathogen-specific treatment, accuracies based on only classifying CM cases above a particular probability threshold were determined. For instance, for CM cases in which either Gram-negative or Gram-positive had a probability >0.90, classification according to the Gram-status reached an accuracy of 97%. We found that the greater the probability for a particular pathogen was for a CM case, the more accurate a classification of this case as being caused by this pathogen. The probability distributions provided by the NBNs as well as the associated accuracies for varying classification thresholds provide the farmer with considerable insight about the most likely causal pathogen for a CM case and the uncertainty involved.

Key words: clinical mastitis, diagnosis, pathogen, dairy

INTRODUCTION

Mastitis is one of the most frequent and costly diseases in a dairy herd (e.g., Halasa et al., 2007). A large proportion of the total cost of mastitis is caused by clinical mastitis (CM) (Huijps et al., 2008). Effective treatment of CM is important to eliminate and prevent recurrence of IMI, and to identify effective methods of control based on limited or no use of antibiotics to reduce the likelihood of resistance (Hillerton and Kliem, 2002).

Knowing the causal pathogen, and subsequently using appropriate treatment, serves to increase the cure rate of CM (Barkema et al., 2006; McDougall et al., 2007). While bacteriological culturing will provide information about the causal pathogen, this information typically comes too late, because CM needs treatment immediately after diagnosis. In practice therefore, a choice of treatment needs to be made in the absence of culture results. Several other sources of information are available on a dairy farm that could aid in indicating the causal pathogen of a CM case to some extent. Particular pathogens more frequently cause CM in some seasons of the year than in other seasons (Makovec and Ruegg, 2003; Olde Riekerink et al., 2007). Also, cow-specific factors such as parity, DIM, test-day SCC and CM history are a valuable source of information (Zadoks et al., 2001; De Haas et al., 2004). Finally, clinical signs such as the appearance of the milk and the demeanor of the cow can be used to aid indicating the causal pathogen (Jones and Ward, 1990; Milne et al., 2003).

In previous studies, various classification models for pathogen identification for CM were constructed. However, the predictive performance of these models varied strongly (e.g., Jones and Ward, 1990; Kim and Heald, 1999; Milne et al., 2003). A further disadvantage of these models was that they only returned the most likely causal pathogen to a farmer. For choosing among treatment options, a probability distribution for the causal pathogens of a CM case would be more informative as it reveals the uncertainty involved in the classification. For instance, almost equal probabilities for 2 or more causal pathogens would support the decision for a broad spectrum antibiotic treatment while a very high probability for a particular pathogen would support the choice for a more specific treatment. As an example, for Gram-negative CM cases (*Escherichia coli* and *Klebsiella* spp.), supportive treatment is more appropriate than antimicrobial treatment (Morin et al., 1998; Pyörälä and Pyörälä, 1998).

Hogeveen et al. (1994) suggested the use of Bayesian networks for the task of pathogen identification for CM. A Bayesian network includes a collection of stochastic variables representing sources of information and can generate posterior probability distributions for any outcome variable of interest. Naive Bayesian networks (NBN) are the simplest type of Bayesian network. These networks are well known for their powerful performance on

classification tasks (Friedman et al., 1997). NBNs have been studied extensively and are being widely applied in human medicine (e.g., Kukar et al., 1999; Blanco et al., 2005; Chapman et al., 2005). Applications are still relatively rare in veterinary medicine, but they are gaining in popularity (McKendrick et al., 2000; Geenen et al., 2005; Kuncheva et al., 2007).

The objective of this study was to illustrate the value of providing probability distributions for the Gram-status and for the causal pathogen for CM cases to a farmer to take a more informed treatment decision. NBNs were used for computing case-specific probability distributions. These networks were created automatically from data. A dataset of 3,833 CM cases was used with information about the causal pathogen and associated information on clinical signs, cow factors and season.

MATERIALS AND METHODS

Available data

The data available for the present study were described in detail elsewhere (Barkema et al., 1998). In short, data on CM were collected from 274 dairy herds entering the study between December 1992 and June 1994; each herd participated for approximately 1.5 years. All herds had an annual milk production quota between 300,000 and 900,000 kg, and had cows of the Holstein-Friesian or Dutch Friesian breeds. The herd size had a mean of 75 cows and ranged from 40 to 143 cows. Lactating cows were housed in a free-stall barn during winter and were milked in a double-herringbone or two-sided tandem parlor. During the study, farmers were asked to collect milk samples from cows with signs of CM. The samples were taken before treatment, stored in a freezer at the farm (at approximately -20°C), and collected every 6 to 8 weeks for bacteriological culturing. For each CM case, the farmer further provided information on cow identification, date of occurrence, infected quarter, and whether the cow was sick at the moment of CM. In the laboratory, bacteriological culturing of the milk samples was performed according to the standards of the National Mastitis Council (Harmon et al., 1990), and the causal pathogen was determined. Furthermore, the texture and color of the milk were scored independently by 2 technicians after thawing and before bacteriological culturing (Barkema et al., 1998). The Dutch national milk recording system (Nederlands Rundvee Syndicaat, Arnhem, The Netherlands) provided information from the 3- or 4-weekly milk production recording, including cow identification, date of milk recording, date of calving, date of drying off, test-day milk yields (kg of milk, fat and protein), and SCC (cells/mL) for all cows in the study.

Data preparation

The originally available dataset consisted of 8,571 CM cases. To ensure that no unrecorded previous cases of CM had occurred within the lactation, only CM cases from lactations that had been recorded from calving onward were eligible for inclusion. From these, CM cases from lactations without any milk production information, or with a calving interval ≤ 320 days or ≥ 600 days were excluded. CM cases during dry-off were also excluded and intervals between pathogen-specific cases of CM in the same quarter had to be at least 14 days for a case to be included in the final dataset. All contaminated samples, culture-negative samples and CM cases where no sample was taken were excluded. The final dataset for our study now consisted of 3,833 CM cases of 3,018 cows in 3,581 different quarters.

Table 1. Number of clinical mastitis cases per dataset.

		Training	Validation
Datasets 1 and 1b[1]	n	n	n
Gram-negative[3]	1,006	685	321
Gram-positive[4]	2,528	1,647	881
Total	3,534	2,332	1,202
		Training	Validation
Datasets 2 and 2b[2]	n	n	n
STREP[5]	962	647	315
STAPH[6]	746	459	287
COLI[7]	979	661	318
Total	2,687	1,767	920

[1]Dataset 1 contained Gram-specific clinical mastitis history, dataset 1b contained information on whether or not the cow had clinical mastitis before the current clinical mastitis in the same lactation. [2]Dataset 2 contained pathogen-specific clinical mastitis history, dataset 2b contained information on whether or not the cow had clinical mastitis before the current clinical mastitis in the same lactation. [3]*Escherichia coli* (n=923), *Klebsiella* (n=56), *Pseudomonas* (n=17) and mixed cultures (n=10). [4]*Streptococcus dysgalactiae* (n=369), *Streptococcus agalactiae* (n=21), *Streptococcus uberis* (n=254), other streptococci (n=288), *Staphylococcus aureus* (n=746), coagulase-negative staphylococci (n=215), *Arcanobacterium pyogenes* (n=27), *Corynebacterium bovis* (n=90) and mixed cultures (n=518). [5]*Strep. dysgalactiae* (n=369), *Strep. agalactiae* (n=21), *Strep. uberis* (n=254), other streptococci (n=288) and mixed cultures of streptococci (n=30). [6]*Staph. aureus* (n=746). [7]*E. coli* (n=923) and *Klebsiella* (n=56)

From the final dataset, 2 different enhanced versions were constructed (Table 1): dataset 1 included an additional variable classifying each CM case according to the causal pathogen's Gram-status (GRAM), and dataset 2 included an additional variable classifying each CM case according to the causal pathogen (PATH). In dataset 1, all CM cases were classified as either Gram-positive CM (*Streptococcus dysgalactiae*, *Streptococcus agalactiae*, *Streptococcus uberis*, other streptococci, *Staphylococcus aureus*, coagulase-

negative staphylococci) or Gram-negative CM (*E. coli*, *Pseudomonas* and *Klebsiella*). Mixed cultures containing 2 Gram-positive pathogens were classified as Gram-positive and those containing 2 Gram-negative pathogens as Gram-negative. All CM cases that could not be classified according to their Gram-status were excluded from dataset 1 (n = 299). In dataset 2, all CM cases were classified into STREP, containing all *Streptococci* (*Strep. dysgalactiae*, *Strep. agalactiae*, *Strep. uberis* and other streptococci), STAPH, containing *Staph. aureus*, and COLI, containing *E. coli* and *Klebsiella*. Mixed cultures containing pathogens from the same class were classified into this class (for example a mixed culture containing *Strep. uberis* and *Strep. dysgalactiae* was classified as STREP). All CM cases that could not be classified into STREP, STAPH or COLI (n = 1,146) were excluded from dataset 2.

Risk- and indicator variables for the Gram-status in dataset 1 and for the pathogen group in dataset 2 were defined using information from the literature and based on the expertise of the authors (Table 2). In both datasets, the parity, month in lactation, location of infected quarter and season of the year were available for each CM case. Three variables on SCC were constructed (e.g., De Haas et al., 2004). If available, the test-day SCC 1-30 days before CM (SCC1) and the closest test-day SCC > 30 days before CM (SCC2) were determined. Additionally, for multiparous cows, geometric mean SCC from all available test-day records from the previous lactation (PrevSCC) was defined. Since continuous variables cannot be handled in standard Bayesian networks, the values of all SCC variables were classified as either < or ≥ 200,000 cells/mL (Dohoo and Leslie, 1991). For both datasets, moreover, pathogen-specific CM history variables were defined (Zadoks et al., 2001). For dataset 1, these variables were defined as the Gram-status of the causal pathogen 1-30 days and > 30 days before the current CM. For dataset 2, these variables were defined as the pathogen itself 1-30 days and > 30 days before the current CM. In view of the projected use of the developed models, however, it is noted that pathogen-specific CM history information often is not available on farms but only when milk samples of previous CM cases have been collected. Therefore, 2 additional datasets were created, containing the same cases as datasets 1 and 2, respectively, but instead of the variables on pathogen-specific CM history, non-pathogen-specific variables were defined. These variables were defined as whether the cow had CM or not in the previous 30 days and > 30 days ago in the current lactation. These datasets will be referred to as dataset 1b and dataset 2b.

Table 2. Description of the study variables with their abbreviation and different levels used for the construction of naive Bayesian networks.

Description	Abbreviation	Nr. of classes	Classes
Gram-status of current CM case	GRAM	2	Gram-negative, Gram-positive
Pathogen of current CM case	PATH	3	STREP[1], STAPH[2], COLI[3]
Parity	PAR	4	1, 2, 3, ≥4
Month in lactation	MONTH	8	1, 2, 3, 4, 5, 6, 7, ≥8
Season of the year	SEAS	4	January – March, April – June, July – September, October – December
Quarter position of the udder	QP	4	right front, left front, right rear, left rear
SCC 1-30 days before current CM	SCC1	2	<200,000 cells/mL, ≥200,000 cells/mL
SCC >30 days before current CM	SCC2	2	<200,000 cells/mL, ≥200,000 cells/mL
Geometric mean SCC in previous lactation	PrevSCC	2	<200,000 cells/mL, ≥200,000 cells/mL
CM history 1-30 days before current CM	CM1	2	no, yes
CM history > 30 days before current CM	CM2	2	no, yes
Gram history 1-30 days before current CM	GRAM1	3	no previous CM, Gram-positive, Gram-negative
Gram history > 30 days before current CM	GRAM2	3	no previous CM, Gram-positive, Gram-negative
Pathogen history 1-30 days before current CM	PATH1	4	no previous CM, STREP, STAPH, COLI
Pathogen history > 30 days before current CM	PATH2	4	no previous CM, STREP, STAPH, COLI
Color of the milk of cow with CM	COLOR	5	normal, yellowish, very yellow, watery, blood
Texture of the milk of cow with CM	TEXT	5	normal, small flakes, big flakes, serous, viscous
Cow sick at moment of CM	SICK	2	not sick, sick

[1]*Streptococcus dysgalactiae*, *Streptococcus agalactiae*, *Streptococcus uberis*, other streptococci and mixed cultures of streptococci. [2]*Staphylococcus aureus.* [3]*Escherichia coli* and *Klebsiella*

Because hardly any CM information from previous lactations was available in the datasets, no such information was taken into account in our study. There were missing values for the variables on SCC1 (26%), SCC2 (40%) and PrevSCC (22%). In all datasets, finally, 3 variables were included with information on the clinical signs (Jones and Ward, 1990; Milne et al., 2003): color of the milk, texture of the milk, and whether or not the cow was sick. These variables had less than 1% missing values each.

Model building

An NBN consists of a single class variable that represents the possible classes for the dependent variable, and a set of feature variables modeling the relevant levels of the independent variables. In datasets 1 and 1b, the variable GRAM was the class variable, in datasets 2 and 2b the variable PATH was the class variable. All other variables in the 4 datasets were potential feature variables. From each of the 4 datasets, an NBN was constructed, resulting in a total of 4 models. These NBNs differed in their class variable and in the feature variables on CM history. For the models NBN_{GRAM1} and NBN_{GRAM1b}, GRAM was taken as the class variable. For constructing NBN_{GRAM1} the feature variables modeling Gram-specific history (GRAM1 and GRAM2) were used, while for constructing NBN_{GRAM1b} the less specific feature variables capturing CM history (CM1 and CM2) were taken into consideration. Similarly, for NBN_{PATH2} and NBN_{PATH2b} the class variable was PATH; for NBN_{PATH2} the feature variable modeling pathogen-specific history (PATH1 and PATH2) were used and for NBN_{PATH2b} the less specific feature variables with CM history (CM1 and CM2).

NBNs are typically constructed from data which consists of determining prior probabilities for the class variable and of estimating conditional probabilities for the feature variables given the possible classes of the dependent variable. In this study, the prior probabilities for the class variables reflect the prevalence of Gram-positive pathogens and Gram-negative pathogens among the CM cases (Gram-positive: 70.6%, Gram-negative: 29.4%), and the prevalence of the 3 pathogen groups (STREP: 36.6%, STAPH: 26.0% and COLI: 37.4%) in the datasets used for construction (see also Table 1). The conditional probabilities for the feature variables are based on frequency counts in the data. For instance, the conditional probability of a cow being sick, given that it is infected with a Gram-negative pathogen, that is, the probability Pr(Sick = yes | Gram-status = negative), was computed as the proportion of sick cows among the CM cases reporting a Gram-negative pathogen.

In essence, all available feature variables can be included in an NBN. Methods exist, however, for selecting only those feature variables that best discriminate between the different classes of a dependent variable, thereby forestalling overfitting of the data (Langley and Sage, 1994). For this study, a wrapper method with forward selection was

used for selecting appropriate feature variables (e.g., Blanco et al., 2005; Geenen et al., 2005). With this method, feature variables were selected to optimize the accuracy of the NBN under construction. The method started with an NBN including just the class variable and no feature variables. In each subsequent step, it computed the accuracy of the NBN with a single feature variable added, for each such variable separately. It then included the feature variable that increased the accuracy the most, if any. The inclusion of feature variables was continued until the accuracy of the NBN no longer improved.

For the purpose of constructing and subsequently validating the 4 NBNs, the datasets were split into a training set and a validation set. From each dataset, 2/3 of the herds were selected randomly for training; the CM cases of the remaining herds were included in a validation dataset (Table 1). Selecting herds rather than separate CM cases was aimed at applicability of the resulting models on farms. The selection process apparently did not result in a bias, as the prevalence of pathogens was approximately equal in the training and validation datasets (Table 1). Construction of the NBNs was done with 10-fold cross validation on the training dataset. The NBN which performed best on the training set during the cross validation was selected for further evaluation on the validation dataset. Constructing the different NBNs, which includes estimating the prior and conditional probabilities, was done by using the Bayesian-network editing package Dazzle (Schrage et al., 2005).

Validation

Obtaining posterior probabilities. The 4 constructed NBNs were used to calculate posterior probability distributions for the class variables (GRAM and PATH) given information on the selected feature variables, for each CM case from the validation dataset. For computing posterior probabilities, an NBN builds upon Bayes's rule together with the assumption that all feature variables are mutually independent given the class variable. More specifically, for computing the posterior probability Pr ($c \mid f_1, \ldots, f_n$) of the class c given levels $f_1,\ldots,f_n$ for its n feature variables, the model uses

$$\Pr(c \mid f_1,\ldots, f_n) = \frac{\prod_{i=1}^{n} \Pr(f_i \mid c) * \Pr(c)}{\sum_{j=1}^{k} \prod_{i=1}^{n} \Pr(f_i \mid c_j) * \Pr(c_j)} \qquad (1)$$

where $c_1,\ldots,c_k$ are the possible classes for the model's class variable. For the class variable GRAM, for example, c_1 equals the class of Gram-negative pathogens and c_2 equals the class of Gram-positive pathogens. For the class variable PATH, c_1 equals STREP, c_2 equals STAPH, and c_3 equals COLI. In the formula, Pr(c) is the prior probability of the CM being caused by the group c of pathogens. Pr($f_i \mid c_j$) are the conditional probabilities of

the level f_i of the i^{th} selected feature variable given that the CM is caused by the group c_j of pathogens. Note that the prior probabilities Pr(c) and the conditional probabilities Pr(fi | cj) for all i and j, have already been estimated from the data upon constructing the NBN and therefore are readily available in the NBN for the computation of the posterior probabilities using formula (1). The Dazzle package could have been used for computing the posterior probabilities for the Gram-status and for the causal pathogen of a CM case but we decided to use Microsoft Excel. For this purpose, a log-odds transform of formula (1) was used, which resulted in a simple additive formula, which in turn could be easily implemented in Microsoft Excel

Model accuracy. From the posterior probability distribution computed for the class variable for a CM case, the predicted class was established. For the GRAM variable, which had 2 possible classes, the predicted class was the Gram-positive class if its posterior probability was > 0.71; otherwise, the predicted class was Gram-negative. The threshold probability of 0.71 was chosen to reflect the prevalence of Gram-positive CM cases in the dataset. For the PATH class variable, having 3 possible classes, the predicted class was the one with highest posterior probability. If 2 or more classes had equal posterior probabilities, ties were broken at random. Subsequently, the predicted class was compared with the known causal pathogen of the case. By performing these computations for each CM case from the validation dataset, the accuracy of the model was established, that is, the percentage of correctly classified cases.

