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1 **Microbial Aetiology of Acute Clinical Mastitis and Agent-Specific Risk Factors**

2

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20

21 **Abstract**

22 A nation wide study on the microbial aetiology of cases of acute clinical mastitis in Swedish  
23 dairy cows was conducted with the aim to investigate changes in the microbial panorama  
24 compared to a previous study performed 1994-1995. Another aim was to investigate some  
25 agent-specific environmental and individual risk factors. Milk samples were collected from  
26 987 udder quarter cases from 829 cows during six 2-month periods from May 2002 to April  
27 2003, and data on risk factors and demography were collected at sampling by means of a  
28 questionnaire. In total, 1056 bacteriological diagnoses were made. The most frequently  
29 isolated bacterial species was *Staphylococcus aureus* constituting 21.3% of the diagnoses,  
30 followed by *Escherichia coli* (15.9%), *Streptococcus dysgalactiae* (15.6%), *Streptococcus*  
31 *uberis* (11.1%), coagulase-negative staphylococci (6.2%), *Arcanobacterium pyogenes* (6.1%)  
32 and *Klebsiella* spp. (4.2%). Samples with no growth or contamination constituted 10.6% and  
33 4.5% of the diagnoses, respectively. A major shift in the panorama of udder pathogens was  
34 not observed compared to the survey in 1994-1995. Isolation of *Klebsiella* spp. was strongly  
35 associated with the use of sawdust as bedding material. On the other hand, using sawdust as  
36 bedding reduced the risk of isolating *S. uberis* relative to using straw or peat. The risk of  
37 isolating *E. coli* increased with increasing milk yield and was higher in loose housing systems  
38 than in tie stalls. Isolation of *S. aureus* was associated with tie stalls, and *A. pyogenes* with  
39 low yielding cows and teat lesions. *S. dysgalactiae* infections were also associated with teat  
40 lesions.

41

42 **Key words:** bovine mastitis, microbial aetiology, risk factors

43

44

## 45 1. Introduction

46 Mastitis is one of the most important diseases in dairy cows and much effort is dedicated to its  
47 prevention. Mastitis is mostly caused by a rather limited spectrum of bacteria, consisting  
48 mainly of staphylococci, streptococci and coliforms, but any bacteria may infect the udder.  
49 The panorama of udder pathogens varies between countries for example due to differences in  
50 management. In Sweden, a nation wide survey of the microbial aetiology of clinical mastitis  
51 performed in 1994-1995 revealed that *Staphylococcus aureus* (*S. aureus*), streptococci and  
52 *Escherichia coli* (*E. coli*) were the dominating findings (Nilsson et al., 1997). Since then, the  
53 proportion of farms with loose housing system has increased markedly from 5-10% in 1995 to  
54 27% in 2003, when the current study was performed, and the average herd size has increased  
55 from 32 cows/herd in 1995 to 45 cows/herd in 2003 (Swedish Dairy Association, 2008). Such  
56 changes in management systems might promote infections with certain agents leading to a  
57 shift in the microbial panorama causing clinical mastitis. Loose housing systems might  
58 increase the risk for mastitis caused by environmental bacteria, whereas contagious udder  
59 pathogens may be harder to eradicate if they become established in loose housing stalls where  
60 it is difficult to keep strict grouping of cows and milking order. Agent-specific risk factors for  
61 clinical mastitis associated with environment factors like housing, but also to individual cow  
62 factors, have been identified in other countries (e.g. Barkema et al., 1999; Olde Riekerink et  
63 al., 2008). However, such studies have not been performed in Sweden.

64

65 The objective of the present study was to investigate whether the microbial panorama  
66 associated with acute clinical mastitis had changed since the previous investigation performed  
67 1994-1995. Moreover, the importance of some environmental and individual factors for the  
68 risk of agent-specific acute clinical mastitis was also studied.

