Risk factors associated with the presence of *Staphylococcus aureus* in milk and on hock skin

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Abstract

Staphylococcus aureus (*S. aureus*) is commonly found on skin and in skin lesions, and is the most common udder pathogen in Swedish dairy cows. As hair loss and/or lesions on hock skin is rather common on cows housed in stalls, we wanted to investigate the association between the presence of *S. aureus* in milk and on hock skin. A total of 177 cows from five Swedish dairy herds with *S. aureus* mastitis problems participated in the study. From each cow, milk and hock skin samples were taken and analyzed for *S. aureus*. Two multivariable logistic regression models were constructed: one with *S. aureus* in milk (yes/no) as the dependent variable, and one with *S. aureus* on hock skin (yes/no) as the dependent variable. Both were handled as a risk factor in the other model. Other risk factors investigated in the models were hock skin condition (graded as normal, hair loss only or with skin lesions), case of clinical mastitis or other disease before the day of sampling, milk yield, milk composition, parity and breed. In the multivariable model with *S. aureus* in milk as the dependent variable, days in milk and *S. aureus* on hock skin were found to be significantly associated with the outcome. In the other multivariable model, with *S. aureus* in the milk, parity, clinical mastitis before the sampling and having hock skin with hair loss or lesions was significantly associated with having *S. aureus* on hock skin. This study shows a strong association between *S. aureus* in milk and *S. aureus* on hock skin.

Keywords: *S. aureus, milk, hock skin, risk factors*

Introduction

In Sweden, *Staphylococcus aureus* is the most commonly isolated bacteria in both clinical and sub-clinical mastitis of dairy cows (Swedish Dairy Association 2008, Ericsson Unnerstad *et al.* 2009). Problem herds are recommended to keep strict milking order and milking routines, and to cull cows with chronic infection. However, sometimes these actions do not result in reduced spread of infection in the herd, due to lack of compliance to the recommendations or indicating sources of infection other than those normally considered.

As *S. aureus* is often found on skin and may cause wound infections (Matos *et al.* 1991), wounds could be a reservoir for mastitis-causing bacteria. Hock injuries and other lesions are common in cows in free-stall housing systems (Weary and Taszkun 2000), and associations between cows with hock injuries or hock swelling and clinical mastitis or increased somatic cell count (SCC) have been found (Sogstad *et al.* 2006, Fulwider *et al.* 2007).

The aim of this study was to investigate associations between the presence of *S. aureus* on hock skin and in milk, and determine whether cow factors were associated with the presence of *S. aureus*.

Materials and methods

Study population

A total of 177 cows from five Swedish herds were included in this study. Herd selection criteria were to have a history of *S. aureus* problems, to have tie-stalls and to be enrolled in the official Swedish milking and animal disease recording schemes. All herds were visited and sampled between December 2006 and March 2007.

Bacteriological sampling

Milk samples were collected aseptically at least 1 hour after morning milking, while hock samples were taken by rolling a sterile swab moistened with saline over the hock. The swab sample was placed on a sterile Vogel-Johnson agar and kept chilled over night. The condition of the hock skin was scored as either intact, with hair loss or with hair loss and lesions. The milk samples were incubated at 37°C for 16–18 hours, followed by culturing 10µL on 5% bovine blood agar with esculin. This method has been shown to increase the frequency of *S. aureus*-positive quarter cultures from milk samples in herds with mastitis problems related to *S. aureus* (unpubl. data). The hock samples were adjusted to room temperatures followed by incubation at 37°C for four hours (Fox *et al.* 1992). The hock samples were then cultured on 5% bovine blood agar with esculin and on modified Baird-Parker agar (MBP). The blood and MBP agar plates were incubated at 37°C for 16–24 hours and 48 hours, respectively. In all samples, growth of *S. aureus* was evaluated in accordance with accredited routines at the laboratory. A sample was classified as positive if at least one colony-forming unit (CFU) of *S. aureus* was isolated.

Cow data

Information about milk production, calvings, diseases and cullings during the lactations most recently before and after the sampling period was obtained from the Swedish official milk and animal disease recording schemes.

Statistical analysis

Logistic regression models, with the herd included as a fixed factor, were used to investigate associations between the dependent variables (presence of *S. aureus* in milk or on hocks) and the explanatory variables (*S. aureus* on hocks, *S. aureus* in milk, days in milk (DIM) at sampling, clinical mastitis (CM) in the two months before sampling, other diseases in the two months before sampling, breed, parity, hock injuries, lnSCC, urea level in milk and energy-corrected milk (ECM) yield on the test day most recently before the sampling). The median sample test day occurred at 126 DIM (50% central range: 58–264 DIM). All explanatory variables were first screened in univariable logistic regression analysis. Co-linearity between variables was assessed pair-wise by calculation of Spearman rank correlations. Two multivariable models, one for each dependent variable, were constructed using manual stepwise backward regression analysis, where variables not significant in the model were re-entered whenever a new variable became significant, or a variable was removed. To remain in the final models, variables had to have $p \leq 0.05$ in the likelihood ratio χ^2 test. Biologically plausible two-way interactions between the main effects were tested in all final models. Due to missing data, only 171 of the 177 cows were included in the final model. Model validation was performed according to Hosmer and Lemeshow (2000). Data editing and all statistical analyses were done using Stata Software (StataCorp. 2003).

Table 1 Final logistic regression model of risk factors associated with the presence of *S. aureus* in milk (n=171).