In practice on a farm, only CM cases with a very high posterior probability for a single pathogen or group of pathogens will be eligible for pathogen-specific treatment; CM cases which resulted in more or less equal posterior probabilities, will be more eligible for broad spectrum use of antibiotics. By providing probability distributions for the causal pathogens or groups of pathogens, the farmer can readily distinguish between such cases. To establish the accuracy of the models for CM cases with a high posterior probability (for instance >0.80) for a single class, all other cases were left unclassified. As an example, suppose a dataset consisting of 1,000 CM cases, 400 of which had a posterior probability for their Gram-status >0.80; the remaining 600 CM cases had posterior probabilities <0.80. The 400 CM cases then were used for determining the model's accuracy. If 325 of these cases were classified correctly, for instance, the accuracy of the model for CM cases with probabilities >0.80 would be 81%. These accuracies will be called stratified accuracies, since they are based upon different strata of the dataset under study. All accuracies were calculated using SAS, version 9.1 (SAS Institute Inc., Cary, NC).

RESULTS

Naive Bayesian networks

A total of 4 NBNs were constructed from the 4 datasets. After feature selection, NBN_{GRAM1} and NBN_{GRAM1b} contained 5 feature variables, NBN_{PATH2} contained 8 feature variables and NBN_{PATH2b} contained 5 feature variables (Table 3). Upon feature selection for NBN_{GRAM1} and NBN_{GRAM1b}, the feature variable SICK, modeling whether or not the cow was sick at the moment of the current CM, increased the accuracy the most and was selected first. Also the feature variable COLOR, capturing the color of the cow's milk, proved to be a relatively strong indicator for the Gram-status of the current CM. For NBN_{PATH2} and NBN_{PATH2b}, the feature variable SCC1 was selected first. Also, the feature variable SICK had an ability to discriminate between the possible causal pathogens for the current CM. For NBN_{GRAM1}, the feature variables capturing Gram-specific CM history information (GRAM1 and GRAM2) were selected (Table 3). Similarly, the feature variables modeling pathogen-specific CM history information (PATH1 and PATH2) were included in NBN_{PATH2} quite early in the feature selection process.

Table 3. Overview of the order in which the feature variables were included in the final naive Bayesian networks[1]

	NBN_{GRAM1}[2]	NBN_{GRAM1b}[3]	NBN_{PATH2}[4]	NBN_{PATH2b}[5]
1st added feature	SICK	SICK	SCC1	SCC1
2nd added feature	GRAM1	COLOR	PATH1	SICK
3rd added feature	COLOR	SCC1	SICK	TEXT
4th added feature	GRAM2	TEXT	PATH2	SEAS
5th added feature	SCC1	SCC2	SCC2	CM2
6th added feature	-	-	TEXT	-
7th added feature	-	-	SEAS	-
8th added feature	-	-	COLOR	-

[1]SEAS = season of the year, SCC1 = SCC 1-30 days before current CM, SCC2 = SCC >30 days before current CM, CM2 = CM history >30 days before current CM, PATH1 = pathogen history 1-30 days before current CM, PATH2 = pathogen history > 30 days before current CM, GRAM1 = Gram history 1-30 days before current CM, GRAM2 = Gram history > 30 days before current CM, COLOR = color of the milk of cow with CM, TEXT = texture of the milk of cow with CM, SICK = whether cow was sick at moment of CM. [2]Naive Bayesian network including Gram-specific clinical mastitis history. [3]Naive Bayesian network including whether or not cow had clinical mastitis before the current clinical mastitis in the same lactation. [4]Naive Bayesian network including pathogen-specific clinical mastitis history. [5]Naive Bayesian network including whether or not cow had clinical mastitis before the current clinical mastitis in the same lactation.

Validation

Obtaining posterior probabilities. For each CM case from the validation datasets, posterior probability distributions were established using the constructed NBNs. For instance, a sick cow with CM, with watery milk in the sample, with an SCC in the last month < 200,000 cells/mL, and in this lactation no CM before the current CM, had a posterior probability of a Gram-negative pathogen causing the CM of 0.74 and a posterior probability of a Gram-positive pathogen causing the CM of 0.26. A cow with CM, which was not sick, with a normal color of the milk, with an SCC in the last month ≥ 200,000 cells/mL, and in this lactation no CM before the current CM, had a posterior probability of a Gram-negative pathogen of 0.33 and a posterior probability of a Gram-positive pathogen of 0.67.

Another example was a cow with CM in April, which was sick, with watery milk and small flakes in the milk, with 2 previous SCC measurements ≥ 200,000 cells.mL. For this lactation, moreover, there was no CM before the current CM. There was a posterior probability for STREP, STAPH and COLI of 0.38, 0.24 and 0.38, respectively. Another cow experienced CM in January, was not sick, had milk with a very yellow color and with big flakes, experienced 2 previous SCC measurements < 200,000 cells/mL, and had no CM before the current CM in this lactation. The posterior probability for STREP, STAPH and COLI was 0.15, 0.06 and 0.79, respectively.

Model accuracy. Using a threshold probability of 0.71 for predicting a CM case as being Gram-positive resulted in accuracies of 73% and 70% for NBN_{GRAM1} and NBN_{GRAM1b}, respectively. The difference in accuracy between the 2 Gram predicting models was not significant (P = 0.15). Table 4 presents, for the NBN_{GRAM1} model, the predicted and actual numbers of cases for each Gram-status. Out of the 1,202 CM cases in the validation dataset, a total of 874 (i.e. 197 + 677) cases were classified correctly by the NBN, which resulted in an accuracy of 73% (i.e. 874 / 1,202). Of the 801 Gram-positive predicted CM cases, 677 cases were indeed caused by Gram-positive pathogens; 85% of the Gram-positive predicted cases were thus classified correctly. Of the 401 Gram-negative predicted CM cases, 197 cases were caused by Gram-negative pathogens; 49% of the Gram-negative predicted cases were thus classified correctly.

Table 4. Predicted and actual numbers of clinical mastitis cases for Gram-negative and Gram-positive pathogens for NBN_{GRAM1}[1].

		Actual		
		Gram-negative	Gram-positive	Total
Predicted	Gram-negative	197 (49%)	204 (51%)	401 (100%)
	Gram-positive	124 (15%)	677 (85%)	801 (100%)
	Total	321	881	1202

[1]Naive Bayesian network including Gram-specific clinical mastitis history.

Using the most likely pathogen as the predicted value, the PATH predicting models gave accuracies of 52% and 48% for NBN_{PATH2} and NBN_{PATH2b}, respectively. The difference in accuracy was significant (P = 0.05). Table 5 presents for the NBN_{PATH2} model, the predicted and actual numbers of cases for each group of causal pathogens. Out of the 920 CM cases in the validation dataset, a total of 480 (i.e. 144 + 126 + 210) cases were classified correctly by the NBN, which resulted in an accuracy of 52% (i.e. 480 / 920). Of the 305 STREP predicted CM cases, 144 cases (i.e. 47%) were indeed caused by STREP. The NBN further classified 31% of these 305 STREP predicted cases incorrectly as STAPH and another 22% as COLI. Of the total of 228 CM cases with STAPH as the predicted causal pathogen, 126 cases (i.e. 55%) were indeed caused by STAPH. 27% of these cases, however, were incorrectly predicted as STREP and another 18% were predicted as COLI. For the 387 CM cases predicted as COLI, the percentage of correctly classified cases was 54%; 29% of these cases were predicted as STREP and the remaining 17% were predicted as STAPH.

Table 5. Predicted and actual numbers of clinical mastitis cases for each group of pathogens using the most likely pathogen as the predicted value for NBN_{PATH2}[1].

		Actual			
		STREP[2]	STAPH[3]	COLI[4]	Total
Predicted	STREP[2]	144 (47%)	94 (31%)	67 (22%)	305 (100%)
	STAPH[3]	61 (27%)	126 (55%)	41 (18%)	228 (100%)
	COLI[4]	110 (29%)	67 (17%)	210 (54%)	387 (100%)
	Total	315	287	318	920

[1]Naive Bayesian network including pathogen-specific clinical mastitis history.
[2]*Streptococcus dysgalactiae*, *Streptococcus agalactiae*, *Streptococcus uberis*, other streptococci and mixed cultures of streptococci.
[3]*Staphylococcus aureus.*
[4]*Escherichia coli* and *Klebsiella*

Stratified accuracies were computed for the GRAM predicting models (Table 6) and for the PATH predicting models (Table 8), using different strata. For instance, with the NBN_{GRAM1} model, a total of 601 CM cases (equal to 50% of the 1202 CM cases in the validation dataset) resulted in a posterior probability of over 0.80 of the CM being caused by a Gram-positive pathogen or by a Gram-negative pathogen alternatively. Of these 601 CM cases, 90% were classified correctly. With the same model, a total of 186 CM cases resulted in a posterior probability of over 0.95 for one of the two possible classes of the Gram-status of the causal pathogen. All these 186 CM cases were classified correctly, resulting in a stratified accuracy of 100%. Table 7 details the stratified accuracy of the NBN_{GRAM1} model for cases with a posterior probability exceeding a threshold value of 0.90. Out of the 342 CM cases in the dataset for which a posterior probability of over 0.90 was computed for a Gram-negative pathogen or for a Gram-positive pathogen alternatively, a total of 330 (i.e. 309 + 21) cases were classified correctly by the NBN, which resulted in a stratified accuracy of 97% (i.e. 330 / 342). Of the 321 Gram-positive predicted CM cases, 309 cases (i.e. 96%) were indeed caused by a Gram-positive pathogen. All the 21 Gram-negative predicted CM cases were indeed caused by a Gram-negative pathogen.

Table 6. Stratified accuracy at different thresholds on the posterior probability of the most likely Gram-status for NBN_{GRAM1} and NBN_{GRAM1b}.

	NBN_{GRAM1}[1]		NBN_{GRAM1b}[2]	
Threshold on posterior probability	Number (%) of CM cases above threshold	Stratified accuracy (%)	Number (%) of CM cases above threshold	Stratified accuracy (%)
0.70	911 (76%)	83	900 (75%)	81
0.75	798 (66%)	86	817 (68%)	83
0.80	601 (50%)	90	660 (55%)	86
0.85	433 (36%)	94	538 (45%)	89
0.90	342 (28%)	97	378 (31%)	90
0.95	186 (15%)	100	168 (14%)	96

[1]Naive Bayesian network including Gram-specific clinical mastitis history.
[2]Naive Bayesian network including whether or not cow had clinical mastitis before the current clinical mastitis in the same lactation.

Table 7. Predicted and actual numbers of clinical mastitis cases for Gram-negative and Gram-positive pathogens with a posterior probability over 0.90 for NBN_{GRAM1}[1].

		Actual		
		Gram-negative	Gram-positive	Total
Predicted	Gram-negative	21 (100%)	0 (0%)	21 (100%)
	Gram-positive	12 (4%)	309 (96%)	321 (100%)
	Total	33	309	342

[1]Naive Bayesian network including Gram-specific clinical mastitis history

Using NBN_{PATH2} with pathogen-specific CM history information, 155 CM cases resulted in a posterior probability distribution in which either STREP, STAPH or COLI had a posterior probability of over 0.80. Of these 155 CM cases, 76% were classified correctly. Table 9 details the stratified accuracy of the NBN_{PATH2} model for cases with a posterior probability exceeding a threshold value of 0.90. Out of the 84 CM cases in the dataset for which a posterior probability of over 0.90 was computed for one of the causal pathogens, a total of 70 (i.e. 1 + 23 + 46) cases were classified correctly by the NBN, which amounts to a stratified accuracy of 83% (i.e. 70 / 84). The STREP predicted CM case was indeed

caused by STREP. Of the 29 STAPH predicted CM cases, 79% were caused by STAPH; 17% of these cases, however, were incorrectly predicted as STREP and another 4% were predicted as COLI. Of the 54 COLI predicted CM cases, 85% were caused by COLI; 9% of these cases, however, were incorrectly predicted as STREP and another 6% were predicted as STAPH.

Table 8. Stratified accuracy at different thresholds on the posterior probability of the most likely causal pathogen for NBN_{PATH2} and NBN_{PATH2b}.

	NBN_{PATH2}[1]		NBN_{PATH2b}[2]	
Threshold on posterior probability	Number (%) of CM cases above threshold	Stratified accuracy (%)	Number (%) of CM cases above threshold	Stratified accuracy (%)
0.70	258 (28%)	73	112 (12%)	73
0.75	207 (23%)	77	89 (10%)	78
0.80	155 (17%)	76	54 (6%)	83
0.85	115 (13%)	81	30 (3%)	97
0.90	84 (9%)	83	16 (2%)	100
0.95	36 (4%)	89	9 (1%)	100

[1]Naive Bayesian network including pathogen-specific clinical mastitis history. [2]Naive Bayesian network including whether or not cow had clinical mastitis before the current clinical mastitis in the same lactation

Table 9. Predicted and actual number of clinical mastitis cases for each group of pathogens with a posterior probability over 0.90 for NBN_{PATH2}[1].

		Actual			
		STREP[2]	STAPH[3]	COLI[4]	Total
Predicted	STREP[2]	1 (100%)	0 (0%)	0 (0%)	1 (100%)
	STAPH[3]	5 (17%)	23 (79%)	1 (4%)	29 (100%)
	COLI[4]	5 (9%)	3 (6%)	46 (85%)	54 (100%)
	Total	11	26	47	84

[1]Naive Bayesian network including pathogen-specific clinical mastitis history
[2]*Streptococcus dysgalactiae*, *Streptococcus agalactiae*, *Streptococcus uberis*, other streptococci and mixed cultures of streptococci.
[3]*Staphylococcus aureus.*
[4]*Escherichia coli* and *Klebsiella*

DISCUSSION

Bayesian networks are known as powerful tools for knowledge representation and probabilistic inference (Jensen, 2001). The networks are flexible in terms of capturing complex dependencies among their variables and allow in essence for computing any prior or posterior probability of interest for the modeled variables. These characteristics favor Bayesian networks in comparison with other statistical methods such as logistic regression, for probabilistic reasoning tasks. Even NBNs, the simplest type of Bayesian network, are surprisingly effective (Langley and Sage, 1994). NBNs are building upon the assumption that all feature variables are mutually independent given the class variable and provide for computing a (posterior) probability distribution for the class variable. This probability distribution then is used for classification purposes. As reported by Friedman et al. (1997), the classification performance of NBNs is good, even if the independence assumption for the feature variables does not hold in the data. Relaxing the independence assumption could in essence lead to more accurate classification models. In tree-augmented NBN (Friedman et al., 1997), for instance, a tree-like dependency structure over the feature variable is constructed: each feature variable then is directly dependent of the class variable and of at most one other feature variable. Creating tree-augmented NBN, however, includes searching for dependencies between feature variables. For accurately recovering these dependencies from a dataset, more data need to be available than for building an NBN. A tree-augmented NBN is often a good compromise between an NBN and a Bayesian network based on causality: the model structure is simple enough to avoid overfitting, but strong dependencies can be taken into account.

In the present study, in addition to the 4 NBNs, tree-augmented NBNs were also constructed (data not shown). These tree-augmented networks had lower accuracies than their matching NBNs. An explanation of these lower accuracies is that the relatively small datasets available in our study caused unreliable estimates upon searching for dependencies and led to an irrelevant dependence structure (Friedman and Goldszmidt, 1996). Another explanation is that the feature variables in our study did not contain enough information to make a clear distinction between the causal pathogens and that the constructed tree-augmented NBNs as a consequence of their larger number of estimates tend to overfit the data. For these reasons, we chose to use NBNs even though their lack of dependencies between the feature variables is somewhat unrealistic. Although some of the modelled independencies are likely to hold, it is questionable, for example, whether the color and the texture of the milk of a cow with CM are independent given the Gram-status of the causal pathogen.

The constructed NBNs can be used to generate a posterior probability distribution of the Gram-status of a CM case or on the causal pathogen. Based upon this distribution, the

farmer can take an informed treatment decision. The Gram-positive pathogens *Strep. uberis* and *Staph. aureus*, for example, need different treatment regimes; especially the duration of the treatment influences the cure rate (Sol et al., 2000; Barkema et al., 2006). Because of the expected low cure rate for *Staph. aureus* (e.g., Sol et al., 2000; Taponen et al., 2003; McDougall et al., 2007), moreover, a decision to cull the cow instead of treating the cow should be considered as well. Posterior probabilities of STREP, STAPH and COLI, as provided by the NBNs, give the farmer insight in the likelihood of *Staph. aureus* compared to for example *Strep. uberis*, allowing him to carefully evaluate and weigh the possible consequences of different treatment regimes.

For the different NBNs, the feature variables SICK and SCC1 were selected first, indicating that these variables served to increase the accuracy the most. Whenever pathogen-specific CM history variables were available in the datasets, these variables were selected for the NBNs, which indicates that these variables have some predictive value for the causal pathogen. In contrast, the feature variables on non-pathogen-specific CM history (CM1 and CM2) were included quite late or not at all in the feature selection process, which indicates that these variables do not contain enough information for distinguishing between the different groups of pathogens. In all NBNs at least one of the feature variables modeling the color and the texture of the milk were selected. Also in previous studies the importance of these variables was reported for pathogen diagnosis of CM cases (e.g., Jones and Ward, 1990; Zadoks et al., 2001, De Haas et al., 2004).