69

70 **2. Material and methods**

71 *2.1 Study design*

72 Milk samples from udder quarters with acute clinical mastitis were collected by field  
73 veterinarians in 51 veterinary practices distributed all over Sweden. Only cases of acute  
74 clinical mastitis in lactating cows were eligible for sampling. Inclusion criteria for acute  
75 clinical mastitis were macroscopic changes in the milk of at least one udder quarter, no  
76 previous record of clinical mastitis during the present lactation period, somatic cell count  
77 (SCC) at the latest monthly milk recording below 200 000 cells/ml and no treatment with  
78 antimicrobials during the previous 30 days. The number of cases of acute clinical mastitis  
79 sampled at each veterinary practice was proportional to the number of dairy cows in the  
80 county and the number of participating practices in the county. In each practice, cases were  
81 enrolled in order of appearance until the sampling quota for the practice was filled during  
82 each of six consecutive two-month periods, from May 2002 to April 2003. The target sample  
83 size was 1000 cases of acute clinical mastitis, which would be sufficient to significantly  
84 detect a 5% change, approximately, in the proportion of *S. aureus* since 1995, and changes  
85 smaller than 5% for pathogens with a lower proportion. Since some practices did not have a  
86 sufficient number of cases that fulfilled the inclusion criteria only 829 cows (987 udder  
87 quarter cases) could be included. The field veterinarian collected aseptic milk samples from  
88 all affected udder quarters. On the sampling occasion the veterinarian also registered data  
89 about the cow and the herd by using a specified questionnaire. Housing system, floor of  
90 cubicles, bedding material, and design of passages and cubicles were recorded as were breed  
91 of the cow, lactation number, date of latest calving, milk yield at latest monthly milk  
92 recording, and presence of teat lesions.

93

94 2.2. *Bacteriological analyses*

95 Milk samples (10 $\mu$ l) were cultured on blood (5% bovine blood) agar plates, incubated at 37°C  
96 for 16-24 hours, and evaluated in accordance with the routines at the participating veterinary  
97 practices. The plates were sent to the National Veterinary Institute, Uppsala, Sweden, where  
98 the growth on the plates was confirmed by additional laboratory tests in accordance with the  
99 routines at the laboratory. *S. aureus* was identified by means of typical colony morphology,  
100 and  $\alpha$ - and  $\beta$ -hemolysis, and, when typical hemolysis zones were not present, coagulase  
101 reaction (coagulase-positive). Coagulase-negative staphylococci (CNS) were identified by  
102 typical colony morphology and coagulase reaction, but were not further characterized.  
103 Streptococci were determined by colony morphology and CAMP-reaction, and 12  
104 biochemical reactions (hippurate, aesculine, salicine, sorbitol, mannitol, raffinose, lactose,  
105 saccharose, inuline, trehalose, starch and glycerine) were used for typing to the species level  
106 (Bergey's manual, 1986; Barrow and Feltham, 1993). For isolates not confirmed with these  
107 methods, Lancefield grouping (Streptex, Murex Biotech Limited, Dartford, UK) was used.  
108 Enterococci were confirmed by Gram-staining and growth of red colonies on SlaBa-plates  
109 (Slanetz & Bartley Medium, Oxoid Ltd., Basingstoke, England). Gram-negative bacteria with  
110 typical colony morphology, and positive for p-nitrophenyl- $\beta$ -D-glucopyranosiduronic acid  
111 (PGUA) and indole were considered as *E. coli*. For other Gram-negative bacteria, oxidase  
112 reaction and API 20 E (bioMérieux, Craponne, France) or API 20 NE (bioMérieux, Craponne,  
113 France) was used. For confirmation of *Arcanobacterium pyogenes* (*A. pyogenes*) colony  
114 morphology, Gram-staining, and culture in 3% CO<sub>2</sub> was performed. *Bacillus* spp., yeasts and  
115 *Corynebacterium* spp. were confirmed by means of colony morphology and Gram staining. A  
116 milk sample was classified as positive if at least one colony-forming unit (CFU) of *S. aureus*  
117 or *S. agalactiae* was isolated. For other agents, the presence of at least three CFU was needed  
118 for positive classification. Samples were classified as contaminated if three or more bacterial

119 types were isolated from one milk sample and growth of a major udder pathogen was not  
120 identified. If moderate to high growth of a major udder pathogen was found in combination  
121 with a few CFU of several contaminating species the sample would be diagnosed as positive  
122 for growth of the major udder pathogen.

