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The prediction of the prevalence and risk factors for subclinical heifer mastitis in Turkish dairy farms

Ayhan BAŞTAN[1,*], Seçkin SALAR[1], Mehmet CENGİZ[2], İsfendiyar DARBAZ[3], Mürşide Ayşe DEMİREL[4], Doğukan ÖZEN[5]
[1]Department of Obstetrics and Gynecology, Faculty of Veterinary Medicine, Ankara University, Ankara, Turkey
[2]Department of Obstetrics and Gynecology, Faculty of Veterinary Medicine, Atatürk University, Erzurum, Turkey
[3]Department of Obstetrics and Gynecology, Faculty of Veterinary Medicine, Near East University, Lefkoşa, Northern Cyprus
[4]Department of Care and Research in Laboratory Animals, Faculty of Pharmacy, Gazi University, Ankara, Turkey
[5]Department of Biostatistics, Faculty of Veterinary Medicine, Ankara University, Ankara, Turkey

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Abstract: The aim of this study was to determine the risk factors for subclinical heifer mastitis and to create a model that can predict the prevalence of subclinical mastitis of pregnant heifers in farms in Turkey. Lacteal secretion samples were taken from 439 pregnant (6–9 months) heifers and bacteriological analyses were performed. In this study, 37.47% of the samples were determined to be infected. In lacteal secretion samples, the isolation rate of coagulase-negative staphylococci and *Staphylococcus aureus* was 44.83% and 35.71%, respectively. The incidence of mastitis was calculated as 42.87 ± 4.12%. An eight-question survey was conducted. Using the data collected, a multiple linear regression analysis using backward stepwise method was used to predict the incidence of mastitis. According to the multiple regression model, the number of animals, well-balanced ration, separating the cows in dry periods into different boxes, and contact of heifers with older cows significantly contributed to the model ($P < 0.05$). Coefficient of determination (R^2) for the model was estimated at 93.8%. Today, with the knowledge of risk factors for pregnant heifers, changes in management would be beneficial to prevent mastitis. Additionally, this study showed that predictive models for the incidence of mastitis could be conducted through comprehensive future studies.

Key words: Heifers, regression model, risk factors, subclinical mastitis

1. Introduction

Heifers are the future milk producers of every dairy herd. Mastitis during development of the mammary gland and in early lactation is hypothesized to adversely affect their milk production and udder health, leading to considerable economic losses for dairy farms (1).

Unfortunately, most producers regard young heifers as uninfected, and the presence of mastitis is not observed until calving or until the first signs of clinical mastitis in early lactation. Thus, an animal may carry an intramammary infection for a year or more before it is diagnosed with mastitis. The greatest development of milk-producing tissue in the udder occurs during the first pregnancy, so it is important to protect the mammary glands from microorganisms to ensure maximum milk production during the first lactation (2).

The rate of intramammary infections in breeding age and pregnant heifers is much higher than previously thought. Many of these infections, which can persist for long periods of time, are associated with somatic cell counts and are likely to impair mammary development during gestation and affect milk production after calving (3).

Many risk factors have been identified for heifer mastitis. Mastitis is a multifactorial disease, requiring exposure to a combination of environmental and pathogenic factors and with variable responses between animals. Identification of factors for mastitis is important for the development of control and prevention strategies (4).

The aim of the present study was to determine the risk factors for subclinical heifer mastitis and to create a model that can predict the prevalence of subclinical mastitis in pregnant heifers on farms in Turkey.

2. Materials and methods

2.1. Herds and heifers

The study was conducted between December 2012 and February 2013, during the late pregnancy period (6–9 months) of 439 dairy heifers from 12 randomly selected herds (average of 36 heifers per herd, ranging between

* Correspondence: abastan@ankara.edu.tr

15 and 90) located in different regions of Turkey such as the Aegean Sea, Black Sea, Central Anatolia, Marmara, and Mediterranean Sea regions. Lacteal secretion samples taken from these heifers and survey data were evaluated. The heifers were chosen if they showed no signs of clinical mastitis, had 4 quarters free of teat abnormalities, and had not received antibiotics or antiinflammatory treatment during the previous 30 days. The period of pregnancy in heifers was confirmed by rectal palpation and insemination data.

The heifers were kept indoors for 1 year and were fed with hay, corn silage, alfalfa, and concentrate feed. The feeding program was usually similar on all farms, although management was different.