The main purpose of this study was to illustrate the value of providing probability distributions for the Gram-status and for the causal pathogen to a farmer to take a more informed treatment decision. A validation of a probability distribution is not possible. It is still valuable, however, to gain at least some insight in the quality of the constructed NBNs. For this purpose, the accuracy was determined of all constructed NBNs. The accuracy was also used as an indication of the model's quality in other studies of NBNs (e.g., Blanco et al., 2005; Geenen et al., 2005) and in studies on other classification models for CM (e.g., Kim and Heald, 1999; Heald et al., 2000; Milne et al., 2003). In other studies classifying CM cases according to their Gram-status (Jones and Ward, 1990; Milne et al., 2003) or into *E. coli* or not (White et al., 1986a, b), accuracy varied between 71 and 79%. There are no related studies reporting accuracies for classifying CM causal pathogens in STREP, STAPH or COLI. It was also reported that trained veterinarians reached an accuracy of 62% in classifying CM cases into *E. coli* or not (White et al., 1986b). Because of the different prevalences of the causal pathogens in these studies, the reported accuracies are not readily compared with the accuracies obtained in the present study. In the present study, the accuracies computed for the NBN_{GRAM1} and NBN_{GRAM1b} models were 73% and 70%, respectively. For the purpose of comparison, note that a simple model using random guessing based upon prevalence would have an accuracy of 58%; a

model which attributes each CM case to a Gram-positive pathogen, in turn, would have an accuracy of 70%. These observations suggest that the feature variables included in the datasets are not indicative enough to make a marked distinction between Gram-positive and Gram-negative pathogens. Stratified accuracies were established to gain some insight in the reliability of high posterior probabilities for a single pathogen. Table 6 shows that the stratified accuracies of the Gram-predicting models increase with greater threshold probabilities and in fact reach very high values. For 28% of the CM cases, for instance, a stratified accuracy of 97% was reached. These high stratified accuracies indicate that the greater a posterior probability computed for either a positive or a negative Gram-status of the causal pathogen, the more the farmer can rely on the classification result. For a CM case with a high probability for the Gram-status, a more specific treatment can thus be decided upon. The results of the exact predictions for CM cases with posterior probabilities over 0.90 indicate that high posterior probabilities on both Gram-positive and Gram-negative pathogens are reliable (Table 7).

The accuracies computed for the NBN_{PATH2} and NBN_{PATH2b} models were 52% and 48%, respectively. Since the three groups of causal pathogens occur in approximately equal numbers in the dataset, these accuracies clearly improve on random guessing, which would result in an accuracy of approximately 30%. Also a model which attributes each CM case to a single fixed pathogen, for instance COLI, would have an accuracy of approximately 30%. These observations suggest that the feature variables included in the datasets carry more information for distinguishing between the different groups of pathogens. Classifying CM cases into STREP, STAPH or COLI moreover resulted in high stratified accuracies. For instance, for 4% of the CM cases a stratified accuracy of 89% was reached. The NBN models with feature variables on pathogen-specific CM history had significant higher accuracies than the NBN models with feature variables on non-pathogen-specific CM history.

The present study was based on the observation that simply classifying CM cases according to their Gram-status or their causal pathogen, i.e. simply presenting the most likely value, does not provide sufficient information to assist a farmer in deciding on the most appropriate treatment for CM. With that method, only the most likely pathogen is presented to a farmer. In our study, classifying (as the accuracy does) was only used to determine the overall performance of the NBNs. In our opinion, a probability distribution for the causal pathogen, revealing the uncertainty involved, provides more information in deciding on the most appropriate treatment for CM. The advantage of providing such probability distributions is that the farmer and his/her advisor(s) could interpret the uncertainty involved and can take a more informed treatment decision. For instance, consider a CM case with STREP for the predicted class, and assume that the posterior probability distribution for the causal pathogens equals 0.45, 0.15 and 0.40 for STREP,

STAPH and COLI, respectively. Providing only the predicted class would ignore the high posterior probability on COLI, which can cause seriously wrong treatment decisions. In practice, only CM cases for which a single pathogen receives a high posterior probability will be eligible for more specific treatment rather than broad spectrum use of antibiotics.

Because training and validation of the 4 NBNs was performed on data coming from different farms, results of this study can be generalized to all farms. In the present study, data from herds with different pathogen prevalences were combined in a single dataset and analysed together. It is likely that performance of the presented NBNs can be improved by performing separate analyses on different groups of farms according to their pathogen prevalences, as was done by Heald et al. (2000), provided that sufficient data are available for each group. The class variable in the NBNs, however, captures the prior probabilities on the causal groups of pathogens (GRAM or PATH) and can be adapted easily to farm-specific pathogen prevalences. For instance, for farms with a known high proportion of *E. coli* cases and consequentially a high prior probability on this pathogen will result in greater posterior probabilities for COLI. The prior probabilities on the causal groups of pathogens can be moreover adapted continually, that is, new evidence on the distribution of pathogens, as a result of bacteriological culturing of CM cases, can be included directly in the NBNs and, posterior probabilities will more reliably reflect reality on the farm.

The NBNs used in this study can be included in automated decision support systems that support the choice and duration of treatment for each CM case separately. Because most information sources used in this study are available on most dairy farms, such systems can be installed at the farm site and used by the farmer. Using the NBNs would not include extra costs as the information is readily available. However, for almost all CM cases there will remain some uncertainty about the causal group of pathogens. Wrong treatment decisions will therefore still be made with economic losses as a consequence. For instance, treating a cow against Gram-negative pathogens while it was infected with Gram-positive pathogens may result in high economic losses due to decreased milk production and infection of other cows. In future work, therefore, economic consequences of the different treatment options will be included in the models to provide an optimal advice on the choice of treatment.

CONCLUSIONS

Using NBNs, a probability distribution for the Gram-status and for the causal group of pathogens can be given for each CM case. To obtain insight in the quality of the constructed NBNs, the accuracy was determined. The accuracy of classifying CM cases into Gram-positive or Gram-negative pathogens was 73%, while the accuracy of classifying CM cases into STREP, STAPH or COLI was 52%. Because only CM cases with a high probability on a single causal pathogen will be considered for pathogen-specific treatment, accuracies based on only classifying CM cases above a particular probability threshold were determined as well. For CM cases with high posterior probabilities a reliable prediction for the causal pathogen can be given. Using the provided probability distribution for the causal pathogen the farmer can interpret the uncertainty involved and can take a more informed decision. On a farm, this information can result in an improvement in comparison with the current situation where information on the causal pathogen is not. The probability distributions were based on information sources which are usually available at a farm. This makes it possible to use results of this study in automated decision support systems to aid decision on choice and duration of treatment for each individual CM case.

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Chapter 6

Cow-specific treatment of clinical mastitis. An economic approach

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ABSTRACT

Under Dutch circumstances, most clinical mastitis (CM) cases of cows on dairy farms are treated with a standard intramammary antimicrobial treatment. Several antimicrobial treatments are, however, available for CM, differing in antimicrobial compound, route of application, duration and costs. Because cow factors (e.g., parity, stage of lactation and somatic cell count history) and the causal pathogen influence the probability of cure, cow-specific treatment of CM is often recommended. The objective of this study was to determine if cow-specific treatment of CM is economically beneficial. Using a stochastic Monte Carlo simulation model, 20,000 CM cases were simulated. For each simulated CM case, the consequences of using different antimicrobial treatment regimes (standard 3 d intramammary, extended 5 d intramammary, combination 3 d intramammary + systemic, combination 3 d intramammary + systemic + 1 d non-steroid anti-inflammatory drugs, and combination extended 5 d intramammary + systemic) were simulated simultaneously. Finally, total costs of the 5 antimicrobial treatment regimes were compared. Some inputs for the model were based on literature information and, if no information was available, assumptions made by the authors were used. Bacteriological cure for each individual cow depended on the antimicrobial treatment regime, the causal pathogen and the cow factors parity, stage of lactation, somatic cell count history, CM history and whether the cow was systemically ill or not. Total costs for each case depended on treatment costs for the initial CM case (including costs for antibiotics, milk withdrawal and labor), treatment costs for follow-up CM cases, costs for milk production losses and costs for culling. Average total costs for CM using the 5 treatments were €176, €194, €199, €204 and €216, respectively. Average probabilities of bacteriological cure for the 5 treatments were 0.53, 0.65, 0.65, 0.68 and 0.75, respectively. For all different simulated CM cases, the standard 3 d intramammary antimicrobial treatment had the lowest total costs. The benefits of lower costs for milk production losses and culling for cases treated with the intensive treatments, did not outweigh the higher treatment costs. The stochastic model was developed using information from literature and assumptions made by the authors. Using these information sources resulted in a difference in effectiveness of different antimicrobial treatments for CM. Based on our assumptions, cow-specific treatment of CM was not economically beneficial.

Key words: clinical mastitis, antimicrobial treatment, economics, dairy cow

INTRODUCTION

Mastitis is one of the most frequent occurring and costly disease in dairy cows (e.g., Halasa et al., 2007). Most mild clinical mastitis (CM) cases are treated with a 3 d intramammary treatment. However, several antimicrobial treatment regimes are available for CM, differing in antimicrobial compound, route of application, duration, probability of cure and costs (e.g., Barkema et al., 2006). Other treatment options are using non-steroid anti-inflammatory drugs (NSAID) in combination with an antimicrobial treatment (McDougall et al., 2009) or immediately culling (Bar et al., 2008a). The probability of cure depends highly on the causal pathogen of CM (e.g., McDougall et al., 2007; Bradley and Green, 2009). The probability of cure is not only determined by the treatment regime and the causal pathogen. Also several cow factors influence the success of treatment. For instance, Sol et al. (2000) and Barkema et al. (2006) mentioned that the probability of cure of *S. aureus* CM depends for instance on the cow factors SCC and parity.

At an increasing number of dairy farms, information on cow factors that may influence the probability of cure is available automatically via management programs. A farmer has to take all information sources into account when making treatment decisions for a cow with CM. Because people can only use a maximum number of information sources to make the optimal decision (Miller, 1956), making the optimal decision for treatment of CM based on all available information sources is very difficult. Combining information on several treatment options and all cure-influencing cow factors in an automated decision support model would be useful to support farmers in their decision on the optimal CM treatment. Bar et al. (2008b) presented a dynamic programming model to support farmers in making CM treatment decisions. In their model, however, it was not possible to choose between different antimicrobial treatment regimes for individual cows.

The importance of optimization the choice of CM treatment is stressed frequently (Barkema et al., 2006; Bar et al., 2008b; Steeneveld et al., 2009). But before developing a computerized decision support model for treatment of CM, it must be investigated whether optimizing the choice of treatment is worthwhile. The optimal CM treatment is often measured in terms of clinical or bacteriological cure. Barkema et al. (2006), however, mentioned that dairy farming is an economic enterprise and that therefore the real measure of cure should be the cost-benefit of treatment. So far, no study has determined whether making cow-specific CM treatment decisions is economically beneficial.

The overall objective of this study was to determine if cow-specific treatment of CM is economically beneficial.

MATERIALS AND METHODS

Model Development

A stochastic Monte Carlo simulation model, at cow level, was built to calculate the costs of CM cases treated with different treatment regimes using Microsoft Excel with @Risk add-in software (Palisade, 2002). Model outcomes were generated in 3 steps. First, each iteration (20,000 in total) during the simulation process provided a specific cow with CM. In the second step, the follow-up of treating the simulated CM case was simulated using 6 defined treatment regimes (5 different antimicrobial treatment regimes and immediate culling). The third step involved calculation of the associated total costs for CM under each of the 6 treatment regimes. All discrete events and variability with regard to the modeled CM cases under the 6 different treatment regimes were triggered stochastically using random numbers drawn from relevant distributions. These distributions were based on knowledge of the model domain, information from literature and, if no other information was available, on assumptions made by the authors.

Simulation of a Cow with Clinical Mastitis

The causal pathogen of the CM case was determined using a discrete probability distribution. In our model, the CM case could be caused by streptococci (*Streptococcus uberis* and *Streptococcus dysgalactiae*) (probability = 0.40), *Staphylococcus aureus* (probability = 0.30) or *Escherichia coli* (probability = 0.30). This pathogen distribution was based on results of Barkema et al. (1998) and Olde Riekerink et al. (2007). The length of the calving interval of the cow with CM was determined with a pert probability distribution with a minimum of 336 d, a most likely value of 410 d and a maximum of 556 d (CRV, 2009). Subsequently, using a dry period of 60 d, the length of lactation was 60 d shorter than the calving interval. The parity of the cow (heifer or older cow), whether the cow was systemically ill or not, and whether the CM case was a repeated case or not were determined with discrete probability distributions, where probabilities were dependent on the causal pathogen. The month in milk of CM (1, 2, 3, …, ≥8) was determined with a discrete probability distribution, where probabilities were dependent on the causal pathogen and parity. When the month in milk was determined, a uniform probability distribution was used to determine the exact day of CM occurrence in that specific month in milk. The 305-d milk production of the cow with CM was modeled assuming a normal distribution. A mean 305-d milk production for heifers and older cows of 7,434 and 8,666 kg was used, respectively (CRV, 2009). The daily milk production at moment of CM and the remaining milk production after the CM case were estimated using Wood's lactation curve (Wood, 1967). The most recent SCC measurement before the CM case depended on the causal pathogen and the parity of the cow. The most recent SCC measurement (categorized into <200,000, 200,000 to 500,000 and >500,000 cells/mL) was determined using a discrete probability distribution.

Probability distributions to determine the parity, month in milk, most recent SCC and whether the cow with CM is systemically ill or not were based on data as used by Steeneveld et al. (2009). The probabilities to determine whether the CM case was a repeated case or not, were based on Döpfer et al. (1999) and Swinkels et al. (2005a, b). All these probabilities are presented in Tables 1a, 1b and 1c.

Simulation of Follow-up of Treatment Regimes for Clinical Mastitis

For each simulated CM case, the follow-up of using different antimicrobial treatment regimes was simulated simultaneously. In total, 5 antimicrobial treatment regimes were defined, differing in route of application (3 d intramammary with antimicrobials (IMM3), 5 d intramammary with antimicrobials (IMM5), 3 d intramammary + systemic with antimicrobials (IMM3_S), 3 d intramammary + systemic with antimicrobials + 1 d NSAID (IMM3_S_NSAID) and 5 d intramammary + systemic with antimicrobials (IMM5_S)), costs, milk withdrawal period and total treatment time (Table 2). The follow-up of the antimicrobial treatment regimes is presented in Fig. 1.

Treatment of the initial CM case (CM1) can result in complete cure (bacteriological + clinical cure), no cure (no bacteriological cure + no clinical cure), or clinical cure but no bacteriological cure (Fig. 1). To determine this cure status, it was first determined whether CM1 was cured bacteriologically or not after antimicrobial treatment.

Table 1a. Probabilities used to determine the parity of the cow, whether the cow with clinical mastitis was systemically ill or not and whether the clinical mastitis case is a repeated case or not, given the causal pathogen of clinical mastitis.

	Parity		Systemically ill		Repeated case	
	1	≥2	yes	no	yes	no
Streptococci	0.19	0.81	0.10	0.90	0.10	0.90
Staphylococcus aureus	0.18	0.82	0.06	0.94	0.12	0.88
Escherichia coli	0.15	0.85	0.31	0.69	0.05	0.95

Table 1b. Probabilities used to determine the month in milk, given the parity of the cow and the causal pathogen of clinical mastitis.

	Parity 1 Month in milk								Parity ≥2 Month in milk							
	1	2	3	4	5	6	7	≥8	1	2	3	4	5	6	7	≥8
Streptococci	0.60	0.13	0.08	0.02	0.05	0.05	0.02	0.05	0.25	0.16	0.16	0.10	0.08	0.06	0.07	0.12
Staphylococcus aureus	0.38	0.10	0.12	0.08	0.11	0.07	0.01	0.13	0.38	0.13	0.15	0.12	0.08	0.08	0.06	0.14
Escherichia coli	0.36	0.16	0.11	0.09	0.03	0.06	0.07	0.12	0.26	0.18	0.14	0.12	0.10	0.08	0.04	0.08

Table 1c. Probabilities used to determine the most recent somatic cell count (SCC) measurement, given the parity of the cow and the causal pathogen of clinical mastitis.

	Parity 1 SCC (*1,000 cells/mL)			Parity ≥2 SCC (*1,000 cells/mL)		
	<200	200-500	>500	<200	200-500	>500
Streptococci	0.34	0.22	0.44	0.41	0.17	0.42
Staphylococcus aureus	0.40	0.25	0.35	0.35	0.20	0.45
Escherichia coli	0.84	0.08	0.08	0.70	0.12	0.18

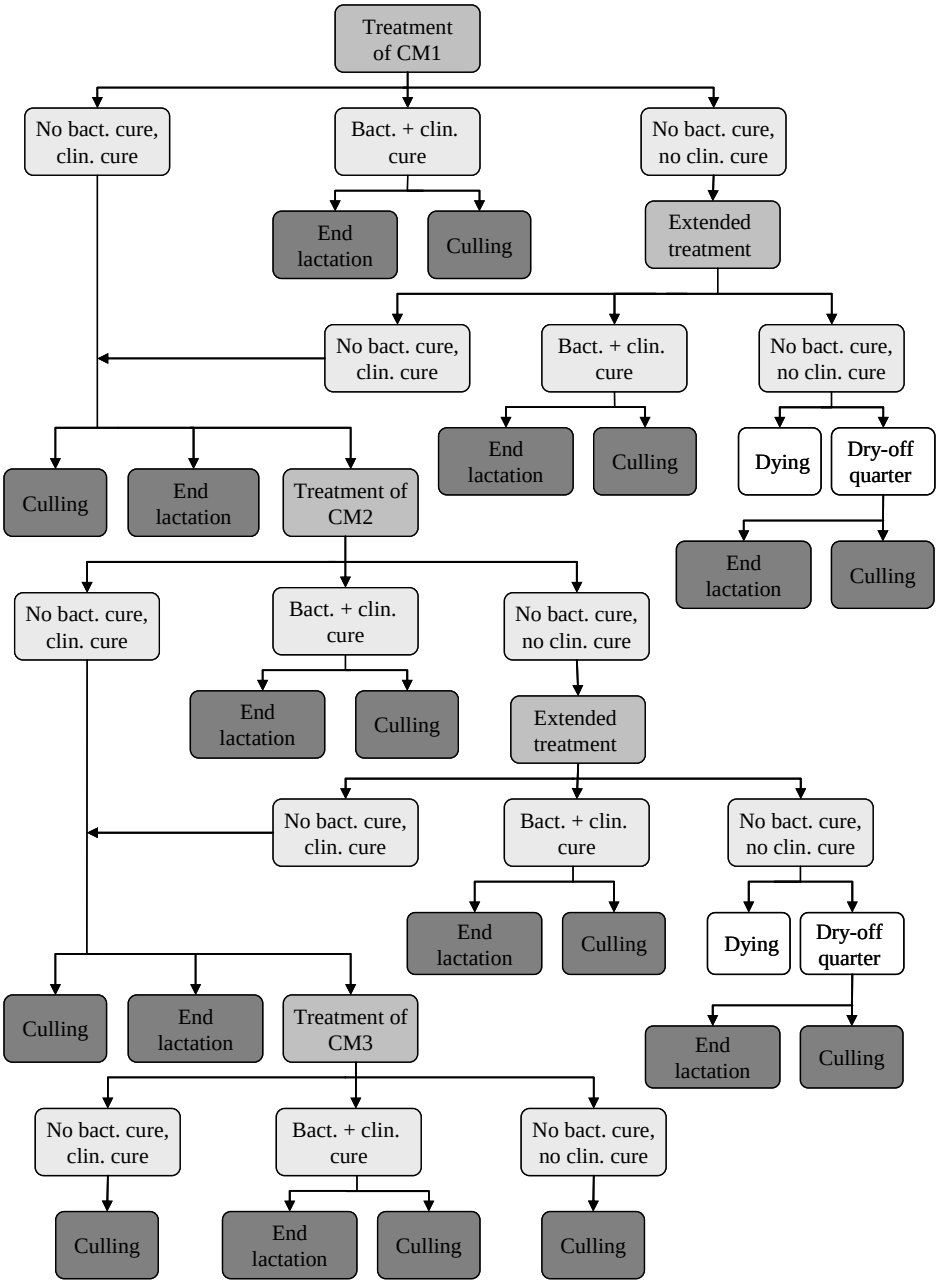

Figure 1. Schematic representation of the simulation model for antimicrobial treatment of a clinical mastitis (CM) case (bact. = bacteriological, clin.= clinical).