123

### 124 *2.3. Statistical analysis*

125 Culture results and questionnaire data were registered in a database (Microsoft® Access,  
126 Microsoft Corp.). For risk factor analyses, contaminated samples (n=48) and samples with no  
127 growth (n=112) were excluded, as were samples lacking herd identity (n=14). Duplicate  
128 records on cows that were infected with the same agent on more than one teat were also  
129 excluded (n=45). The resulting data set consisted of 837 unique isolates from 814 udder  
130 quarters in 734 cows and 571 herds. Information on daily milk yield and days in milk (DIM)  
131 was only available for 455 and 702 cows, respectively. Quarter-level data was summarised at  
132 the cow-level, creating a 2-level dataset with 734 records. Four variables describing quarter  
133 level features were generated in this manner; teat lesion (on any teat), number of quarters  
134 infected in total, number of quarters with agent in question, and number of diagnoses per  
135 quarter. Descriptive statistics of all herd variables, including year and season, and cow  
136 variables is given in Tables 1 and 2, respectively. The variables housing and bedding were  
137 coded in two different ways to emphasise different aspects of the risk factors (e.g. different  
138 types of tie stalls versus loose housing) and at the same time avoiding to produce covariate  
139 groups with too few observations.

140

141 The odds of a certain agent being isolated from a case of clinical mastitis given the  
142 presence/absence of different risk factors was estimated using logistic regression. We  
143 accounted for repeated observations within herd by applying the cluster option in Stata, in



144 order to obtain robust variance estimates. However, the potential effect of clustering was  
145 limited (average number of cows sampled per herd = 1.28).

146

147 The dependent variables in each of seven models indicated whether the cow was a case  
148 (1=yes, 0=no) with respect to the following agents: *E. coli*, *S. aureus*, CNS, *A. pyogenes*,  
149 *Klebsiella* spp, *Streptococcus dysgalactiae* (*S. dysgalactiae*) and *Streptococcus uberis* (*S.*  
150 *uberis*). In other words, we defined seven different mastitis outcomes (similar to defining  
151 different disease outcomes in a set of individuals, such as coughing, lameness or diarrhea).  
152 All cows were represented once in all models, either as cases or as non-cases. A cow from  
153 which more than one bacterial species was isolated appeared as a case in the model for each  
154 of the species, and as a control otherwise. The group of comparison (“non-cases”) in each  
155 model consisted of cows with acute clinical mastitis involving other pathogens than the one of  
156 interest.

157

158 Univariate associations (at  $p < 0.15$ ) were used to identify candidate predictors for the  
159 multivariable models. The sets of candidate variables for each agent model are indicated in  
160 Tables 3 and 4. Whenever variables with substantial amount of missing data (DIM and daily  
161 milk yield,  $n=454$  with daily milk yield in the model and  $n=444$  with both variables included)  
162 were significant in the univariate analyses, the analysis was first run with these variables  
163 included. If they were not significant, the model was reassessed without including them.

164 There were also 2 missing observations for the variables Housing 1 and 2; consequently only  
165 732 records were included for agents where they were considered in the initial model. All  
166 two-way interactions between candidate predictors were included in the initial multivariable  
167 models and tested for significance. Non-significant ( $p > 0.05$ , likelihood ratio test) variables  
168 were eliminated in the multivariable analysis using a stepwise backward procedure. Model fit

169 was assessed using the Hosmer-Lemeshow goodness-of-fit-test. All analyses were performed  
170 using the statistical software Stata, version 8 (StataCorp, College Station, TX, USA).