2.2. Initial data set and data handling

The study was conducted on the farms due to the farmers' willingness to cooperate with us. Before sampling, we visited all farms with an attendant and surveyed the farmers to obtain information about risk factors. The risk factor criteria that we used included the following: herd size, nutritional management, usage of calf pens, housing of dry cows, absence of fly control, feeding calves with waste milk, heifer contact with older cows, and type of bedding material.

2.3. Sampling

Quarter secretion samples were taken from pregnant heifers according to the recommended procedures for milk samples (5). Before sampling, teat ends were dipped into antiseptic solution (DeLaval easy foam EF300, DeLaval) and dried. After these procedures, teat ends were cleaned with 70% ethyl alcohol-soaked gauze. A few streams of milk were discarded to reduce the number of contaminating bacteria in the teat canal. Plastic tubes were held as horizontally as possible and milk samples of approximately 10 mL were collected into sterile plastic tubes using gentle milking. After sampling, teat ends were dipped into 1% iodine solution, and vials of tubes were tightly closed, refrigerated at 4 °C, and transported to the laboratory into cold chain.

2.4. Bacteriological examination

Before culturing, all samples were homogenized at room temperature. Following this procedure, bacteriological tests were performed according to the National Mastitis Council procedures (5). The samples (100 µL) were spread on blood agar and MacConkey agar plates, and were then incubated under aerobic conditions at 37 °C. Each plate was examined at 24 and 48 h after inoculation. Bacteria were identified in each colony by Gram stain. Catalase test was performed for gram-positive cocci. Catalase-positive and -negative colonies were determined as *Staphylococcus* spp. and *Streptococcus* spp., respectively. Coagulase tests were used for the differentiation of *Staphylococcus aureus* and coagulase-negative staphylococci (CNS) colonies. *S. aureus* colonies had coagulase-positive reactions. Streptococci were classified according to colony morphology, hemolytic properties, CAMP test, Lancefield group, and hydrolysis of esculin and hippurate. *Streptococcus dysgalactiae* had CAMP-negative reaction and was positive for Lancefield group C. *Streptococcus agalactiae* was positive for group B and the hippurate test. *Streptococcus uberis* hydrolyzed the esculin. *Escherichia coli* showed positive reactions to catalase, indole, methyl red, and lactose tests. *Bacillus* spp. and *Corynebacterium bovis* were identified by time of appearance on incubated plates, colony morphology, and Gram stain. According to the results, prevalence and risk factors of mastitis in heifers prior to first parturition were detected.

2.5. Statistical analysis

Multiple linear regression analyses using backward stepwise method were used to predict the prevalence of mastitis. Multiple regression equation of Y on X1, X2, … Xk is given by:

$$Y = \beta 0 + \beta 1 \times X1 + \beta 2 \times X2 + \ldots + \beta k \times Xk,$$

where y is a dependent variable; β 0 is a constant; and β 1, β 2… β k are coefficients of independent variables (X1, X2, Xk). SPSS 14.1 (SPSS Inc.) was used for statistical analysis.

3. Results

In this study, 37.47% of the lacteal secretion samples taken from 1736 quarters of udders, which belonged to 439 heifers, were determined to be infected. The mean prevalence of mastitis on the farms was calculated as 42.87 ± 4.12%. Isolation rates and pathogens isolated from lacteal secretion samples are shown in Table 1.

Responses to survey questions are shown in Table 2.

According to the multiple regression model, the number of animals in the herd, a well-balanced ration, separating the cows in dry periods into different boxes, and the status of contact of heifers with older cows contributed to the model significantly ($P < 0.05$; Tables 3 and 4). Coefficient of determination (R^2) for the model was estimated as 93.8% (Table 4). Multiple regression analysis using backward elimination method was carried out in 5 steps. Variables such as 'separate calf pens', 'feeding of waste milk to calves', 'fly control', and 'using organic bedding material' were removed from the model in each step, respectively. The fitted model included 'number of animals (<50), 'feeding with well-balanced ration', 'dry cows housed in different groups', 'heifers', and 'contact with older cows' variables.

The findings of this study showed that factors such as the number of animals in the herd (<50) and heifers' contact with older cows increased the incidence of subclinical mastitis; feeding with a well-balanced ration and dry cows being housed in different groups decreased

Table 1. Results of bacteriological analyses.