Variable	β	SE	OR	95% CI (OR)	p-value
Intercept	−0.76	0.49			-
Days in milk at sampling	−0.004	0.002	0.59[a]	0.37;0.94	0.03
S. aureus present on hock skin					
No	Ref.[b]	-	-	-	-
Yes	1.06	0.43	2.90	1.26;6.69	0.01

[a] Odds ratio is calculated for an increase in DIM from median DIM (126) to the fourth quartile day (264 DIM)
[b] Reference category

Results

Staphylococcus aureus was isolated from milk and hock skin samples of 49 (28%) and 43 (24%) of the cows, respectively. In 55% of the cows with *S. aureus* in their milk, the bacteria was also found on hock skin, while 45% of the cows with *S. aureus* present on hock skin also had it in their milk.

In 83 (48%) cows the hock skin was intact, while hair loss was observed in 57 (33%) cows and 34 (19%) cows had both hair loss and lesions. *S. aureus* was found in 20 (24%) of the samples from intact hock skin, 21 (37%) of those from hock skin with hair loss, and 20 (59%) of those from hock skin with hair loss and lesions.

In the univariable analysis of variables associated with the risk of *S. aureus* in milk, 7 out of 11 variables were significant ($p<0.25$) and included in the multivariable analysis. In the final model, only DIM at sampling and *S. aureus* on hock skin remained ($p<0.05$; Table 1). Increasing DIM at sampling reduced the risk of finding *S. aureus* in milk, while cows with *S. aureus* present on hock skin had three times higher risk of also having it in their milk, compared to cows without *S. aureus* on hock skin.

In the univariable analysis of variables associated with the risk of finding *S. aureus* on hock skin, 8 out of 12 variables were significant ($p<0.25$) and included in the multivariable analysis. In the final model of variables associated with *S. aureus* on hock skin, *S. aureus* in their milk, parity, hock skin condition and treatment of CM before sampling remained significantly associated with the outcome ($p<0.05$; Table 2). Cows with *S. aureus* in their milk and cows with their hock skin scored as having hair loss or hair loss and lesions were at a higher risk of having *S. aureus* on hock skin, compared to cows without *S. aureus* in their milk and had intact hock skin. Cows in parity ≥2 and cows that had been treated for CM before the sampling were less likely to have *S. aureus* on hock skin compared, to first-parity cows and cows without treatment of CM before the sampling.

Discussion

The results indicate a strong association between the presence of *S. aureus* in milk and on hock skin. However, since causal relationships were not investigated in this study, the origin of the infections is not known. The *S. aureus* isolates found in milk and hock skin samples have been genotyped using pulsed-field gel electrophoresis (Capurro *et al.* 2009). According to these results, most milk and hock skin isolates were of the same genotype within cow, indicating a relationship between *S. aureus* in milk and on hock skin. There is a possibility that hock skin may

Table 2 Final logistic regression model of risk factors associated with the presence of *S. aureus* on hock skin (n=168).

Variable	β	SE	OR	95% CI (OR)	p-value
Intercept	−1.03	0.64	-	-	-
S. aureus present in milk samples					
No	Ref.[a]				
Yes	1.21	0.46	3.34	1.37; 8.17	0.008
Parity					
First-parity	Ref.[a]	-	-	-	-
Second parity	−0.95	0.49	0.39	0.15; 1.01	0.05
≥ third parity	−1.05	0.48	0.35	0.14; 0.90	0.03
Hock injuries					
No	Ref.[a]	-	-	-	-
Yes	1.06	0.43	2.87	1.24; 6.67	0.01
Treatment of CM before sampling					
No	Ref.[a]	-	-	-	-
Yes	−1.26	0.64	0.28	0.08; 0.96	0.04

[a] Reference category

be contaminated with *S. aureus* by milk leakage. Milk leakage is more common when cows lie down than when they are standing (Persson Waller *et al.* 2003), increasing the probability that the milk contaminates the legs and hocks. On the other hand, infected hock lesions might in turn contaminate the teats and udder when the cow lies down. In different situations, either one may be the reservoir of *S. aureus* causing the infection of the other. Reducing the risk of milk leakage and hock lesions could be a way of reducing circulating infections in the herd.

In the present study we found an increased risk of finding *S. aureus* on hock skin if the hock skin was not intact, but an association between non-intact hock skin and *S. aureus* in milk was not found. This, in combination with the strong association between *S. aureus* in milk and on hock skin, indicates that it is the presence of *S. aureus* on hock skin that is associated with *S. aureus* in milk, and that hock lesions might be of minor importance. However, as hair loss or lesions were of importance in finding *S. aureus* on hock skin, improving hock skin condition would result in a reduced risk of the hock skin acting as a reservoir for *S. aureus* infections. In a study by Fulwider *et al.* (2007), a positive correlation was found between hock lesions and SCC.

The association between increasing DIM at sampling and a reduction in *S. aureus* findings in milk in the present study is not in agreement with Piccinini *et al.* (2007), who showed an increase of subclinical intramammary infections with increasing DIM, or Zadoks *et al.* (2001), who did not find any association between *S. aureus* findings and DIM. More studies are needed to clarify this relationship.

The fact that *S. aureus* was less likely to be found on hock skin in older cows than in younger cows was surprising. The reasons for this finding are not known. According to other results from the study, *S. aureus* was also isolated in body samples from young heifer calves and from heifers in late pregnancy (Capurro *et al.* 2009).

The reduced risk of finding *S. aureus* on hock skin in cows that had been treated for CM within two months of sampling may be a result of the antimicrobial treatment itself; most mastitis cases

in Sweden are treated systemically, and there was no association between treatment of CM and findings of *S. aureus* in milk. More studies are needed to investigate this relationship further.

Conclusion

There was a strong association between findings of *S. aureus* in milk and on hock skin. Prevention of hock skin lesions should reduce the risk of finding *S. aureus* on hock skin, but not necessarily in milk.

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