171

### 172 **3. Results**

#### 173 *3.1. Distribution of udder pathogens*

174 In total, 987 udder quarter cases from 829 cows were sampled. At least one microbial species  
175 supposedly associated with the case of mastitis was isolated from 827 (84%) of the quarter  
176 samples. One species was isolated from 761 (92%) of those samples, two species were  
177 isolated from 63 (8%) samples, and three species were isolated from 3 (<1%) samples. The  
178 distribution of microbial diagnoses is shown in Table 5. In total, 1056 microbial diagnoses  
179 were obtained from 987 udder quarter samples. No growth was yielded from 112 samples  
180 (11.3% of the samples, 10.6% of the microbial diagnoses) while 48 samples (4.9% of the  
181 samples, 4.5% of the microbial diagnoses) were contaminated. The most frequently isolated  
182 bacterial species was *S. aureus*, which accounted for 21.3% of the diagnoses followed by *E.*  
183 *coli* (15.9%), *S. dysgalactiae* (15.6%) and *S. uberis* (11.1%). Less frequent findings were  
184 CNS, *A. pyogenes* and *Klebsiella* spp. *Streptococcus agalactiae* (*S. agalactiae*), other  
185 streptococci, enterococci, *Bacillus* spp., *Corynebacterium* spp., yeasts, *Pseudomonas*  
186 *aeruginosa*, *Mannheimia haemolytica*, *Enterobacter* spp., *Serratia* spp., *Citrobacter freundii*  
187 and *Proteus mirabilis* each composed less than 1% of the diagnoses.

188

#### 189 *3.2. Associations between prevalence of udder pathogens, and herd and individual risk* 190 *factors*

191 The study population consisted of cows with acute clinical mastitis and risk factors must  
192 therefore be interpreted accordingly. A significant risk factor does not suggest that there is a  
193 higher risk of contracting mastitis. Rather, the risk factor is associated with a higher

194 likelihood of isolating a certain pathogen, given that a cow is infected. Thus, we do not look  
195 primarily at factors to be targeted for prevention, rather factors that are involved in the  
196 etiology. The results of the multivariable analyses are presented in Tables 3 and 4. The  
197 following variables were not significant in any of the final models; Floor of cubicle, Bedding  
198 2, Year, Parity, Number of quarters infected with the agent in question, and DIM.

199

200 The risk of isolating *S. aureus* (rather than any other udder pathogen) was associated with  
201 being housed in tie stalls, and *S. aureus* infection was relatively more common during the  
202 housing season (Nov-Apr) than in May-Oct. The probability of isolating CNS was relatively  
203 lower in cases with teat lesions, and CNS infection was associated with infections in several  
204 udder quarters. The risk of isolating *E. coli* was lower in short tie stalls than in loose housing,  
205 and higher in cows with high milk yield. The probability of isolating *E. coli* was also  
206 relatively lower in cases with teat lesions. *Klebsiella* spp. was strongly correlated with the use  
207 of sawdust as bedding material. The probability of finding *Klebsiella* spp was four times  
208 higher when sawdust was used as bedding compared to straw or peat. *A. pyogenes* was  
209 associated with low milk yield, and both *A. pyogenes* and *S. dysgalactiae* were associated  
210 with teat lesions. *S. uberis* was more common during early compared to late housing season.  
211 Using sawdust as bedding reduced the risk of isolating *S. uberis* relative to using straw or  
212 peat. The likelihood of isolating *S. uberis* (rather than any other pathogen) was higher in  
213 Holstein cows. In all infections, except *Klebsiella* spp., there was a strong likelihood of  
214 diagnosing more than one infection at the same time.

215

#### 216 **4. Discussion**

217 To get reliable data on microbial aetiology in mastitis, representative surveys must be  
218 performed. Since the cases of mastitis in the present study were sampled under strict inclusion

219 criteria and originated from all parts of the country the isolates should represent a random  
220 sample of pathogens causing acute clinical mastitis, defined by no previous record of clinical  
221 mastitis during the lactation period and SCC below 200 000 cells/ml at the latest monthly  
222 milk recording, in Sweden in 2002-2003. A national survey on clinical mastitis was also  
223 performed in 1994-1995 (Nilsson et al., 1997). Relative to that survey, a major shift in the  
224 panorama of udder pathogens causing acute clinical mastitis was not found. The proportions  
225 of the two most common udder pathogens, *S. aureus* and *E. coli*, were almost identical in the  
226 two investigations despite differences in housing and herd size.