Bacteria	Farm 1 (n = 74)	Farm 2 (n = 31)	Farm 3 (n = 37)	Farm 4 (n = 31)	Farm 5 (n = 90)	Farm 6 (n = 38)	Farm 7 (n = 40)	Farm 8 (n = 26)	Farm 9 (n = 15)	Farm 10 (n = 15)	Farm 11 (n = 17)	Farm 12 (n = 25)	Total (n = 439)
S. aureus	39	14	20	5	51	28	25	5	21	9	13	5	235 (35.71%)
CNS	54	27	20	20	23	31	23	35	10	12	20	20	295 (44.83%)
Bacilli spp.	9	1	1	0	0	4	13	0	8	0	2	4	42 (6.38%)
Acinetobacter spp.	2	0	0	3	0	1	0	0	0	2	0	0	8 (1.21%)
E. coli	0	1	1	3	0	3	0	1	2	3	0	4	18 (2.73%)
Streptococci spp.	0	2	3	5	5	6	1	1	5	6	2	7	43 (6.53%)
Corynebacterium spp.	0	2	0	0	0	0	0	0	0	0	0	0	2 (0.3%)
Other	2	0	0	4	3	4	0	0	0	0	0	2	15 (2.27%)
Total	106/296 (35.8%)	47/124 (37.9%)	45/148 (30.4%)	40/124 (32.2%)	82/360 (22.2%)	77/152 (50.6%)	62/160 (38.7%)	42/104 (40.3%)	46/60 (76.6%)	32/60 (53.33%)	37/68 (54.4%)	42/100 (42%)	658/1756 (37.47%)

Table 2. Results of survey question responses.

		Yes	No
1-	Number of animals (<50)	10 (83.33%)	2 (16.67%)
2-	Feeding with well-balanced ration	9 (75%)	3 (25%)
3-	Separate calf pens	7 (58.33%)	5 (41.67%)
4-	Separating the cows in dry periods into different boxes	9 (75%)	3 (25%)
5-	Fly control	3 (25%)	9 (75%)
6-	Using organic bedding material	10 (83.33%)	2 (16.67%)
7-	Feeding waste milk to calves	7 (58.33%)	5 (41.67%)
8-	Contact of heifers with older cows	6 (50%)	6 (50%)

the incidence of subclinical mastitis ($P < 0.01$). Practices such as separate calf pens, fly control, and using organic bedding material on farms were not statistically significant in this predictive modeling (Tables 3 and 4).

4. Discussion

Mastitis is a multifactorial disease, requiring exposure to a combination of environmental and pathogenic factors and with variable responses between animals. Identification of risk factors for heifer mastitis is important for the development of control and prevention strategies (6). In Turkey, there is no report on the prevalence and risk factors for heifer mastitis.

The overall prevalence of subclinical mastitis in heifers was 37.47% at quarter level, and the mean incidence of mastitis on farms was calculated as 42.87 ± 4.12% in this study. This level is both lower (7–9) and higher (10–12) than several other studies' findings. Variation in the prevalence of subclinical heifer mastitis between this study and others (7–12) might be related to differences in management and to environmental factors.

CNS are opportunistic pathogens causing mastitis and can be colonized on the teat skin. Therefore, CNS are the most frequently isolated and important bacteria of heifer mastitis (13–17). In the current study, CNS were the most prevalent bacteria isolated from heifer subclinical mastitis. Following CNS, *S. aureus* was the most frequent. The high prevalence of CNS in this study is similar to other researchers' results (13–17). The high prevalence of CNS may contribute to the presence of these microorganisms on the skin of teats saprophytically.

S. aureus accounts for the most frequently isolated bacteria from mammary glands of heifers in mastitis cases after the CNS species. *S. aureus* causes significant losses

Table 3. Sources of variation.

Source	Sum of squares	Degrees of freedom	Mean square	F	P
Adjusted model	2098.123	4	524.531	26.659	<0.001
Interception	4983.903	1	4983.903	253.301	<0.001
Number of animals (<50)	354.368	1	354.368	18.01	0.004
Feeding with well-balanced ration	541.068	1	541.068	27.499	0.001
Dry cows housed in different groups	406.708	1	406.708	20.67	0.003
Heifers' contact with older cows	395.842	1	395.842	20.118	0.003
Error	137.731	7	19.676		
Total	24,289.039	12			
Adjusted total	2235.853	11			

Table 4. Coefficients of the variables in the model.

Model*	B	Std. error	Standardized beta	Tolerance	VIF
(Constant)	23.877	6.903			
Number of animals (<50)	15.816	3.727	0.432**	0.85	1.176
Feeding with well-balanced ration	–20.729	3.953	–0.658**	0.56	1.787
Dry cows housed in different groups	–17.971	3.953	–0.570**	0.56	1.787
Heifers' contact with older cows	15.76	3.514	0.577**	0.531	1.882

* R^2= 0.938; adjusted R^2: 0.903, ** P < 0.01

in milk yield, increasing somatic cell counts and chronic infections compared to other *Staphylococcus* species (1,18). Previous studies showed that the prevalence of *S. aureus* in heifer mastitis was between 3% and 44% (19–22). In our study, the isolation rate of *S. aureus* was similar to those of previous studies. The similarities between the present study and the aforementioned studies might be related to the lack of heifer mastitis control programs.