To make the probability of bacteriological cure cow-specific, first maximum probabilities of bacteriological cure were determined for each causal pathogen treated with each of the defined antimicrobial treatment regimes. Probability of bacteriological cure was highest (=maximum) for a heifer, not systemically ill, in the first 60 DIM, with a most recent SCC < 200,000 cells/mL and no CM history (Table 2). Because such maximum probabilities of bacteriological cure were not available in literature, values from the following sources were adapted: McDougall et al. (1998), Sol et al. (2000), McDougall (2003), Oliver et al. (2003), Wraight et al. (2003), Serieys et al. (2005), McDougall et al. (2007) and Bradley and Green (2009). Literature information on the probability of bacteriological cure was limited for the intensive antimicrobial treatment regimes. Therefore, assumptions made by the authors were used to fill in all remaining maximum probabilities of bacteriological cure (Table 2). Subsequently, in comparison with the defined maximum probabilities of bacteriological cure, lower probabilities for older cows, for cows >60 DIM, for systemically ill cows, for cows with most recent SCC between 200,000 and 500,000 cells/mL, for cows with most recent SCC >500,000 cells/mL, and for cows with a repeated CM case were assumed (Table 3). Information from the peer-reviewed literature and assumptions made by the authors was used to determine the effect of cow factors on the probability of bacteriological cure (Table 3). A cow-specific probability of bacteriological cure was calculated for each simulated cow with CM treated with each of the 5 defined antimicrobial treatment regimes using the defined maximum probability of bacteriological cure and the defined effects of the cow factors on the probability of bacteriological cure. The cow-specific probability of bacteriological cure ($p_{Bact.cure}$) was calculated with the following equation, using a logit formula:

$$P_{bact.cure} = \frac{1}{(1+Exp(-1*((\ln(\frac{C_{max}}{1-C_{max}})+\ln(\frac{C_{Parity}}{C_{max}})+\ln(\frac{C_{Sys}}{C_{max}})+\ln(\frac{C_{DIM}}{C_{max}})+\ln(\frac{C_{SCC}}{C_{max}})+\ln(\frac{C_{R}}{C_{max}})))}$$

where $C_{max.}$ is the maximum probability of bacteriological cure for the combination of causal pathogen and treatment regime (Table 2). C_{parity}, C_{Sys}, C_{DIM}, C_{SCC} and C_R are the maximum probability of bacteriological cure (Table 2) minus the effect of the cow factors (parity, systemically ill, DIM, most recent SCC and repeated CM) on the probability of bacteriological cure (Table 3).

Subsequently, it was determined whether the CM case was cured bacteriologically or not using a discrete probability distribution. Each bacteriologically cured CM case was assumed to be cured clinically as well. A discrete probability distribution was used to determine whether non-bacteriological cured CM cases were cured clinically or not. These probabilities of clinical cure are presented in Table 2.

Table 2. Characteristics of 5 defined clinical mastitis (CM) antimicrobial treatment regimes.

	Antimicrobial treatment regime				
	IMM3	IMM5	IMM3_S	IMM3_S_NSAID	IMM5_S
Application and duration (d)	Intramammary (3)	Intramammary (5)	Intramammary (3) + systemic (3)	Intramammary (3) + systemic (3) + NSAID (1)	Intramammary (5) + systemic (3)
Total costs antibiotics (€)	15	25	45	54	55
Milk withdrawal (d)	5	7	5	5	7
Total treatment time (min)[1]	42	62	45	48	65
Probability of bacteriological cure[2]					
Streptococcus dysgalactiae or *uberis*	0.70[3]	0.80[6]	0.80[8]	0.83[8]	0.90[8]
Staphylococcus aureus	0.40[4]	0.60[7]	0.60[8]	0.63[8]	0.70[8]
Escherichia coli	0.80[5]	0.80[8]	0.80[8]	0.80[8]	0.80[8]
Probability of clinical cure for non-bacteriological cured CM cases	0.80[8]	0.80[8]	0.80[8]	0.80[8]	0.80[8]

[1]Includes time for treatment and time for milk withdrawal
[2]Probability of bacteriological cure assumed for heifers, not systemically ill, <60 DIM, with somatic cell count <200,000 cells/mL and no previous clinical mastitis
[3]After McDougall et al., 1998; McDougall (2003); Oliver et al., 2003; Wraight et al., 2003; Serieys et al., 2005; McDougall et al., 2007, Bradley and Green, 2009
[4]After Sol et al., 2000; McDougall (2003); Wraight et al., 2003; Serieys et al., 2005; McDougall et al., 2007, Bradley and Green, 2009
[5]After Serieys et al., 2005; Bradley and Green, 2009
[6]After Oliver et al., 2003
[7]After Sol et al., 2000
[8]Estimated by the authors

Completely non-cured CM cases received an extended treatment with the initial treatment regime. Non-bacteriologically, but clinically cured CM cases could get clinical flare-up cases (CM2 and CM3). These clinical flare-up cases were caused by the same pathogen as CM1 and were again treated with the initial treatment regime. In addition, the follow-up of the extended treatment and the treatment of CM2 and CM3 were simulated (Fig. 1). The follow-up was simulated using discrete probability distributions; the probabilities are presented in Table 3.

A cow with a completely non-cured CM case after an extended treatment could die or the quarter of the cow could be dried-off. For cows with dried-off quarters it was determined whether this cow was culled in the remaining of lactation or not (Table 3). A cow that cured completely had a probability of being culled, which depended on whether the CM case was the first, second or third case (Table 3). A cow with a non-bacteriologically, but clinically cured CM case had a probability of being culled, a probability of getting a clinical flare-up, and a probability of reaching the end of lactation. The probability of being culled increased with the number of CM cases (Table 3). The probabilities of being culled were 0.10 higher when the cow with CM was systemically ill.

Table 3. Values and source of parameters used in the simulation model for different antimicrobial treatment regimes for clinical mastitis (CM).

	Value	Source
Input parameters for simulation treatment of CM		
Decrease in probability of bacteriological cure[1]		Expertise
parity ≥ 2	0.10	
days in milk ≥ 60	0.10	
systemically ill	0.20	
SCC 200,000-500,000 cells/mL	0.10	
SCC >500,000 cells/mL	0.20	
repeated case	0.20	
Probability of being culled for non-bacteriological cured CM cases		After Bar et al., 2008a
Initial CM case	0.16	
First clinical flare-up case	0.20	
Probability of being culled for completely cured CM cases		After Bar et al., 2008a
Initial CM case	0.05	
First clinical flare-up case	0.15	
Second clinical flare-up case	0.25	
Probability of mortality for non-clinical cured CM cases	0.05	After Bar et al., 2008a
Probability of drying-off quarter for non-clinical cured CM cases	0.95	Expertise
Probability of being culled for cows with dried-off quarters	0.33	Expertise
Increase in all culling probabilities when cow with CM is systemically ill	0.10	Expertise
Probability of clinical flare-up for non-bacteriological cured CM cases		
Streptococcus dysgalactiae or *uberis*	0.10	Swinkels et al., 2005b
Staphylococcus aureus	0.12	Swinkels et al., 2005a
Escherichia coli	0.05	Döpfer et al., 1999
Increase in milk production losses per month in lactation (%)[2]		After Gröhn et al., 2004;
Staphylococcus aureus	2	Hagnestam et al., 2007;
Escherichia coli	4	Schukken et al., 2009
parity ≥ 2	5	
systemically ill	5	
non-bacteriologically cured	5	
Economic input parameters		
Economic value discarded milk (€/kg)	0.17	Huijps et al., 2008
Economic value milk production losses (€/kg)	0.12	Huijps et al., 2008
Labor costs (€/hour)	18	Huijps et al., 2008
Culling costs (€)	Retention	Houben et al., 1994
	pay-off	Van der Walle, 2004

[1]In comparison with a heifer, not systemically ill, <60 DIM, with somatic cell count <200,000 cells/mL and no previous clinical mastitis

[2]In comparison with a bacteriologically cured CM case caused by streptococci in heifers which were not systemically ill

A pert distribution was used to determine the day of occurrence of the potential first and second clinical flare-up cases. The minimum interval between clinical (flare-up) cases was 14 d, the most likely interval between clinical flare-up cases was 30 d and the last day of occurrence of a clinical flare-up was the last day of lactation. For CM2 and CM3, the daily milk production at the day of occurrence (using Wood's lactation curve), the most recent SCC measurement and whether the cow is systemically ill or not were determined again. It was assumed that a cow not cured bacteriologically after CM3 was culled 7 d after the day of treatment. For all other culled cows, the day of culling was in the remaining of lactation and was determined with a uniform probability distribution.

Calculation of Costs

The total costs for the defined CM treatment regimes included costs for treatment of CM1 (including antimicrobials, discarded milk and labor), costs for treatment of follow-up treatments (for extended treatments and treatments of clinical flare-up cases, including costs for antimicrobials, discarded milk and labor), costs for milk production losses and costs for culling.

Costs for antimicrobials for all defined treatment regimes are presented in Table 2. Total costs of discarded milk were determined by the daily milk production on the day of CM (estimated with Wood's lactation curve), milk withdrawal time (Table 2), and the economic value of discarded milk (Table 3). Total labor costs were determined by treatment time (for treating and milk withdrawal) (Table 2) and the hourly cost of labor (Table 3).

Total costs for milk production loss were determined by the remaining milk production after the CM case (estimated with Wood's lactation curve), the percentage of milk production loss per month after CM, and the economic value of milk production loss (Table 3). The percentage of milk production loss per month after CM depended on the causal pathogen (Gröhn et al., 2004; Schukken et al., 2009), parity (Gröhn et al., 2004; Hagnestam et al., 2007; Schukken et al., 2009), month after CM (Schukken et al., 2009), whether the cow was systemically ill or not, and whether the cow was cured bacteriologically or not (Table 3). Milk production losses ranged from 6% (month 1) to 1% (month ≥8) per month after CM for bacteriologically cured CM cases caused by streptococci in heifers which were not systemically ill. An additional milk production loss per month after CM was added for CM caused by *S. aureus* and *E. coli*, for CM in older cows, for non-bacteriologically cured CM cases and for systemically ill cows (Table 3). Cows with a dried-off quarter had a total 15% milk production loss in the remaining of lactation. After a clinical flare-up case, milk production loss decreased with the same amount as after CM1 (Schukken et al., 2009). In the current study, a percentage milk production loss per month after CM was needed for a combination of cow factors. Because

in most studies estimates on milk production losses were given in kg, it was needed to adapt these values from literature to percentages.

Culling costs were determined for culled cows and were expressed using retention pay-off (RPO). The RPO-values were calculated using a stochastic model developed by Houben et al. (1994). The model outcomes were updated by Van der Walle (2004) using values for prices and production level from the year 2003. With this model, culling costs were based on the specific cow factors parity, month of lactation, pregnancy status and production level. As an example, the culling costs for a pregnant cow in her second lactation with a very high production level were €766, while the culling costs for a non-pregnant heifer with a very low production level were €115.

Validation and Sensitivity Analysis

Because data were not available for an external validation of the model, an internal validation was performed. A large number of inputs were compared to the output to check the consistency and the credibility of the model output. A sensitivity analysis was performed to verify the values of the input parameters and to assess the effect of varying the input values on the outcome total costs of the treatment regime. Values for input variables in the sensitivity analysis were based on information in the literature. Assumptions made by the authors were used if no information in literature was found. The sensitivity analysis was performed on the pathogen distribution, the probability of culling, the percentage milk production losses, probabilities of bacteriological cure, costs for culling, costs for labor, costs for milk production losses and costs for milk withdrawal.

RESULTS

The outcome of treatment specific parameters under default circumstances is presented in Table 4. On average, the overall probability of bacteriological cure for treatment of CM1 with IMM3, IMM5, IMM3_S, IMM3_S_NSAID and IMM5_S was 0.53, 0.65, 0.65, 0.68 and 0.75, respectively. On average, the overall probability of clinical cure for treatment of CM1 with IMM3, IMM5, IMM3_S, IMM3_S_NSAID and IMM5_S was 0.90, 0.93, 0.93, 0.93 and 0.95, respectively. Intensive antimicrobial treatment regimes resulted in less culled cows, less CM cases with an extended treatment and less clinical flare-up cases. The total amount of milk production losses due to CM was approximately the same for all 5 antimicrobial treatment regimes.

On average, total costs for a CM case treated with IMM3 were €176 (Table 5), and consisted of costs for the initial treatment (€52), follow-up treatments (€7), milk production losses (€75) and culling (€42). The total costs of CM treated with the intensive

antimicrobial treatments were €194, €199, €204 and €216 for IMM5, IMM3_S, IMM3_S_NSAID and IMM5_S, respectively. With increasing intensity of the antibiotic treatment regime, the costs for milk production losses and culling decreased. These benefits of the intensive antimicrobial treatment regimes did, however, not outweigh the increasing costs for the initial treatment (costs for antibiotics, milk withdrawal and labor). For instance, in comparison with IMM3, the average benefits of IMM5 were €8 (€4 for less milk production losses and €4 for less culling), while the average extra costs were €26 (€10 for antibiotics, €10 for milk withdrawal and €6 for labor). In Table 5, the variability in outcomes is shown using 5th and 95th percentiles. The range in outcomes was smaller for the intensive antimicrobial treatment regimes.

Table 4. Average non-economic output of the Monte Carlo simulation model.

	Antimicrobial treatment regime[1]				
	IMM3	IMM5	IMM3_S	IMM3_S_ NSAID	IMM5_S
Probability of bacteriological cure for CM1[2]					
Streptococcus dysgalactiae or *uberis*	0.60	0.73	0.73	0.76	0.86
Staphylococcus aureus	0.22	0.47	0.47	0.50	0.60
Escherichia coli	0.74	0.74	0.74	0.74	0.74
Overall	0.53	0.65	0.65	0.68	0.75
Probability of clinical cure for CM1[2]					
Streptococcus dysgalactiae or *uberis*	0.92	0.95	0.95	0.96	0.97
Staphylococcus aureus	0.84	0.89	0.89	0.90	0.91
Escherichia coli	0.94	0.94	0.94	0.94	0.94
Overall	0.90	0.93	0.93	0.93	0.95
Overall probability of bacteriological cure for CM2 and CM3[3]	0.44	0.57	0.57	0.68	0.61
Culled cows (%)	12	10	10	9	8
Milk production losses (%)	7	6	6	6	6
Extended treatments (%)	10	7	7	5	3
CM2 and CM3[3] (%)	4	3	3	2	2

[1]IMM3 = standard 3 d intramammary treatment with antimicrobials, IMM5 = extended 5 d intramammary treatment with antimicrobials, IMM3_S = 3 d standard intramammary + systemic treatment with antimicrobials, IMM3_S_NSAID = standard 3d intramammary + systemic with antimicrobials + 1d NSAID, and IMM5_S = extended 5 d intramammary + systemic with antimicrobials.
[2]Treatment of the initial clinical mastitis case
[3]Treatment of the clinical flare-up cases

Table 5. Average total costs (€) for the 6 clinical mastitis treatment regimes, including costs for the different cost factors (5th and 95th percentiles given between brackets).

	Treatment regime[1]					
	IMM3	IMM5	IMM3_S	IMM3_S_ NSAID	IMM5_S	Culling
Treatment CM1[2]						
Antibiotics	15	25	45	54	55	0
Milk withdrawal	24	34	24	24	34	0
	(9; 33)	(13; 47)	(9; 33)	(9; 33)	(13; 47)	
Labor	13	19	14	14	20	0
Treatment CM2	7	7	8	7	6	0
and CM3[3]	(0; 55)	(0; 76)	(0; 87)	(0; 90)	(0; 87)	
Milk production	75	71	71	70	68	0
losses	(20; 151)	(19; 145)	(18; 145)	(19; 142)	(19; 120)	
Culling	42	38	37	35	33	556
	(0; 428)	(0; 380)	(0; 382)	(0; 350)	(0; 221)	(152; 962)
Total costs	176	194	199	204	216	556
	(62; 561)	(81; 566)	(91; 567)	(100; 561)	(110; 552)	(152; 962)

[1]IMM3 = standard 3 d intramammary treatment with antimicrobials, IMM5 = extended 5 d intramammary treatment with antimicrobials, IMM3_S = 3 d standard intramammary + systemic treatment with antimicrobials, IMM3_S_NSAID = standard 3d intramammary + systemic with antimicrobials + 1d NSAID, and IMM5_S = extended 5 d intramammary + systemic with antimicrobials.
[2]Initial clinical mastitis case of the cow
[3]Costs for antibiotics, milk withdrawal and labor of the clinical flare-up cases

The causal pathogen and several cow characteristics influenced the total costs of CM treated with different treatment regimes (Table 6). On average, the total costs of a CM case caused by streptococci and treated with IMM3 was €154, while the total costs of a *S. aureus* CM case and treated with IMM3 was €200. The costs of CM increased with increasing daily milk production at moment of CM, increasing relative production value, increasing parity number and decreasing month in milk. Also, for repeated CM cases and CM cases in systemically ill cows, the costs were higher than for non-repeated cases and CM cases in not systemically ill cows, respectively. Antimicrobial treatment of CM with IMM3 resulted in the lowest total costs for all causal pathogens and for all cow characteristics. There was only one exception, immediately culling resulted in the lowest total costs for cows with a low relative production value (<80).