227  
228 The panorama of udder pathogens causing clinical mastitis in Sweden is quite different  
229 compared to some other countries. In UK, for example, environmental pathogens such as *E.*  
230 *coli* and *S. uberis* are most common (e.g. Milne et al., 2002; Bradley et al., 2007).  
231 Environmental pathogens also pre-dominate in US according to Neuder et al. (2003), but  
232 Bezek (1998) reported more staphylococci and streptococci than coliforms. In a recent  
233 Canadian study, however, *S. aureus* was the most frequently isolated pathogen, followed by  
234 *E. coli* and *S. uberis* (Olde Riekerink et al., 2008). Moreover, in a Norwegian study on clinical  
235 mastitis in heifers, *S. aureus* was the dominating finding followed by *S. dysgalactiae* (Waage  
236 et al., 1999). When comparing studies differences in the definition of clinical mastitis must be  
237 considered. In the present study, cases of exacerbative and chronic clinical mastitis were  
238 excluded, which may have resulted in a different distribution of pathogens than if all clinical  
239 cases were included.

240  
241 The reasons for the pre-dominance of *S. aureus* in clinical mastitis in Sweden are not clear,  
242 but housing system and herd size are factors that might be of importance. Although the  
243 Swedish dairy industry has moved towards larger herds and more loose housing systems in

244 recent years relatively small herds with tie stalls still dominate. Consistent with this an  
245 association between tie stalls and *S. aureus* was seen in the present study. This finding is in  
246 line with those of a recent Canadian study (Olde Riekerink et al., 2008). If strict milking order  
247 is not accomplished, spread of contagious bacteria, like *S. aureus*, cannot be stopped in any  
248 housing system. Many countries use blanket dry cow therapy, which may reduce the  
249 occurrence of *S. aureus*, as long as the bacteria are susceptible to the antimicrobial used. In  
250 Sweden, dry cow therapy is used selectively on cows having increased milk SCC. It is  
251 estimated that approximately 20-25% of the cows are dry cow treated with antimicrobials  
252 each year. It was more likely to find *S. aureus* in the housing season than in the pasture  
253 season, which is in line with a recent Canadian study with similar housing and pasture  
254 conditions (Olde Riekerink et al., 2007). *S. aureus* is also associated with high SCC, which  
255 may be reflected in the bulk milk SCC. The geometric mean of the bulk milk SCC of Swedish  
256 herds decreased, however, from 190 000 cells/ml in 1995 to 169 000 cells/ml in 2003  
257 (Swedish Dairy Association, 2008).

258  
259 Another important housing factor found in the present study was the association between  
260 isolation of *E. coli* in cases of clinical mastitis and loose housing, which is in line with  
261 findings by Olde Riekerink et al. (2008). Thus, the increasing proportion of loose housing  
262 herds has so far not lead to a shift towards more environmental infections in Sweden, but as  
263 the proportion of loose housing herds is increasing steadily this may be different in the future.  
264 Isolation of *E. coli* was also associated with high daily milk yield. High milk production may  
265 contribute to increased risk of milk leakage, which may lead to open teat canals and larger  
266 risk for infections by environmental bacteria like *E. coli*. The percentage of cows leaking milk  
267 in a herd was found to be a risk factor for clinical *E. coli* mastitis in a study by Schukken et al.  
268 (1991).

269

270 The proportion of *S. uberis* tended to be lower compared to the investigation in 1994-1995,  
271 and this type of infection was not associated with housing system. A higher incidence of *S.*  
272 *uberis* in cows in tie stalls than in cows in free stalls was, however, found by Olde Riekerink  
273 et al. (2008). In the present study, isolation of *S. uberis* was less common in cases where cows  
274 were bedded on sawdust compared to if straw was used. Sawdust was, however, strongly  
275 associated with isolation of *Klebsiella* spp., which is in line with other studies (Bramley and  
276 Neave, 1975; Oz et al., 1985). Zdanowicz et al. (2004) also found more *Klebsiella* spp. on teat  
277 ends of cows housed on sawdust compared with on teat ends of cows housed on sand. They  
278 also found that *Streptococcus* spp. was isolated more often from teat ends of cows housed on  
279 sand compared with cows housed on sawdust. Sand was not used in any of the farms in the  
280 present study. We also found that isolation of *S. uberis* was more common in early housing  
281 season than in late housing season, but the reason for this is not known. In a Dutch study, the  
282 highest incidence of clinical *S. uberis* mastitis was found in summer (Olde Riekerink et al.,  
283 2007). In addition we found a higher likelihood to isolate *S. uberis* in Swedish Holstein cows  
284 than in Swedish Red cows. When considering all veterinary-treated cases of clinical mastitis a  
285 higher incidence of clinical mastitis is found in Swedish Holstein cows than in Swedish Red  
286 cows (Persson Waller et al., 2008).