Potential risk factors such as herd size, housing, feeding with mastitic milk, fly control, keeping the pregnant heifers with dry cows, and contact with older cows and their relation to the prevalence of subclinical mastitis in heifers were examined in this study. The results indicated that herd size, feeding with a balanced ration, keeping the pregnant heifers with dry cows, and contact with older cows were the risk factors for subclinical heifers mastitis.

Studies indicated that feeding with a well-balanced ration was among the risk factors for subclinical heifer mastitis (23). Generally, well-balanced feeding is fundamental for optimal immunity and the ability of animals to resist diseases (24). In our study, it was found that feeding with a well-balanced ration was an important risk factor as it reduced the incidence of mastitis in heifers. The reason why such a result was obtained can be explained by the fact that the rate of udder edema is low and the udder immune system is strong in herds fed with well-balanced rations. Udder edema is a well-recognized risk factor for mastitis in heifers (4). A combination of genetics and ration generally contributes to the development of udder edema in heifers (4).

Feeding of waste milk to calves is another well-recognized risk factor for mastitis in heifers, and this practice should be discouraged unless the milk can be pasteurized prior to feeding. The exact mechanism through which the organism is transferred to the udder is unknown, but it is likely to be related to the colonization of the teat skin and inner thighs with mastitis-causing organisms (23). Previous studies showed that the feeding of mastitic milk to calves can result in increased risk of mastitis caused by *S. agalactiae* (6). On the contrary, the feeding of mastitic milk to calves was not determined as a risk factor in our study. Additionally, the rate of mastitis caused by *Streptococcus* spp. was lower on farms (6.43%). The reason for this outcome could be related to the low incidence of *S. agalactiae*.

The results of the present study revealed that herd size affected the incidence of subclinical heifer mastitis. This outcome can be associated with the fact that management becomes easier when herd size is smaller.

Several studies have shown that contact of heifers with older cows before calving (even housing in the same barn) increased the risk of clinical mastitis after calving, and separation of heifers from older cows has generally been recommended (18,23). In our study, we determined that heifers' contact with older cows increased the rate of subclinical mastitis. This result seems to confirm the aforementioned information. Furthermore, contact with mastitic cows can increase the transmission of bacteria to pregnant heifers.

Studies have shown that the horn fly (*Hameotobia irritans*) is an important vector for mastitis by causing *S. aureus* in heifers. *Hameotobia irritans* can be colonized with *S. aureus* during feeding and can remain colonized for several days. Incidence of infections caused by the fly in heifers having abrasion and wounds on the teat skin (70%) is higher than heifers with healthy teats (40%) (2). Decreasing fly populations on heifers and in barns is important to help reduce new intramammary infections (3,17,25). In our study, fly control was not determined as a risk factor. The reason for this outcome could be attributed to the fact that the present study was conducted in the autumn and winter (flies are not at a mature stage in this period).

Calves that are group-housed have the opportunity for cross-suckling, thus resulting in increased risk of transmission of contagious pathogens (4), especially *S. agalactiae* (6). In the present study, we determined that using calf pens did not affect the incidence of subclinical heifer mastitis. Additionally, the rate of mastitis caused by *Streptococcus* spp. was lower on farms (6.43%). The number of calves kept in calf pens may have affected this result.

The bedding type, which has low humidity and a lower amount of nutrients available to bacteria, and is also made of inorganic material, is ideal for dairy cows. Inorganic bedding material houses fewer coliform bacteria compared to organic material (6). In the present study, we determined that using organic bedding material did not affect the incidence of subclinical heifer mastitis. The cause of this result may include the following: 1) *E. coli* can cause more cases of clinical mastitis during early lactation, 2) there was regular replacement of the bedding material on the farms, and 3) the study was conducted during the late pregnancy (6–9 months) period (level of lactoferrin is high in this period).

Today, with the knowledge of environmental risk factors for pregnant heifers with subclinical mastitis, which has a great importance in the dairy cattle industry, changes should be made in the management that would be beneficial in preventing subclinical heifer mastitis.

Additionally, this study showed that predictive models for the incidence of subclinical heifer mastitis could be carried out with future comprehensive studies. Important results may be obtained through future research in this area by utilizing different statistical methods.

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