The association between total average costs and the probability of bacteriological cure is presented in Fig. 2. Treatment of all simulated CM cases with IMM3 resulted in the lowest total average costs but also in the lowest probability of bacteriological cure (0.53) (Fig. 2). The total costs and the probability of cure increased with the intensive antimicrobial treatments.

Table 6. Average total costs (€) for the 6 treatment regimes for all cow characteristics.

	Treatment regime[1]					
	IMM3	IMM5	IMM3_S	IMM3_S_ NSAID	IMM5_S	Culling
Overall	176	194	199	204	216	556
Causal pathogen						
Streptococcus dysgalactiae or *uberis*	154	173	178	185	193	550
Staphylococcus aureus	200	204	207	216	225	545
Escherichia coli	181	213	220	226	243	579
Daily milk production (kg)						
<20	110	122	136	151	139	354
20-25	129	145	151	159	163	426
25-30	140	159	170	178	185	419
30-35	152	172	178	185	200	412
35-40	176	197	200	211	223	535
>40	223	242	243	249	260	753
Systemically ill						
No	167	184	188	196	206	555
Yes	230	256	267	272	286	561
Previous clinical mastitis						
No	175	194	199	207	218	559
Yes	184	198	199	202	214	545
Parity						
1	123	142	152	158	170	242
≥2	187	206	210	217	228	625
SCC (*1,000 cells/mL)						
<200	173	197	200	207	221	554
200-500	174	189	197	206	214	545
>500	182	194	201	208	216	563
Relative production value						
<80	95	115	127	137	149	94
80-90	122	142	147	155	169	213
90-100	156	175	182	190	201	432
100-110	186	205	209	216	226	616
110-120	212	226	228	237	248	759
>120	237	258	234	257	259	924
Month in milk						
1	202	222	226	234	246	658
2	205	225	223	234	246	614
3	189	210	211	217	227	584
4	178	191	199	208	219	533
5	151	171	178	187	199	485
6	140	156	168	176	179	441
7	125	145	151	158	166	453
≥8	121	136	145	142	164	434

[1]IMM3 = standard 3 d intramammary treatment with antimicrobials, IMM5 = extended 5 d intramammary treatment with antimicrobials, IMM3_S = 3 d standard intramammary + systemic treatment with antimicrobials, IMM3_S_NSAID = standard 3d intramammary + systemic with antimicrobials + 1d NSAID, and IMM5_S = extended 5 d intramammary + systemic with antimicrobials.

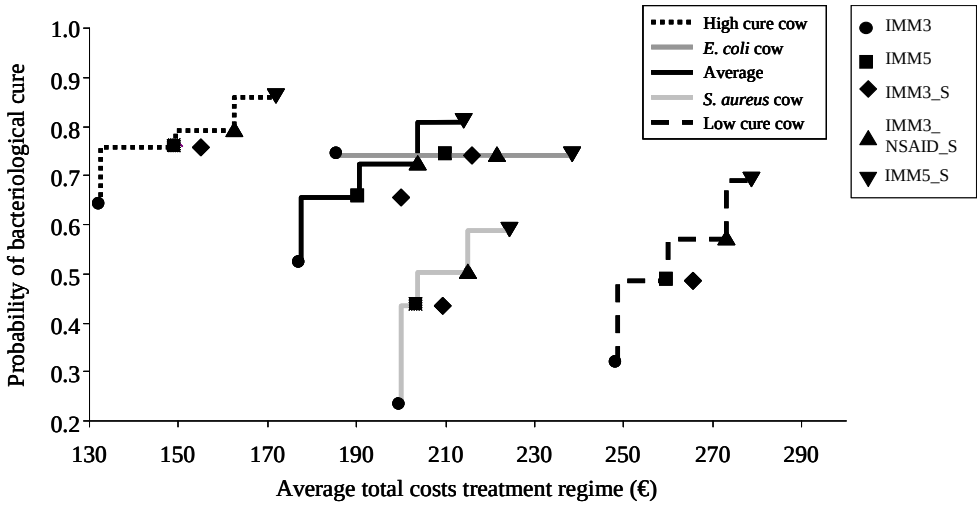

Figure 2. Average total costs for each antimicrobial treatment regime with their associated average probability of bacteriological cure for different cows (IMM3 = standard 3 d intramammary treatment with antimicrobials, IMM5 = extended 5 d intramammary treatment with antimicrobials, IMM3_S = 3 d standard intramammary + systemic treatment with antimicrobials, IMM3_S_NSAID = standard 3d intramammary + systemic with antimicrobials + 1d NSAID, and IMM5_S = extended 5 d intramammary + systemic with antimicrobials). The lines resemble the least-cost frontier of treatment for the different clinical mastitis cases.

Treatment regimes included on the least-cost frontier resembles those treatment options that are the most cost effective. Because treatment with IMM3_S resulted in higher costs but the same probability of cure as with treatment with IMM5, the IMM3_S treatment was not cost-effective and therefore not included on the least-cost frontier. Total costs and probability of cure are also presented for an *E. coli* CM case, a *S. aureus* CM case, a CM case with a low probability of cure (older cow, >60 DIM, SCC>500,000 cells/mL, a previous CM case and systemically ill), and for a CM case with a high probability of cure (heifer, <60 DIM, SCC<200,000 cell/mL, no previous CM and not systemically ill) (Fig. 2). Treatment with IMM3 resulted in the lowest total costs for all these specific cows. The difference in total average costs between the antimicrobial treatment regimes was smaller for a cow with *S. aureus* CM than for a cow with a high probability of cure.

Results of the sensitivity analysis are given in Tables 7 and 8. The pathogen distribution, the probability of culling a cow, the amount of milk production losses, the costs for culling, the costs for milk production losses, costs for discarded milk and costs for labor influenced the total costs for CM treated with different treatment regimes. For instance, increasing the economic value of milk with €0.05 did increase the costs of CM treated

with IMM3 with €39. Treatment of CM with IMM3 resulted in the lowest total costs for all situations presented in Table 7.

Increasing probabilities of bacteriological cure resulted in lower costs for CM for all defined antimicrobial treatment regimes, while decreasing probabilities of bacteriological cure resulted in higher costs for CM (Table 8). By increasing the probability of bacteriological cure of streptococcal CM with 0.10 for all defined antimicrobial treatment regimes, the total costs of CM treated with IMM3 decreased with €7 in comparison with the default situation. By increasing the probability of bacteriological cure of *S. aureus* and *E. coli* CM with 0.10 for all defined treatment regimes, the total costs of CM treated with IMM3 decreased with €8 and €7 in comparison with the default situation, respectively. By increasing the probability of bacteriological cure of the intensive antimicrobial treatment regimes (IMM5, IMM3_S, IMM3_S_NSAID and IMM5_S) with 0.10, treatment of CM with IMM5 resulted in the same total costs as treatment with IMM3.

Table 7. Sensitivity of the total costs (€) of clinical mastitis (CM) treated with 6 different treatment regimes for different input values.

	Value	Default	Treatment regime[1]					
			IMM3	IMM5	IMM3_S	IMM3_S_ NSAID	IMM5_S	Culling
Default situation			176	194	199	204	216	556
Pathogen distribution (Streptococci; *S. aureus*; *E. coli*)	0.60; 0.20; 0.20	0.40; 0.30; 0.30	172	188	196	200	208	556
	0.20; 0.60; 0.20	0.40; 0.30; 0.30	184	199	203	210	224	556
	0.20; 0.20; 0.60	0.40; 0.30; 0.30	184	198	205	216	229	556
Probability of culling for non-bacteriological cured CM cases	0.21; 0.25[2]	0.16; 0.20[2]	186	200	205	212	219	556
	0.11; 0.15[2]	0.16; 0.20[2]	171	191	196	205	219	556
Probability of culling for completely cured CM cases	0	0.05; 0.15; 0.25[3]	166	178	182	190	200	556
Milk production losses per month in lactation (%)	6-30[4]	1-25[4]	204	219	226	237	247	556
	1-20[4]	1-25[4]	147	164	170	180	191	556
Costs culling (€)	RPO +100	RPO[5]	188	204	208	217	223	659
	RPO -100	RPO	165	179	187	192	208	455
Economic value milk (€/kg)	0.17[6]; 0.22[7]	0.12[6]; 0.17[7]	215	232	237	245	256	556
	0.07[6]; 0.12[7]	0.12[6]; 0.17[7]	138	150	159	171	179	556
Costs labor (€/hour)	0	18	168	178	185	195	198	556
	9	18	169	182	194	200	208	556

[1]IMM3 = standard 3 d intramammary treatment with antimicrobials, IMM5 = extended 5 d intramammary treatment with antimicrobials, IMM3_S = 3 d standard intramammary + systemic treatment with antimicrobials, IMM3_S_NSAID = standard 3d intramammary + systemic with antimicrobials + 1d NSAID, and IMM5_S = extended 5 d intramammary + systemic with antimicrobials.
[2]Dependent on culling due to the first clinical mastitis case or due to the first clinical flare-up case
[3]Dependent on culling due to the first clinical mastitis case, due to the first clinical flare-up case or due to the second clinical flare-up case
[4]Dependent on the causal pathogen, parity, month after CM case, whether cow is systemically ill or not and whether CM is cured bacteriologically or not
[5]Retention pay-off
[6]Economic value for milk production losses
[7]Economic value for discarded milk.

Table 8. Sensitivity of the total costs (€) of clinical mastitis (CM) treated with 5 different treatment regimes for input values on probability of bacteriological cure.

	Change in probability of bacteriological cure	Antibiotic treatment regime[1]				
		IMM3	IMM5	IMM3_S	IMM3_S_ NSAID	IMM5_S
Default probability of bacteriological cure[2]						
Streptococcus dysgalactiae or *uberis*		0.70	0.80	0.80	0.83	0.90
Staphylococcus aureus		0.40	0.60	0.60	0.63	0.70
Escherichia coli		0.80	0.80	0.80	0.80	0.80
Default situation		176	194	199	204	216
Probability of bacteriological cure of Streptococci CM	+ 0.10	169	184	191	200	244
	- 0.10	177	189	197	206	218
Probability of bacteriological cure of *Staphylococcus aureus* CM	+ 0.10	168	189	194	196	211
	- 0.10	176	194	201	208	219
Probability of bacteriological cure of *Escherichia coli* CM	+ 0.10	169	185	190	200	208
	- 0.10	179	192	200	207	219
Probability of bacteriological cure of IMM5, IMM3_S, IMM3_S_NSAID and IMM5_S[1]	+ 0.05	176	185	190	200	206
	+ 0.10	176	176	184	193	234
	- 0.05	176	196	207	210	221
	- 0.10	176	206	215	220	231

[1]IMM3 = standard 3 d intramammary treatment with antimicrobials, IMM5 = extended 5 d intramammary treatment with antimicrobials, IMM3_S = 3 d standard intramammary + systemic treatment with antimicrobials, IMM3_S_NSAID = standard 3d intramammary + systemic with antimicrobials + 1d NSAID, and IMM5_S = extended 5 d intramammary + systemic with antimicrobials.

[2]Probability of bacteriological cure assumed for heifers, not systemically ill, <60 DIM, with somatic cell count <200,000 cells/mL and no previous clinical mastitis

DISCUSSION

In this study, total average costs for a CM case treated with IMM3 were €176 (Table 5), and consist of costs for the initial treatment (€52), the follow-up treatments (€7), milk production losses (€75) and culling (€42). The total costs increased with the more intensive antibiotic treatment regimes. In comparison with IMM3, treating with the intensive antibiotic treatment regimes resulted in benefits (Table 5), resulting from less follow-up treatments, less milk production losses and less culling (Table 4). These benefits did, however, not outweigh the extra treatment costs (costs for antibiotics, milk withdrawal and labor) (Table 5). Additionally, for all different cow characteristics (Table 6 and Fig. 2) and under different circumstances (Table 7), treatment of CM with IMM3 always resulted in the lowest total costs. For all different cow characteristics, the benefits of the intensive antibiotic treatment regimes on decreased costs for follow-up treatments, milk production losses and culling did not outweigh the extra treatment costs for the initial CM case. Based on our assumptions, cow-specific treatment of CM was not economically beneficial.

Results of the current study are highly influenced by the used assumptions, and therefore the results will not be applicable to all farm situations. Because of lack of detailed information, as was needed for our model, several assumptions had to be made. These assumptions included for instance the pathogen distribution used, the probabilities of cure and culling used, and an assumption that all defined treatments are effective against both Gram-positive and Gram-negative pathogens. All probabilities of cure used in the current simulation model were based as much as possible on information in the peer-reviewed literature. Although many clinical trials on treatment of CM have been performed (e.g., Serieys et al., 2005; McDougall et al., 2007; Bradley and Green, 2009), detailed information on the probability of cure necessary for the current simulation model was not available in literature. Pathogen-specific probabilities of bacteriological and clinical cure were available for treatment with IMM3 (e.g., McDougall, 1998; McDougall, 2003; Wraight, 2003), but for the more intensive antibiotic treatment regimes these pathogen-specific probabilities of cure were only reported in 3 studies (Sol et al., 2000; Oliver et al., 2003; Taponen et al., 2003). Moreover, it was necessary to adapt available average probabilities of cure to maximum probabilities of cure because cow-specific probabilities were needed for the simulations. Also, estimates on the effect of cow factors on the probability of cure were necessary. Although some studies indicated that cow factors influenced the probability of cure (Sol et al., 2000; Bradley and Green, 2009), usable estimates on the effects were not available. Assumptions made by the authors were used to determine all missing probabilities of cure, to adapt all average probabilities to maximum probabilities of cure, and to determine the effect of cow factors on the probability of cure.

There was a lot of uncertainty about the used probabilities of cure, and thus the sensitivity analysis was important to verify our estimates.

The probabilities of bacteriological cure used, influenced the total costs of CM. Higher probabilities of cure resulted in lower costs for CM. Increasing or decreasing the probabilities of bacteriological cure, however, did not result in lower total costs for the intensive antibiotic treatment regimes than for the standard antibiotic treatment regime (Table 8). Increasing the probability of bacteriological cure for IMM5 with 0.10 resulted in equal total costs (€176) for treatment with IMM3 and IMM5 (Table 8). This implies that the benefits of treating with IMM5 did almost outweigh the extra treatment costs. So, especially the difference in probability of bacteriological cure between the defined treatment regimes influences whether cow-specific treatment is economically beneficial. Using our defined values on the probabilities of bacteriological and clinical cure, cow-specific treatment of CM is not economically beneficial. But this conclusion may change when results of future clinical trials on intensive antibiotic treatment regimes will give much higher probabilities of cure.

To determine if cow-specific treatment of CM is economically beneficial, treatment regimes were defined which were used by Dutch dairy farmers. Also, Dutch economic values were used, for instance on costs for culling, discarding milk and labor. Other studies using Dutch input values also estimated the costs for CM cases treated with standard treatment regimes (Huijps et al., 2008; Halasa et al., 2009). The costs for a CM case of €176 were lower than the €210 found by Huijps et al. (2008). This was especially due to the used probability of culling in the current model, which was based on whether the CM case was the first case in the lactation or a follow-up one (Bar et al., 2008a), whether the cow was systemically ill or not and the cure status. The costs for CM caused by streptococci, *S. aureus* and *E. coli* of €154, €199 and €188 (Table 6) are in agreement with estimates found by Halasa et al. (2009). Milk production losses and culling have a high contribution to the total costs of CM (Table 5), as was also found by Huijps et al. (2008) and Halasa et al. (2009). In other countries, the costs for culling, discarding milk and labor are different. Including different economic input values influenced the total costs for CM, as was found in the results from the sensitivity analysis. Varying these economic input values did, however, not influence the result that treating CM with IMM3 resulted in the lowest total costs. To adapt the model to other countries, country-specific economic input values are needed.

Including culling in the current model was difficult. Culling is a decision of the dairy farmer and several factors are involved. Not only the udder health status, but also factors such as milk production, reproduction status (e.g., Lehenbauer and Oltjen, 1998), the milk quota situation of the farm and the availability of replacement heifers play a role as well in

the decision to cull a cow. Including all these factors is very difficult and was not included in our study.

The costs of culling have a high contribution to the total costs of CM and influence the amount of benefits of the intensive antibiotic treatment regimes (Table 5). The costs of culling were based on the RPO-value of the cow, this value represents the amount of money that should be spent in trying to keep the cow in case of health problems (e.g., Groenendaal et al., 2004). The RPO-value of a cow varies between farms and estimations of the RPO-value are difficult to make. The results of the sensitivity analysis demonstrate, however, that increasing or decreasing the costs of culling a cow did not result in lower costs for the intensive antibiotic treatment regimes than for the standard antibiotic treatment regime. Average total costs for immediately culling of a cow with CM were €556. This value was much higher than the average total costs for the 5 antimicrobial treatment regimes (Table 5) and therefore immediately culling of a cow with CM is unprofitable.

It will be interesting to include the no treatment scenario in our model. It was decided not to include this scenario for 2 reasons. First, in the Netherlands almost all CM cases are treated with antimicrobials. No treatment of CM cases is very rare, and therefore including no treatment does not reflect reality for Dutch farms. Secondly, for our model very detailed information was needed, for instance for the bacteriological cure. There is hardly any experience with leaving CM cases untreated (Guterbock et al., 1993; Roberson et al., 2004). Therefore, also for the authors (and other experts) it was very difficult to give cure estimates for the no treatment scenario. Because of the lack of available information about the no treatment scenario we decided not to include this scenario. We expect that including the no treatment scenario based on best guesses will not reflect reality and can result in wrong conclusions.