287

288 Both *A. pyogenes* and *S. dysgalactiae*, unlike *E. coli* and CNS, are prone to colonise wounds,  
289 which may explain their association with teat lesions (Pyörälä and Honkanen-Buzalski, 1995).  
290 *A. pyogenes* is also often isolated in mixed cultures with *S. dysgalactiae* in the summer  
291 mastitis complex (Schwan and Holmberg, 1979). An explanation for the association between  
292 isolation of *A. pyogenes* and low milk yield may be that this type of infection is likely to be  
293 chronic, which may lead to a decrease in milk production.

294

295 As management and milking systems are continuously changing in the Swedish dairy  
296 industry, changes in microbial patterns might occur in the future. Therefore, studies like the  
297 present should be repeated regularly to update the knowledge of trends in the panorama of  
298 microorganisms causing clinical mastitis. National surveys on microbial aetiology of  
299 subclinical bovine mastitis have not been performed in Sweden. The distribution of udder  
300 pathogens causing subclinical mastitis is most likely different from the microbial distribution  
301 in clinical mastitis. Thus, such investigations are warranted.

302

### 303 **5. Conclusions**

304 In Sweden, a major shift in the distribution of udder pathogens in acute clinical mastitis was  
305 not found in 2002-2003 compared to a similar survey performed in 1994-1995. *S. aureus* is  
306 still the most frequently isolated pathogen followed by *E. coli*. Thus, a shift towards  
307 environmental pathogens could not be confirmed under Swedish conditions despite an  
308 increasing number of cows per herd and more herds having loose housing systems. Using  
309 sawdust as bedding was identified as a risk factor for isolating *Klebsiella* spp., but sawdust  
310 reduced the risk of isolating *S. uberis*. *E. coli* infections were associated with loose housing  
311 systems and *S. aureus* infections with tie stalls. Both *A. pyogenes* and *S. dysgalactiae* were  
312 associated with teat lesions.

313

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316

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364 Table 1.  
 365 Descriptive statistics of variables reflecting environmental conditions for 734 cows with acute  
 366 clinical mastitis in 571 Swedish dairy herds, from May 2002 to April 2003. The variables  
 367 were tested for association with seven different udder pathogens (*Staphylococcus aureus*,  
 368 *Arcanobacterium pyogenes*, *Escherichia coli*, *Klebsiella* spp, coagulase-negative  
 369 staphylococci, *Streptococcus dysgalactiae* and *Streptococcus uberis*).

Variable	Category	Frequency (%)
Housing 1 <sup>1,2</sup>	Loose (w. cubicles)	242 (33)
	Tie stalls, long	141 (19)
	Tie stalls, short/Other	349 (48)
Housing 2 <sup>1,2</sup>	Loose (w. cubicles), cold	57 (8)
	Loose (w. cubicles), warm	185 (25)
	Tie stalls	490 (67)
Floor of cubicle	Rubber mat/Cubicle mattress	517 (70)
	Concrete/Concrete+rubber mat	196 (27)
	Other	21 (3)
Bedding 1 <sup>2</sup>	Straw/Peat	268 (36)
	Mixed	217 (30)
	Saw dust	249 (34)
Bedding 2 <sup>2</sup>	Straw	255 (35)
	Mixed	27 (4)
	Saw dust/Shavings	452 (62)
Year	2002	481 (66)
	2003	253 (34)
Season	Early housing season (Sep-Dec)	256 (35)
	Late housing season (Jan-Apr)	249 (34)
	Pasture (May-Aug)	229 (31)
Season – <i>S. aureus</i> <sup>3</sup>	May-Nov	349 (48)
	Dec-April	385 (52)

370 <sup>1</sup> Data was missing from 2 cows.