Benefits of CM treatment also include prevention of mastitis in other cows at the farm. Halasa et al. (2009) mentioned the importance of transmission of pathogens for the costs of CM. Van den Borne et al. (accepted) showed that lactational treatment of contagious subclinical mastitis seems economically beneficial in dairy herds that implement management measures to decrease the transmission of contagious mastitis pathogens such as *S. aureus*. The intensive antibiotic treatment regimes resulted in higher probabilities of bacteriological cure than the standard treatment regime (Table 4), and these intensive treatment regimes will therefore prevent more new CM cases in other cows than the standard treatment regime. It is expected that also including these benefits will favor the intensive antibiotic treatment regimes more. To include these benefits, the within-herd dynamics of infection must be included and developing a herd-level model is necessary.

The model developed in our study simulated single CM cases, and it was not possible to properly include the within-herd dynamics of infection.

Dairy farmers do not always take cost-effective decisions. Also other factors than the costs influence mastitis management decisions (Valeeva et al., 2007). For specific cows with CM, farmers will prefer a treatment which results in the highest probability of cure instead of the lowest total costs. Taking these decisions will not result in a cost-effective decision, but for some farmers a cured cow is more important. For some specific CM cases, the extra amount of money needed to reach a higher probability of cure is small. For instance, treating with IMM5 instead of IMM3 for a *S. aureus* CM will result in an additional €4, but the probability of cure will increase with 0.25 (Fig. 2). This implies that using the intensive antibiotic treatments for *S. aureus* CM is more economically beneficial than for CM cases caused by other pathogens. Bacteriological culture results comes too late available, therefore, a choice of treatment needs to be made without knowing the causal pathogen. Several models, however, were developed that can aid diagnosis of the causal pathogen (Jones and Ward, 1990; Heald et al., 2000; De Haas et al., 2004; Steeneveld et al., 2009).

Recently, promising results are presented about on-farm culture systems to get insight in the causal pathogen of CM (Lago et al., 2009; Keefe et al., 2010). For mild or moderate CM cases there was no difference in probability of cure (Keefe et al., 2010), CM recurrence, SCC, milk production and culling (Lago et al., 2009) between CM cases treated immediately and cases treated 24 hours after detection. It would be very interesting to evaluate the use of these on-farm diagnostic tests economically. However, to evaluate the economic consequences of using on-farm diagnostic tests a different model is needed. With that model all possible outcomes of on-farm bacteriological culture must be modeled. Thereafter, according to the test result all possible actions of the farmer must be modeled.

With increasing automation on dairy farms, the possibilities for automated individual cow management have also increased. Automated decisions support on choice of CM treatment for individual cows is an example of automated individual cow management. To determine whether it is worthwhile to include choice of treatment in decision support models, it is needed to determine if choosing different antibiotic treatment for different cows is economically beneficial. Because of the cow-specific probabilities of cure, it was expected that cow-specific treatment would be economically beneficial. Based on our assumptions, cow-specific treatment of CM was not economically beneficial.

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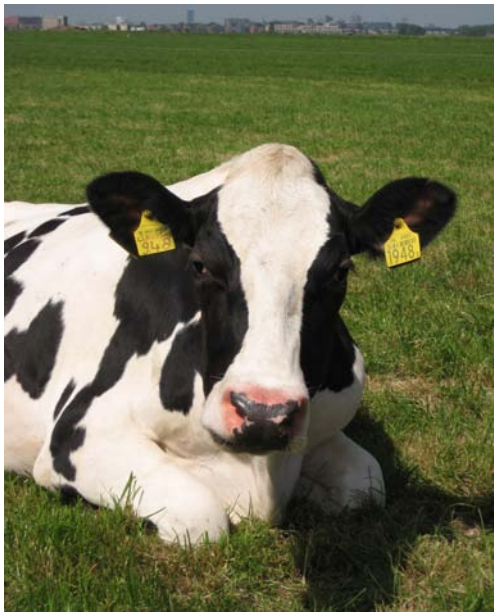
Chapter 7

General discussion



Information obtained from precision dairy farming technologies is only useful if it is interpreted and utilized effectively in decision making (Bewley, 2010). Support systems will be needed to take the best individual cow decisions. Because of the continuous monitoring of cows on farms with an automatic milking system (AMS), especially on these farms there are possibilities for such support. To maintain a good udder health on dairy farms with an AMS, two individual cow decisions must be made. First, it must be decided which alerted cows need to be checked visually for clinical mastitis (CM). Currently, the management system of the AMS just provides alert lists containing cows suspected of having CM. It is believed that by just providing alert lists, the management system is underutilized. It is expected that by combining sensor information and non-AMS cow information CM detection can be improved (e.g., Mottram, 1997; Chagunda et al., 2006). The second decision to make is a treatment for each cow with CM. It is expected that individual cow information and information from the visual inspection can be used to improve such decisions.

This thesis was conducted with the objectives (i) to improve CM detection with AMS by combining sensor and non-AMS cow information and (ii) to improve CM treatment decisions. In this chapter, the main results of this thesis are discussed. Additionally, the datasets and methodologies used are discussed. Finally, future prospects for detection and treatment of CM are described.

MAIN RESULTS OF THE STUDY

Improving CM detection with AMS by combining sensor and non-AMS cow information

It was expected that by combining sensor information and non-AMS cow information, CM detection could be improved. All non-AMS cow information variables studied (parity, month in lactation, season of the year, somatic cell count (SCC) history and CM history) influenced the probability of having CM (Chapter 2). It seemed that a priority list containing the cows with the highest priority for visual checking for CM could be made (Chapter 3). Using data from farms with an AMS, however, it turned out that the non-AMS cow information had no additional value for CM detection (Chapter 4b). Moreover, adding non-AMS cow information to the already available alert information did not improve the ability to discriminate between true-positive and false-positive mastitis alerts (Chapter 4a). It was unexpected that adding non-AMS cow information can not be used to further distinguish between milkings with and without CM. The unexpected result can perhaps be explained by the large number of cows having subclinical mastitis, the non-discriminative power of SCC obtained from the four-weekly milk recording, and the correlation between electrical conductivity and SCC. These explanations are discussed in more detail below.

Cows with subclinical mastitis. Subclinical mastitis has several risk factors on non-AMS cow information in common with CM, such as an increased parity (e.g., Busato et al., 2000; Zadoks et al., 2001b). Moreover, both CM and subclinical mastitis are characterized by an increased SCC (e.g., Djabri et al., 2002a). Therefore, by adding non-AMS cow information also cows suspected of having subclinical mastitis get increased posterior probabilities for having CM. The objective of the current study was to improve CM detection. Because cows with subclinical mastitis are not clinically diseased and show normal milk, the increased posterior probabilities of cows having high SCC and thus suspected of having subclinical mastitis are considered unjustified.

Non-discriminative power of SCC. Although cows with an increased SCC are at higher risk of getting CM (e.g., Green et al., 2004; Whist and Østerås, 2006; Breen et al., 2009), which was also reported in our study (Chapter 2), high SCC was not discriminative enough to detect cows with CM given that they were already alerted. In the datasets used for the current study, many cows were present with high SCC. Of all alerted milkings on the research farm, in total 2,217 had a SCC ≥500,000 cells/mL in the previous 30 days, while for only 34 milkings CM was reported (Chapter 4a). On the commercial farms, in total 53,566 milkings had a SCC ≥200,000 cells/mL in the previous 30 days, while for only 130 milkings CM was reported (Chapter 4b). Using the presented method, for all these milkings increased posterior probabilities of having CM were calculated. Apparently, the majority of the cows with increased posterior probabilities due to high SCC did not get CM. So, for the majority of the milkings, adding SCC resulted in an unjustified increased posterior probability.

Probably, the SCC variables did not contain enough discriminative power because these variables are not measured in the milk during the milking with the AMS. The SCC-values came from the four-weekly milk recording, and the SCC-values include the values from the previous 30 days, before the previous 30 days and the mean of the previous lactation. Because of the long time-window for the SCC history variables, wrong conclusions on the SCC-status can be made. For instance, a cow having a high SCC in the previous 30 days, but a low SCC at the moment of the milking, had a high posterior probability on having CM. This wrong conclusion on the current SCC-status of the cow will cause a wrong indication about the current CM-status of the cow. Besides the SCC information variables, also the other non-AMS cow information variables did not improve the ability to discriminate between milkings with and without CM. Also a French study (Djabri et al., 2002b) concluded that taking parity and stage of lactation into account had a very limited impact on the accuracy of detection intramammary infections.

Correlation between electrical conductivity and SCC. Because SCC and electrical conductivity are correlated (e.g., Kamphuis et al., 2008), the discriminative power of SCC is probably also limited. The correlation indicates that electrical conductivity and SCC are partly describing the same information. This can explain why adding SCC information, in presence of electrical conductivity information, did not improve the ability to discriminate between milkings with and without CM.

Improving CM treatment decisions

It was expected that CM treatment decisions can be improved by taking into account cow information and the information from the visual inspection. These information sources can indeed be used to give a probability distribution on the causal pathogen (Chapter 5). For CM cases with a very high probability on Gram-positive or Gram-negative pathogens (>0.90) a very reliable indication about the Gram-status can be given. In the current study, 28% of the CM cases had such a high probability for the Gram-status of the pathogen. This information can be used to take more informed decisions on the most appropriate treatment for 28% of the CM cases. On a farm, this information can result in an improvement in treatment success in comparison with the current situation where information on the Gram-status is not provided at all.

By taking into account cow information and information from the visual inspection, a differentiation can be made in the expected probability of cure of different antimicrobial treatment regimes. Differentiation of treatments for different CM cases does, however, not provide economic benefits. Treating each cow with CM with a standard intramammary treatment resulted in the lowest total costs, which will support the decision to treat each cow with CM with the standard treatment regime (Chapter 6). Not only economics, however, influence the treatment decision of a farmer. It is previously reported that other factors than economics influence mastitis management decisions (Huijps et al., 2010; Valeeva et al., 2007). Results of a Dutch survey show that the most annoying aspects of mastitis were the uncertainty of a cows' recovery (31%), the extra labor (24%) and the financial consequences (20%) (Jansen et al., 2009). These results indicate that for some farmers a cured cow is more important than achieving the lowest total costs. Results of this thesis show that for some specific CM cases, the extra amount of money needed to reach a higher probability of cure is small. For instance, treating with the 5 day intramammary treatment instead of the standard intramammary treatment for a *S. aureus* CM case will result in only an additional €4, but the probability of cure will increase with 0.25. Using the intensive antibiotic treatments for *S. aureus* CM is more economically beneficial than for CM cases caused by other pathogens (Chapter 6). To support treatment decisions, both the expected probability of cure and the expected total costs of different treatment regimes must be presented to the dairy farmer.

DATA USED IN THIS THESIS

In our work, several datasets were used. All datasets used had their advantages and disadvantages. To determine which combination of non-AMS cow factors influence the probability of having CM (Chapter 2), a dataset was used which was collected in the years 1992-1994 on 274 farms in the northern part of the Netherlands (Barkema et al., 1998). This dataset included a large variety of cows (n = 22,860) and CM cases (n = 5,363), but the dataset was also relatively old and not collected on farms with an AMS. A more recent dataset collected on farms with an AMS would have been preferred, but was not available. We believe, however, that the dataset used was appropriate for the conducted study. It is likely that the effect of cow factors (parity, month in lactation, SCC history and CM history) on CM did not change since 1992-1994, and are more or less equal on farms with and without AMS. For instance, a cow with a high SCC was and still is at higher risk of having CM on every farm, independent of the milking system used.

Chapter 3 was used for exploration and illustration of combining a probability of having CM (based on non-AMS cow information) and the test characteristics of the CM detection system of the AMS. For this study, the same dataset was used as for Chapter 2. Chapter 3 was used as a proof of principle, and therefore it was not necessary to have recent data from farms with an AMS.

For validation of a CM detection model, it was necessary to use data from farms with an AMS. To validate whether sensor data and non-AMS cow information can be used to discriminate between true-positive and false-positive mastitis alerts from an AMS, data from a single research farm with four AMSs was used (Chapter 4a). To validate the performance of a CM detection model using sensor data and non-AMS cow information, data from nine commercial farms with in total 12 AMSs was used (Chapter 4b). A disadvantage of these datasets was that on these farms not all mastitis alerts were checked visually. Because of this, it was not possible to calculate the exact sensitivity (SE) and specificity (SP). Also some true-positive alerts for CM may have been missed and consequently, some alerts may have been incorrectly assigned as false-positive alerts. A dataset including milkings which were all checked visually will be perfect for development of a CM detection model. The results of our work show very clearly that adding non-AMS cow information does not improve CM detection. It is expected that adding non-AMS cow information to CM detection models based on perfect data will also not result in an improved CM detection.

For providing a probability distribution on the causal pathogen (Chapter 5), the same dataset was used as in Chapter 2 and 3. The final dataset included 3,534 CM cases with information on the Gram-status. The results show that cow information and information

from the visual inspection can be used to provide a probability distribution on the Gram-status of a CM case. Although it is not likely that the pathogenesis of CM cases has changed very much, it will be worthwhile to repeat the study based on more recent data. The level of acceptance of a Gram-classification model will be higher when it is based on recent data.

To determine whether cow-specific treatment is economically beneficial (Chapter 6), information from several studies was used. To simulate the CM cases, information from the dataset used in Chapter 5 was used. Therefore, the simulation was based on detailed information from a large variety of CM cases. To simulate the consequences of different antimicrobial treatment regimes, very detailed information was needed, for instance on the probability of cure, probability of culling and the amount of milk production losses. For the standard treatment regime, information on the probability of cure was available in literature (e.g., McDougall et al., 2007; Bradley and Green, 2009). For the intensive antimicrobial treatments, however, the information in literature on the probability of cure was limited. Therefore, it was necessary to use authors' expertise to fill in all needed probabilities of cure. There is hardly any experience with leaving CM cases untreated (Guterbock et al., 1993; Roberson et al., 2004). Consequently, also for experts it will be difficult to give estimates on the probability of cure. Therefore, non-treatment of CM was not included in the stochastic model described in Chapter 6. The usefulness of a simulation model is as good as the input used. To improve the usefulness, it will be needed to include real farm data, preferably from the Netherlands. Currently, registration of antibiotics for individual cows is mandatory, and there are increasing possibilities to do the registration automatically via management systems. Instead of setting up clinical trials, data from these management systems can probably be used to evaluate several antimicrobial treatments. This data will be useful for our developed simulation model.

METHODOLOGIES USED IN THIS THESIS

Naive Bayesian networks

For our application, the preferred outcome of the CM detection model was a probability of having CM based on sensor measurements and non-AMS cow information. Bayesian networks are powerful tools for the representation of probability distributions and for probabilistic reasoning (Jensen, 2001). For this thesis, the simplest type of Bayesian networks (naive Bayesian networks (NBN)) were constructed and validated. These networks are well known for their powerful performance on classification tasks (Friedman et al., 1997). Moreover, they are flexible in terms of handling missing values and they can be adapted easily when new information becomes available. These advantages were important for our application. Datasets collected in the field contain missing values, new

variables on non-AMS cow information might become available in the near future, and probabilities for the included variables can be adapted according to changing circumstances on the farm.

The main characteristic of an NBN is the assumption that all feature variables are mutually independent given the class variable. This implies that all possible interactions present in the dataset are ignored. Consequently, the classification performance can be underestimated. For the illustration of combining non-AMS cow information and AMS information, the more complex tree-augmented NBN were constructed (Chapter 3). By performing validation using AMS data, however, it turned out that the tree-augmented NBN did not result in a better performance than the simpler NBN (Chapter 4a).

Instead of NBN, also more sophisticated classes of Bayesian networks could have been used for our application. These Bayesian networks can also provide a probability on the variable of interest, and they are known to result in higher accuracies than NBN. Constructing sophisticated Bayesian networks, however, has several disadvantages. The graphical structure of these networks must be constructed by hand and is difficult. To make the graphical structure, detailed information is needed on underlying processes. Also intermediate variables, which are variables for which no values can be measured, must be included in sophisticated Bayesian networks. Subsequently, for all variables in the network probability estimates must be included. Literature information or estimates from experts are needed to estimate all probabilities. However, collecting probability estimates for all variables, especially for the intermediate variables, is very difficult and time consuming. Moreover, a general experience is that the level of acceptance of models by end users is low if expertise is used. The acceptance is generally higher for models based on field data.

Also other methods could have been used for our application, for instance, neural networks, time series analysis and regression analysis. These methods can provide a probability for an outcome variable as well. None of these techniques can, however, handle missing values and they are less flexible than NBN. Adapting the developed models to new information is difficult and time consuming. Fuzzy logic is also used frequently for classifying milkings with and without CM (De Mol and Woldt, 2001; Cavero et al., 2006; Kamphuis et al., 2008; Kramer et al., 2009). For our application, however, this method has several disadvantages. Fuzzy logic can not provide a probability as an outcome, can not handle missing values and can not be adapted easily when new information becomes available. Moreover, with fuzzy logic the development of membership functions is difficult, and the outcome is highly dependent on the definition of membership functions.

Stochastic modeling

To determine whether cow-specific treatment of CM is economically beneficial, stochastic modeling was used (Chapter 6). We believe that providing both the expected probability of cure, and the expected total costs of different treatment regimes is useful for treatment decisions. Stochastic modeling can give both outcomes, and also the variation in outcomes is visible. Previously, decision support models for culling and insemination were developed with optimization techniques (e.g., Houben et al., 1994; Groenendaal et al., 2004; Bar et al., 2008). With the optimization model of Bar et al. (2008) it was also possible to estimate the costs of CM for different cows. With their model, however, it was not possible to choose between different antimicrobial treatment regimes for individual cows. Optimization could have been used as well for supporting treatment decisions. A disadvantage of optimization, however, is that a definition of the goal function must be determined, and variation in the outcome is not visible.

FUTURE PROSPECTS FOR DETECTION AND TREATMENT OF CM

Using probabilities for CM detection

To support decisions on which mastitis alerts to check visually for CM, the way the information is presented to the farmer is important. Although, all kind of information is currently presented to the farmer (electrical conductivity, color and milk yield), all alerts are presented without any priority on the likelihood of being a true-positive alert. Moreover, a binary mastitis alert can be interpreted by the farmers as a cow having CM or not. Consequently, the farmer will be disappointed about the large number of visual checks that turn out to be unnecessary, and will lose confidence in the detection model.

By presenting a probability of being a true-positive alert for CM, it will become more clear that alerted cows are suspected of having CM. A farmer can then decide which mastitis alerts have the highest priority for visual checking. Also previously it was reported that using a degree of mastitis will give a better indication about the udder health status than providing a binary outcome (Højsgaard and Friggens, 2010). A probability based on a combination of currently available alert information variables will give a reliable probability. Adding non-AMS cow information will not improve that reliability (Chapter 4a). By presenting a probability, risk-seeking farmers have the opportunity to check alerts with high probabilities only, and thus taking the risk of missing or detecting later CM cases. These farmers take the risk that there might be increased costs due to a decreased probability of cure and a higher SCC for cases detected later. Risk-averse farmers have the opportunity to check also alerts with low probabilities, and thus decreasing the risk of missing CM cases. These farmers will have increased labor costs due to checking of many false-positive alerts. By presenting a probability, the risks and potential costs can be taken

into consideration by the farmers. Consequently, the farmers can take decisions by taking into account their risk attitude. By presenting a probability, it is also possible to adapt the checking behavior of the farmer to current farm circumstances. For instance, alerts with relatively low probabilities can be checked if the bulk milk SCC is trending upwards or when clots are found on the filter sock.

For some farmers, however, dealing with probabilities will be difficult. Especially for farmers starting milking automatically, guidance will be needed. These farmers can be guided by the manufacturer, or they can enter specific study groups. But also the veterinarian can be involved in guiding the farmers in how to use the mastitis alert list. During routine visits, for instance, the checking decisions made can be evaluated using mastitis records of the farm. Subsequently, checking decisions can be adapted accordingly. Additionally, the usefulness of providing probabilities must be evaluated by using mastitis data from farms with and without provided probabilities.

Other possibilities for improving CM detection with AMS

Adding non-AMS cow information to the available sensor information did not improve CM detection, and did not improve the possibilities for management by exception. Other options to improve CM detection with AMS are the development of improved CM detection models based on the currently available sensors, or the development of new sensors.

The current CM detection performance of an AMS can be summarized in an SE of 36.8% and an SP of 97.9% (Mollenhorst and Hogeveen, 2008). To improve CM detection with AMS, several CM detection models were developed based on the current available sensors with the goal to improve performance (De Mol and Ouweltjes, 2001; De Mol and Woldt, 2001; Cavero et al., 2006, 2007, 2008). These models used different statistical methods, different definitions of CM and different time-windows, which makes comparison difficult. Moreover, none of the studies mentioned used data from commercial dairy farms, which could be the reason why the models were never implemented on commercial dairy farms. The only CM detection model based on data of commercial Dutch dairy farms (n=9), and including a large number of CM cases (n=348), was described by Kamphuis et al. (2010). Their CM detection model showed an SE of 40% combined with an SP of 99%. In comparison with other developed CM detection models, a very narrow time-window of less than 24 hours was used. Especially this narrow time-window explains the relatively low SE. A narrow time-window, however, will be of more practical use for dairy farmers (Sherlock et al., 2008). It will give them more confidence in the detection model, especially because fewer alerts are given before clinical signs appear.

To improve CM detection with AMS, also newly developed sensors can be used. Recently, the test characteristics of a CM detection model based on electrical conductivity and improved sensors on the colors blue, green, red and near infrared were presented (SE of 83.3% and SP of 99.4%) (Song et al., 2010). These test characteristics are, however, based on a smaller number of farms and CM cases than in the study of Kamphuis et al. (2010). But most importantly, a wide time-window of four days was used, which at least partly explains the high SE. In-line measuring of SCC is a promising new technique for CM detection with AMS, especially when combined with the already available information on electrical conductivity (Kamphuis et al., 2008). Also biosensors, such as L-lactate dehydrogenase and N-acetyl-β-D-glucosaminidase, can potentially improve CM detection (Brandt et al., 2010; Viguier et al., 2009). Currently, the use of in-line SCC and biosensors is limited, possibly due to the costs involved for each measurement.

Most CM cases are characterized by showing flakes and/or clots in the milk. For most farmers, finding cows with flakes has the highest priority, because these cows need antibiotic treatment. Therefore, a sensor measuring flakes and/or clots will be appropriate for CM detection. So far, however, such a sensor is not available.

Detection of CM versus detection of subclinical mastitis

Treatment of CM during lactation is one of the key elements of mastitis control, and is included in the "5 point program" developed by Neave et al. (1969). Also in the extended 10 point "recommended mastitis control program" of the NMC (www.nmconline.org), treatment of CM during lactation was included. In the Netherlands, the Dutch udder health center developed a treatment protocol for CM (www.ugcn.nl). For mastitis control on a farm, treatment decisions are needed immediately after detection of CM. Therefore, in this thesis, the objective was to improve CM detection with AMS. CM included a wide variety of cases, varying from milk with some small flakes to very severe cases. It is questionable whether the cows with only small flakes need antimicrobial treatment immediately after detection. For mild or moderate CM cases there was no difference in probability of cure (Keefe et al., 2010), CM recurrence, SCC, milk production and culling (Lago et al., 2009) between CM cases treated immediately and cases treated 24 hours after detection. Also the consequences of leaving CM cases caused by *E. coli* or cases with no significant bacterial growth untreated were investigated. No significant differences in probability of cure are reported between the described treatment protocol and treating all cases with antimicrobials. However, a reduction in use of antibiotics of 36% was reported (Keefe et al., 2010). These findings would reduce the importance of early detection of mild and moderate CM cases. The consequences of treating severe CM cases 24 hours after detection were not investigated in both studies.

Because no immediate treatment decisions need to be made for subclinical mastitis, detection of subclinical mastitis with AMS was not evaluated in this thesis. Subclinical mastitis causes production losses (Halasa et al., 2009a; Reksen et al., 2007; Whist et al., 2007, 2009), and higher probabilities of clinical flare ups and culling (Reksen et al., 2006; Whist et al., 2007, 2009). Additionally, subclinical mastitis can be a source of infection for other cows because pathogens can be transmitted between cows (Zadoks et al., 2001a, 2002). Consequently, subclinical mastitis results in considerable economic losses in dairy herds (Halasa et al., 2007). Although not implemented in mastitis control programs, antimicrobial treatment of subclinical mastitis during lactation is suggested to improve udder health on dairy farms (Barkema et al., 2006). Previously, different outcomes on whether treatment of subclinical mastitis is economically beneficial are reported (Swinkels et al., 2005a, 2005b; Steeneveld et al., 2007). Recently, a specific bio-economic model capable of simulating transmission of pathogens was developed (Halasa et al., 2009b). Using this model, it was reported that lactational treatment of contagious subclinical mastitis seems economically beneficial on farms milking conventionally (Van den Borne et al., 2010). On farms with an AMS, more cows are milked with the same milking cluster. Consequently, the transmission of pathogens might be increased, and the economic consequences of treatment might be even more beneficial on farms with an AMS. Post-milking teat disinfection has been shown to be an effective preventive measure for new intramammary infections (e.g., Lam et al., 1997). On farms with an AMS, however, on average, in 18 % of the milkings the teats were not covered with teat disinfecting spray at all after milking. This percentage was positively related to the percentage of new cows with high SCC (Dohmen et al., 2010). So, neglecting to spray teats after milking with AMS can increase the number of cows with high SCC even more.

Therefore, subclinical mastitis might probably have a higher priority of being treated, and thus have a higher priority of being detected than CM. Especially on farms with a high bulk milk SCC the need for detection and treatment of cows with subclinical mastitis increases. The ability of electrical conductivity to separate subclinically infected cows from healthy cows is not satisfactory (Norberg et al., 2004). To detect cows with subclinical mastitis, it is expected that SCC is a much better indicator. A new sensor which estimates the SCC based on viscosity measurements (Whyte et al., 2004) gives opportunities to improve automatic detection of subclinical mastitis. Previously, high correlation coefficients have been reported between in-line SCC determined with the new sensor and a laboratory determined SCC (Leslie et al., 2007; Kamphuis et al., 2008). Especially high correlations are reported for laboratory determined SCC-values above 200,000 cells/mL (Kamphuis et al., 2008).

The mastitis detection models for AMSs currently used (Mollenhorst and Hogeveen, 2008), and the recently developed mastitis detection models, are validated using CM cases

as the gold standard (e.g., De Mol and Ouweltjes, 2001; Kamphuis et al., 2010; Song et al., 2010). To detect also subclinical mastitis cases, another gold standard must be established. Probably, separate detection models for CM and for high SCC must be developed. In that way, cows suspected of having CM and cows having high SCC, and thus suspected of having subclinical mastitis, can be presented to the farmer separately. Subsequently, the farmer can take different decisions for cows suspected of having CM and cows having high SCC. The first goal must remain the detection of as many as possible to be treated CM cases, preferably reaching a SE of at least 70% and a SP of 99% (Mein and Rasmussen, 2008). In addition, the detection model must be able to differentiate between milkings with and without high SCC. To detect high SCC, the SP should be high, but there are no high requirements for SE or time windows. Most likely, the SCC sensor described by Whyte et al. (2004) can be used to detect cows with high SCC. Because a SCC measurement costs approximately €0.02 per cow milking, it is of great interest to explore several implementation strategies for the in-line SCC sensor. Probably it is enough to perform in-line SCC measurements only once a week, or only for cows suspected of having high SCC, for instance cows in beginning of lactation or cows just recovered from CM. Detection of cows with high SCC is only beneficial if after detection appropriate actions are taken, such as performing bacteriological culturing and subsequently treatment or culling.

OUR WORK IN THE FIELD OF PRECISION DAIRY FARMING

Precision dairy farming includes management by exception, and consequently taking individual cow decisions. The AMS is one of the best examples of a precision dairy farming technology. On farms with an AMS, the availability of sensors measuring milk components continuously and the alert lists as the only information source about individual cows, results in management by exception. The results described in this thesis contribute to the research on possibilities on management by exception and taking individual cow decisions.

In this thesis, it was investigated whether non-AMS cow information can be used to improve management by exception. Non-AMS cow information was not useful to decide which cows have the highest priority for visual checking for CM. A combination of alert variables, however, turned out to be useful to decide which cows have the highest priority for visual checking. For supporting individual treatment decisions for cows with CM, the expected probability of cure and the expected total costs will be useful.

CONCLUSIONS

The following main conclusions can be drawn from this thesis.

- CM detection with AMS cannot be improved by adding non-AMS cow information. CM detection models based on only sensor measurements have higher detection performance than CM detection models based on sensor measurements and non-AMS cow information. Moreover, non-AMS cow information cannot be used to discriminate between true-positive and false-positive mastitis alerts from an AMS.

- A combination of alert variables can be used to decide which mastitis alerts need to be checked visually for CM. By presenting a probability of being true-positive for CM, based on a combination of alert variables, a farmer can decide which mastitis alerts have the highest priority for visual checking. Moreover, risk-seeking and risk-averse farmers can make different decisions.

- Non-AMS cow information and information from the visual inspection can be used to give a reliable probability on the Gram-status of CM cases. This probability distribution can be used to support treatment decisions. In the current study, for 28% of the CM cases Gram-status could be determined with a very high probability.

- Cow-specific treatment is not economically beneficial. For all CM cases, the benefits because of lower costs for milk production losses and culling for CM cases treated with the intensive treatments, did not outweigh the higher treatment costs. Presenting cow-specific treatment information is interesting for the farmer, because not only economical information influences the treatment decision.

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Summary

On farms with an automatic milking system (AMS), the cows are milked automatically, and the dairy farmer is not present during the milking process. Sensors on electrical conductivity, color and milk production are used for detection of clinical mastitis (CM). Based on these sensor measurements, mastitis alert lists are generated by the AMS to present to the dairy farmer the cows suspected of having CM. The performance of current CM detection models is suboptimal. Therefore, the number of false-positive alerts is too high.

For an optimal mastitis management on farms with an AMS, two individual cow decisions are important and can be supported automatically. First, there is a need for decision support on which mastitis alerts have the highest priority for visual checking for CM. In essence, all cows with mastitis alerts have to be checked visually for CM. Because of the suboptimal detection performance and therefore the annoyance about the large number of visual checks that turn out to be unnecessary, in practice farmers do not check all mastitis alerts. Non-AMS information about the cow, such as parity, stage of lactation and somatic cell count (SCC) history influence the probability of having CM. It is expected that adding non-AMS cow information can be used to support decisions on which mastitis alerts have the highest priority for visual checking. The second important decision that needs support is the choice of treatment for detected CM cases. Different antimicrobial treatments are available for CM on Dutch dairy farms. These treatments differ in antimicrobial compound, route of application, duration, probability of cure and costs. However, most CM cases receive a standard intramammary treatment. Additional information sources are hardly used to differentiate in choice of treatment for different cows with CM. Several cow factors (e.g., parity and SCC history) and information from the visual inspection influence the success of treatment. These information sources can be used to support CM treatment decisions. Decision support on choice of treatment can be performed by presenting an expected probability of cure for different treatment regimes. It is expected that for different cows, different treatment regimes have the lowest costs. In addition, therefore also the expected costs of different treatment regimes can be used to decide upon the optimal treatment regime for CM for individual cows.

The objectives of the research described in this thesis were (i) to improve automated CM detection by combining sensor and non-AMS cow information and (ii) to improve CM treatment decisions.

Chapter 2 describes which combination of non-AMS cow factors influence the probability of having CM. Data were used from 274 Dutch dairy herds that recorded CM over an 18-month period. The final dataset contained information on 28,137 lactations of 22,860 cows of different parities. In total 5,363 CM cases were recorded. The cow factors parity, month in lactation, season of the year, information on SCC from monthly test-day

records and CM history were included in the logistic regression analysis. Separate analyses were performed for heifers and multiparous cows in both, the first month of lactation, and from the second month of lactation onwards. All non-AMS cow factors together (parity, month in lactation, season of the year, SCC in previous month, geometric mean SCC in previous lactation and CM history) significantly influenced the probability of having CM. The probability of having CM differed considerably among cows. In particular, previous CM cases, a high SCC in the previous month and a high mean SCC in the previous lactation increased the probability of having CM in the current month of lactation.

Chapter 3 illustrates the idea of using the probability of having CM, based on non-AMS cow information, to provide a rank-order on the mastitis alerts from an AMS. A tree-augmented naive Bayesian network was constructed from available data to determine these cow-specific probabilities of having CM. The graphical structure of the network and the probability tables for the variables in the network were based on the same dataset as was used in chapter 2. The available non-AMS cow information (parity, month in lactation, season of the year, SCC history and CM history) were included as variables in the network. By combining the obtained cow-specific probabilities of CM with the sensitivity and specificity of the CM detection system of the AMS, each mastitis alert had a different success rate (positive predictive value). So, each mastitis alert had a different probability of being a true-positive alert for CM. This information can be used to discriminate between CM alerts. Our illustrations indicated that the success rate might range from 3% to 84%, while assuming an equal overall probability for all cows resulted in a success rate of 21%. Using the computed success rates, the CM alerts on an alert list can be ranked-ordered, thereby providing the dairy farmer information about which cows have the highest priority for visual checking for CM.

To validate the effect of adding non-AMS cow information, data from a single research farm with in total 4 AMS was used. The overall objective of **chapter 4a** was to investigate whether by using non-AMS cow information a selection of the alerted cows that need further investigation for CM can be made. In addition, it was also investigated whether alert information itself can be used to make a selection of alerted cows that need further investigation. During a 2-year study period, a total of 11,156 alerts for CM, including 159 true-positive alerts, were collected at one farm in the Netherlands. Non-AMS cow information on parity, month in lactation, season of the year, SCC history and CM history was added to each alert. In addition, six alert information variables were defined. These were the height of electrical conductivity, the alert origin (electrical conductivity, color, or both), whether or not a color alert for mastitic milk was given, whether or not a color alert for abnormal milk was given, the deviation from the expected milk yield and the number of alerts of the cow in the preceding 12-96 hours. Subsequently, 3 naive Bayesian

networks were constructed to compute the probability of an alert being truly positive based on only non-AMS cow information, based on only alert information, and based on both types of information. The network that includes both types of information had the highest area under the receiver operating characteristic curve, followed by the network that includes only alert information and the network including only non-AMS cow information. The use of a combination of alert information variables was the best way to make a selection of alerted cows that need further investigation for CM. The effect of adding non-AMS cow information to make a distinction between true-positive and false-positive alerts was minor.

It was unexpected that non-AMS cow information could not be used to discriminate between true-positive and false-positive mastitis alerts. To confirm results found in chapter 4a, a dataset including more farms with an AMS was used as well (**chapter 4b)**. Based on data of 9 Dutch commercial dairy farms, a CM detection model was developed. The model provided for each cow milking the prior probability that the cow milking is a mastitis one, based on sensor measurements of electrical conductivity, color and milk yield. In total, 47,049 cow milkings, including 99 milkings for which CM was reported, were available with a prior probability of having CM based on the sensor measurements. To each of these cow milkings, non-AMS cow information (parity, month in lactation, season, SCC history and CM history) was added. After adding the non-AMS cow information, a posterior probability of having CM was calculated for each cow milking. The CM detection performance based on the prior probability (based on sensor measurements alone) was compared with the CM detection performance based on the posterior probability (based on the sensor measurements and the added non-AMS cow information). The CM detection model based on only sensor measurements had the highest area under the receiver operating characteristic curve. Results show that addition of non-AMS cow information to all milkings did not improve CM detection performance. So, if probabilities based on sensor measurements are available, adding non-AMS cow information showed no additional value for the detection of CM.

For detected CM cases a decision on the best treatment regime has to be made. Having an indication about the causal pathogen, and subsequently using appropriate treatment, serves to increase the cure rate of CM. The objective of **chapter 5** was to determine probability distributions for the Gram-status for CM cases. Data were used from 274 Dutch dairy herds in which the occurrence of CM was recorded. For in total 3,833 CM cases the Gram-status could be determined. Two-thirds of the dataset were used for the construction process and one-third was retained for validation. Information about the cow with CM was included in a naive Bayesian network (parity, month in lactation, season of the year, position of the infected quarter, SCC history and CM history, being sick or not, and color and texture of the milk). Accuracy was calculated to obtain insight in the quality of the

constructed network. The accuracy of classifying CM cases into Gram-positive or Gram-negative pathogens was 73%. Since only CM cases with a high probability for a single causal pathogen will be considered for pathogen-specific treatment, accuracies based on only classifying CM cases above a particular probability threshold were determined. For instance, for CM cases in which either Gram-negative or Gram-positive had a probability > 0.90, classification according to the Gram-status reached an accuracy of 97%. We found that the greater the probability for a particular pathogen was for a CM case, the more accurate a classification of this case as being caused by this pathogen. The probability distributions provided by the naive Bayesian networks provide the farmer with considerable insight about the most likely causal pathogen for a CM case.