371 <sup>2</sup> The variables Housing and Bedding were coded in two different ways (1 and 2) to  
 372 emphasize different aspects of the risk factors (e.g. higher resolution on tie stalls/loose  
 373 housing, or focus on saw dust/straw as bedding material).

374 <sup>3</sup> Only used in the *S. aureus* model.

375

376 Table 2.  
 377 Descriptive statistics of categorical and continuous variables reflecting individual features of  
 378 734 cows with acute clinical mastitis in 571 Swedish dairy herds, from May 2002 to April  
 379 2003. The variables were tested for association with seven different mastitis pathogens  
 380 (*Staphylococcus aureus*, *Arcanobacterium pyogenes*, *Escherichia coli*, *Klebsiella* spp,  
 381 coagulase-negative staphylococci, *Streptococcus dysgalactiae* and *Streptococcus uberis*).

Variable	Category	Frequency (%)
<i>Categorical variables</i>		
Breed	Swedish Red	310 (42)
	Swedish Holstein	401 (55)
	Other	23 (3)
Parity	1	257 (35)
	2	165 (22)
	3	139 (19)
	>3	173 (24)
Recent environment (last 3 weeks) <sup>1</sup>	Pasture	178 (25)
	Pasture and indoors	37 (5)
	Indoors	505 (70)
Number of diagnoses per quarter	1	637 (87)
	2-3	97 (13)
Number of quarters infected in total	1	666 (91)
	>1	68 (9)
Diagnosed with teat lesions	No	626 (85)
	Yes	108 (15)
Number of quarters infected with agent in question	1	702 (96)
	2-3	32 (4)
<i>Continuous variables</i>		<i>Median (5<sup>th</sup>; 95<sup>th</sup> percentile)</i>
Daily milk yield (kg) (n=455)		31 (15; 46)
Days in milk (n=702)		48 (1; 284)

382 <sup>1</sup>Data was missing from 14 cases.

383

384 Table 3.

385 Odds ratio (OR) estimates with 95% confidence intervals (CI) for the risk of isolating *Staphylococcus (S.) aureus*, coagulase-negative  
 386 staphylococci (CNS) and *Escherichia (E.) coli* from cases of acute clinical mastitis in 734 Swedish dairy cows, during 2002-2003. Shaded areas  
 387 denote candidate variables (univariate association with  $p < 0.15$ ) that were excluded from the final models due to lack of significance ( $p > 0.05$ ).

Variable	Levels	<i>S. aureus</i> <sup>1</sup>			CNS <sup>2</sup>			<i>E. coli</i>		
		OR	CI	p	OR	CI	p	OR	CI	p
	# of cases	211			59			161		
Housing 1	Loose (w. cubicles)							1.00	-	-
	Tie stalls, long							0.90	0.49-1.67	0.75
	Tie stalls, short /Other							0.51	0.31-0.86	0.011
Housing 2	Loose (w. cubicles), cold	1.00	-	-						
	Loose (w. cubicles), warm	1.93	0.82-4.53	0.13						
	Tie stalls	2.47	1.10-5.57	0.029						
Season <i>S. aureus</i>	May-Oct	1.00	-	-						
	Nov-Apr	1.72	1.23-2.41	0.002						
Teat lesion	No				1.00	-	-	1.00	-	-
	Yes				0.26	0.08-0.90	0.034	0.25	0.12-0.53	<0.001
# diagnoses per quarter	1	1.00	-	-	1.00	-	-	1.00	-	-
	2-3	3.34	2.12-5.24	<0.001	2.44	1.17-5.09	0.017	2.78	1.36-5.74	0.005
# quarters infected in total	1				1.00	-	-			
	>1				2.43	1.15-5.13	0.020			
Daily milk yield (kg) <sup>3</sup>								1.037	1.014-1.06	0.003

388 <sup>1</sup> Additional candidate variables not indicated in this table were floor of cubicle, bedding, breed and days in milk<sup>3</sup>.389 <sup>2</sup> Additional candidate variables not indicated in this table were bedding, year, parity, and days in milk<sup>3</sup>.