In **chapter 6**, the economic aspects of cow-specific treatment of CM are described. Using a stochastic Monte Carlo simulation model, 20,000 CM cases were simulated. For each simulated CM case, the consequences of using different antimicrobial treatment regimes (standard 3 d intramammary, extended 5 d intramammary, combination 3 d intramammary + systemic, combination 3 d intramammary + systemic + 1 d non-steroid anti-inflammatory drugs, and combination extended 5 d intramammary + systemic) were simulated simultaneously. Finally, total costs of the 5 antimicrobial treatment regimes were compared. All input for the model was based on literature information and, if no information was available, on authors' knowledge. Bacteriological cure for each individual cow depended on the antimicrobial treatment regime, the causal pathogen and the cow factors parity, stage of lactation, SCC history, CM history and whether the cow was systemically ill or not. Total costs for each case included treatment costs for the initial CM case (including costs for antibiotics, milk withdrawal and labor), treatment costs for follow-up CM cases, costs for milk production losses and costs for culling. Average total costs for CM using the 5 treatments were €176, €194, €199, €204 and €216, respectively. Average probabilities of bacteriological cure for the 5 treatments were 0.53, 0.65, 0.65, 0.68 and 0.75, respectively. For all different simulated CM cases, the standard 3 d intramammary antimicrobial treatment had the lowest total costs. The benefits of lower costs for milk production losses and culling for cases treated with the intensive treatments, did not outweigh the higher treatment costs. In conclusion, although effectiveness of different antimicrobial treatments does vary between cows, differentiation of treatments for different CM cases currently does not provide economic benefits under current Dutch circumstances.

In the general discussion (**chapter 7**), the results of the previous chapters are discussed. Possible explanations for the unexpected result that non-AMS cow information cannot be used to improve CM detection were given. These explanations include the large number of cows having subclinical mastitis, the non-discriminative power of SCC obtained from the four-weekly milk recording, and the correlation between electrical conductivity and SCC.

Also future prospects for detection and treatment of CM are described, such as the development of new sensors and improved detection algorithms, and the detection of subclinical mastitis with AMS.

This thesis proved that CM detection with AMS could not be improved by adding non-AMS cow information. In addition, cow-specific treatment was not economically beneficial.

Samenvatting

Op melkveebedrijven met een automatisch melk systeem (AMS) worden de koeien gemolken zonder dat de veehouder aanwezig is. Voor het controleren van de melk hebben sensoren de rol van de veehouder overgenomen. Verschillende sensoren (geleidbaarheid, kleur en melkproductie) worden gebruikt om klinische mastitis (KM) te detecteren. Afwijkende sensormetingen worden aan de veehouder gepresenteerd op een mastitis attentielijst. Op deze lijst staan de koeien die verdacht worden van KM. Veel attenties staan echter onterecht op de lijst. De geattendeerde koeien hebben geen KM, en de mastitis attenties worden door de veehouder gezien als onterechte attenties.

Voor een optimaal mastitis management op bedrijven met een AMS zijn er twee individuele koe beslissingen belangrijk, en beide beslissingen hebben ondersteuning nodig. Ten eerste is er ondersteuning nodig om te bepalen welke geattendeerde koeien gecontroleerd moeten worden voor KM. Eigenlijk moeten op bedrijven met een AMS alle mastitis attenties door de veehouder gecontroleerd worden. Maar door de niet optimale detectie, en de ergernis over de vele vals-positieve attenties voor KM, controleren veehouders niet alle mastitis attenties. Het zou daarom het nuttig zijn om een lijst aan de veehouder te kunnen presenteren waarop staat welke mastitis attenties de grootste kans hebben om een terechte attentie voor KM te zijn. Verschillende koe factoren die niet door het AMS gemeten worden (zoals de pariteit, lactatiestadium en celgetal historie) beïnvloeden de kans op KM. Het is daarom te verwachten dat het toevoegen van deze niet-AMS koe informatie gebruikt kan worden om te bepalen welke koeien met een mastitis attentie de hoogste prioriteit hebben om gecontroleerd te worden. De tweede individuele koe beslissing die ondersteund kan worden is de keuze voor een behandeling voor elke koe met KM. Voor KM zijn verschillende antibiotica behandelingen beschikbaar, die verschillen in werkzame stof, manier van toediening, lengte van behandeling, kans op genezing en kosten. De meeste KM gevallen worden behandeld met een standaardbehandeling in het uier. Informatie over de koe wordt amper gebruikt om verschil te maken in de keuze voor een behandeling. Verschillende koe factoren (zoals pariteit en celgetal historie) en visuele kenmerken van de melk beïnvloeden echter de kans op genezing. Die informatie kan gebruikt worden om de beslissing voor een KM behandeling te ondersteunen. Deze beslisondersteuning kan bestaan uit het presenteren van een verwachte kans op genezing voor verschillende KM behandelingen. Het is te verwachten dat voor verschillende koeien verschillende behandelingen resulteren in de laagste kosten. Daarom zouden ook de verwachte kosten van verschillende behandelingen gebruikt kunnen worden om de beslissing voor een KM behandeling te ondersteunen.

De doelen van het in dit proefschrift beschreven onderzoek waren (i) het verbeteren van KM detectie door sensorinformatie en niet-AMS koe informatie te combineren, en (ii) het verbeteren van KM behandel beslissingen.

Hoofdstuk 2 beschrijft welke combinatie van niet-AMS koe informatie de kans op KM beïnvloeden. Een dataset bestaande uit gegevens van 274 melkveebedrijven uit het noorden van Nederland was gebruikt. Op deze bedrijven was gedurende 18 maanden informatie over KM bijgehouden. De uiteindelijke dataset bestond uit informatie over 28.137 lactaties van 22.860 koeien van verschillende pariteiten. In totaal bevatte de dataset 5.363 KM gevallen. Informatie over pariteit, maand in lactatie, seizoen van het jaar, celgetal historie en KM historie zijn opgenomen in een logistische regressie analyse om te bepalen of al deze informatie invloed had op de kans op KM. Aparte analyses zijn uitgevoerd voor vaarzen en oudere koeien, beide voor de eerste maand van lactatie en de rest van lactatie. Alle koe informatie samen (pariteit, maand in lactatie, seizoen van het jaar, celgetal in vorige maand, geometrisch gemiddelde celgetal van de vorige lactatie en het aantal vorige KM gevallen) hadden een significant effect op de kans op KM. Deze kans varieerde sterk tussen verschillende koeien. Vooral het aantal vorige KM gevallen, een hoog celgetal in de vorige maand, een hoog geometrisch gemiddeld celgetal in de vorige lactatie liet de kans op KM in de huidige maand van lactatie stijgen.

Hoofdstuk 3 illustreert het gebruik van een kans op KM, gebaseerd op niet-AMS koe informatie, om te bepalen welke mastitis attenties de hoogste prioriteit hebben om gecontroleerd te worden voor KM. Op basis van de beschikbare data was een “tree-augmented naive Bayesian network” gemaakt om een koe-specifieke kans op KM te bepalen voor elke koe. De grafische structuur van het netwerk en de kanstabellen voor de variabelen in het netwerk zijn gebaseerd op dezelfde dataset zoals gebruikt in hoofdstuk 2. Niet-AMS koe informatie (pariteit, maand in lactatie, seizoen van het jaar, celgetal historie en KM historie) zijn als variabelen opgenomen in het netwerk. De koe-specifieke kansen voor KM zijn gecombineerd met de sensitiviteit en specificiteit van het KM detectiesysteem van de AMS. Vervolgens kon voor iedere koe met een mastitis attentie de positief voorspellende waarde bepaald worden. Deze waarde geeft aan wat de kans is dat een mastitis attentie een terechte attentie is. Onze illustraties laten zien dat de positief voorspellende waarde varieerde tussen 3% en 84%. Als er een gelijke kans op KM voor alle koeien wordt aangenomen zou de positief voorspellende waarde voor alle koeien 21% zijn. Door gebruik te maken van de berekende positief voorspellende waarde kan er een volgorde in de mastitis attenties aangebracht worden. Op deze manier kan een veehouder zien welke koeien met een mastitis attentie de hoogste prioriteit hebben om gecontroleerd te worden voor KM.

Het doel van **hoofdstuk 4a** was om te bepalen of niet-AMS koe informatie gebruikt kan worden om onderscheid te maken in mastitis attenties die wel en niet gecontroleerd moeten worden voor KM. Daarnaast was er ook bepaald of er op basis van attentie informatie zelf al bepaald kan worden welke attenties de hoogste prioriteit hebben om gecontroleerd te worden. Er was gebruik gemaakt van data van een onderzoeksbedrijf met

4 AMS. Gedurende 2 jaar is er op dat bedrijf informatie verzameld over 11.156 mastitis attenties, waarvan er 159 terecht positief voor KM waren. Niet-AMS koe informatie (pariteit, maand in lactatie, seizoen van het jaar, celgetal historie en KM historie) was toegevoegd aan elke mastitis attentie. Daarnaast waren er verschillende attentie variabelen gedefinieerd, zoals de hoogte van de geleidbaarheid, het type alert (attentie gebaseerd op geleidbaarheid, op kleur of op beide), het verschil met de verwachte melkgift en het aantal attenties van de koe in de afgelopen 12-96 uur. Vervolgens zijn er 3 "naive Bayesian networks" gebouwd om de kans te bepalen dat een mastitis attentie terecht positief is voor KM. Eén netwerk op basis van niet-AMS koe informatie, één netwerk op basis van de gedefinieerde attentie informatie, en één netwerk op basis van beide informatiebronnen. De resultaten lieten zien dat de niet-AMS koe informatie niet gebruikt kan worden om onderscheid te maken tussen mastitis attenties die wel en niet gecontroleerd moeten worden voor KM. De gedefinieerde attentie informatie kan wel gebruikt worden om te bepalen welke mastitis attenties de hoogste prioriteit hebben om gecontroleerd te worden.

Het was onverwacht dat de niet-AMS koe informatie niet gebruikt kan worden om onderscheid te maken in mastitis attenties die wel en niet gecontroleerd moeten worden voor KM. Om te bevestigen of niet-AMS koe informatie inderdaad niet nuttig is, is er ook data gebruikt die informatie bevatte van meerdere bedrijven met een AMS (**hoofdstuk 4b**). Gebaseerd op informatie van 9 Nederlandse melkveebedrijven met een AMS, was er een KM detectiemodel ontwikkeld. Gebaseerd op sensormetingen voor geleidbaarheid, kleur en melkproductie gaf het model voor iedere koemelking een kans op KM. In totaal waren er voor 47.049 koemelkingen, waarvan 99 melkingen met KM, een kans op KM op basis van sensormetingen beschikbaar. Met behulp van een "naive Bayesian network" is deze kans gebaseerd op alleen sensorinformatie vervolgens aangepast met niet-AMS koe informatie (pariteit, maand in lactatie, seizoen van het jaar, celgetal historie en KM historie). De resultaten lieten zien dat een detectiemodel gebaseerd op sensorinformatie en niet-AMS koe informatie resulteert in een slechtere detectie van KM dan een detectiemodel gebaseerd op alleen sensorinformatie. Ook de resultaten van dit hoofdstuk lieten zien dat de niet-AMS koe informatie geen toegevoegde waarde heeft voor de detectie van KM met een AMS.

Voor gedetecteerde KM gevallen moet er een beslissing genomen voor de behandeling. Als de veehouder een goede indicatie heeft over de veroorzakende bacterie van KM, dan kan er vervolgens een gerichte behandeling gestart worden, wat leidt tot een hogere kans op genezing. Het doel van **hoofdstuk 5** was om op basis van koe informatie een kansverdeling voor de Gram-status van de veroorzakende bacterie van KM te geven. Een dataset met KM informatie van 274 Nederlandse melkveebedrijven was gebruikt. In totaal kon er voor 3.833 KM gevallen bepaald worden of KM veroorzaakt was door Gram-positieve of Gram-negatieve bacteriën. In totaal is 2/3 van de data gebruikt om een "naive

Bayesian network" te bouwen. Informatie over de koe met KM (pariteit, maand in lactatie, seizoen van het jaar, celgetal historie, KM historie, of de koe ziek is ja of nee, en de visuele kenmerken van de melk (bijvoorbeeld kleur)) is opgenomen in het netwerk. De overige 1/3 van de dataset is gebruikt voor validatie. Voor ieder KM geval in de validatie dataset was vervolgens de kans dat KM veroorzaakt was door Gram-positieve of Gram-negatieve bacteriën bepaald. Het totaal aantal correct geclassificeerde KM gevallen in Gram-positieve of Gram-negatieve bacteriën was 73%. In de praktijk komen alleen KM gevallen met erg hoge kansen voor Gram-positieve of Gram-negatieve bacteriën in aanmerking voor een bacterie specifieke behandeling. Daarom was ook het aantal correct geclassificeerde KM gevallen bepaald voor de KM gevallen met een kans op Gram-positieve of Gram-negatieve bacteriën boven 0.90. Voor alle KM gevallen met een kans dat KM veroorzaakt was door Gram-positieve of Gram-negatieve bacteriën boven de 0.90 was 97% correct geclassificeerd. De kansverdelingen voor de Gram-status van de veroorzakende bacterie van een KM geval kan de veehouder gebruiken om een beslissing te nemen voor een behandeling. Vooral erg hoge kansen (>0.90) gaven een betrouwbaar inzicht in de Gram-status van de veroorzakende bacterie.

In **hoofdstuk 6** zijn de economische aspecten van koe-specifiek behandelen beschreven. Met een stochastisch Monte Carlo simulatie model zijn 20.000 KM gevallen gesimuleerd. Voor ieder gesimuleerd KM geval zijn de consequenties van het gebruik van verschillende behandelingen gesimuleerd. De gedefinieerde behandelingen zijn een 3-daagse behandeling in het uier, een verlengde behandeling in het uier, een combinatie van een behandeling in het uier en de nek, een combinatie van een behandeling in het uier en de nek en pijnstillers, en een combinatie van een verlengde behandeling in het uier en de nek. De totale kosten van deze 5 behandelingen zijn met elkaar vergeleken. Alle input voor het model was gebaseerd op informatie vanuit literatuur en kennis van de auteurs. De kans op bacteriologische genezing was afhankelijk van de behandeling, de veroorzakende bacterie en de koe factoren pariteit, lactatiestadium, celgetal historie, KM historie en of de koe met KM ziek was ja of nee. Totale kosten bestonden uit kosten voor de behandeling (antibiotica, melk weggooien en arbeid), kosten voor behandeling van herhalingsgevallen, kosten voor melkproductie verliezen en kosten voor afvoer. De gemiddelde totale kosten voor KM met de 5 gedefinieerde behandelingen waren €176, €194, €199, €204 en €216, respectievelijk. De gemiddelde kans op bacteriologische genezing voor de 5 gedefinieerde behandelingen waren 0.53, 0.65, 0.65, 0.68 en 0.75, respectievelijk. Voor alle gesimuleerde KM gevallen gaf de 3-daagse behandeling in het uier de laagste totale kosten. De verminderde kosten voor lagere melkproductieverliezen en minder afvoer voor KM gevallen behandeld met de meer intensieve behandelingen wogen niet op tegen de extra kosten voor behandeling. Onder de huidige Nederlandse omstandigheden levert verschil maken in behandeling voor verschillende KM gevallen geen economische voordelen op.

In de algemene discussie (**hoofdstuk 7**) zijn de resultaten van de voorgaande hoofdstukken bediscussieerd. Mogelijke verklaringen waarom niet-AMS koe informatie niet gebruikt kan worden om de KM detectie te verbeteren zijn gegeven. Deze verklaringen bevatten de grote hoeveelheid koeien in de dataset met subklinische mastitis, het beperkte discriminerend vermogen van celgetal afkomstig vanuit de 4-wekelijke melkcontrole en de correlatie tussen geleidbaarheid en celgetal. Ook zijn nieuwe mogelijkheden voor detectie en behandeling van KM met een AMS beschreven, zoals de ontwikkeling van nieuwe sensoren en detectiemodellen, en de detectie van subklinische mastitis met een AMS.

De resultaten van dit proefschrift laten zien dat KM detectie met AMS niet verbeterd kon worden door het toevoegen van niet-AMS koe informatie. Koe-specifiek behandelen was economisch niet aantrekkelijk.

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Curriculum vitae

ABOUT THE AUTHOR

Wilma Steeneveld was born on October 26st 1981 in Nootdorp. In 2000 she finished highschool at Christelijk Lyceum Delft. In 2001 she started the study Animal Sciences at Wageningen University.

For her minor thesis at the Business Economics Group she studied the economic consequences of treatment of subclinical mastitis caused by *Streptococcus uberis.* For her major thesis at the group of Animal Breeding and Genetics she investigated the possibilities for a claw health index for dairy cattle. During her internship at the NRS (now CRV) she estimated genetic parameters for clinical mastitis for Dutch Holstein cattle.

After graduating in June 2006 she started as a PhD student at the Department of Farm Animal Health of the faculty of Veterinary Medicine of Utrecht University.

OVER DE AUTEUR

Wilma Steeneveld werd geboren op 26 oktober 1981 in Nootdorp. In 2000 behaalde zij haar VWO diploma aan het Christelijk Lyceum Delft. Vervolgens begon ze in 2001 aan de studie Dierwetenschappen aan Wageningen Universiteit.

Tijdens haar afstudeervak bij de leerstoelgroep Bedrijfseconomie onderzocht ze de economische consequenties van het behandelen van subklinische mastitis veroorzaakt door *Streptococcus uberis*. Vervolgens onderzocht ze tijdens een afstudeervak bij de leerstoelgroep Fokkerij en Genetica de mogelijkheden voor een klauwgezondheidsindex voor melkvee. Tijdens haar stage bij het NRS (nu CRV) schatte ze genetische parameters voor klinische mastitis bij Nederlands melkvee.

Na haar afstuderen in juni 2006 begon ze als promovenda bij het departement Gezondheidszorg voor Landbouwhuisdieren van de faculteit Diergeneeskunde van de Universiteit Utrecht.

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