390 <sup>3</sup> Data on daily milk yield and days in milk were not available for all cases. Thus, for models where daily milk yield and days in milk were  
 391 significant in the univariate screening, a reduced dataset was used (n=454).

392 Table 4.

393 Odds ratio (OR) estimates with 95% confidence intervals (CI) for the risk of isolating *Klebsiella* spp, *Arcanobacter pyogenes*, *Streptococcus*  
 394 *dysgalactiae* and *Streptococcus uberis* from cases of acute clinical mastitis in 734 Swedish dairy cows, during 2002-2003. Shaded areas denote  
 395 candidate variables (univariate association with  $p < 0.15$ ) that were excluded from the final models due to lack of significance ( $p > 0.05$ ).

Variable	Levels	<i>Klebsiella</i> spp. <sup>1</sup>			<i>A. pyogenes</i> <sup>1</sup>			<i>S. dysgalactiae</i> <sup>2</sup>			<i>S. uberis</i> <sup>2</sup>		
		OR	CI	p	OR	CI	p	OR	CI	P	OR	CI	p
	# of cases	42			57			150			111		
Bedding 1	Straw/Peat	1.00	-	-							1.00	-	-
	Mixed	1.99	0.77-5.16	0.16							0.89	0.55-1.42	0.62
	Sawdust	3.98	1.74-9.09	0.001							0.47	0.27-0.82	0.008
Season	Early housing season (Sep-Dec)										1.00	-	-
	Late housing season (Jan-Apr)										0.52	0.31-0.88	0.014
	Pasture (May-Aug)										1.09	0.67-1.77	0.73
Breed	Swedish Red										1.00	-	-
	Swedish Holstein										1.72	1.12-2.66	0.014
	Other										1.88	0.59-6.00	0.28
Teat lesion	No				1.00	-	-	1.00	-	-			
	Yes				2.75	1.07-7.02	0.035	2.66	1.67-4.20	<0.001			
# diagnoses per quarter	1				1.00	-	-	1.00	-	-	1.00	-	-
	2-3				4.61	1.73-12.27	0.002	3.06	1.88-4.98	<0.001	2.44	1.43-4.16	0.001
Daily milk yield (kg) <sup>4</sup>					0.95	0.91-0.99	0.015						

396 <sup>1</sup> Additional candidate variables not indicated in this table were parity and days in milk<sup>3</sup>.397 <sup>2</sup> Additional candidate variable not indicated in this table was # of teats infected with the agent in question.398 <sup>3</sup> Additional candidate variable not indicated in this table was year.

399 <sup>4</sup> Data on daily milk yield and days in milk were not available for all cases. Thus, for models where daily milk yield and days in milk were  
400 significant in the univariate screening, a reduced dataset was used (n=454).

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401 Table 5.  
 402 Distribution of 1056 bacteriological diagnoses (number (n), percentage, 95% confidence  
 403 interval (CI)) from 987 udder quarters from 829 cows with acute clinical mastitis in 2002-  
 404 2003. For comparison, the results (n and %) of a Swedish investigation (n=837 diagnoses)  
 405 performed 1994-1995 (Nilsson et al. 1997) are also presented.

Diagnosis	2002-2003			1994-1995	
	n	%	CI	n	%
<i>Staphylococcus aureus</i>	225	21.3	18.9-23.9	181	21.6
Coagulase-negative staphylococci	65	6.2	4.8-7.8	35	4.2
<i>Streptococcus dysgalactiae</i>	165	15.6	13.5-18.0	113	13.5
<i>Streptococcus uberis</i>	117	11.1	9.3-13.2	128	15.3
<i>Streptococcus agalactiae</i>	6	0.6	0.2-1.3	4 <sup>3</sup>	0.5 <sup>3</sup>
Other streptococci	9	0.9	0.4-1.7	7	0.8
Enterococci	8	0.8	0.4-1.5	7	0.8
<i>Arcanobacterium pyogenes</i>	64	6.1	4.7-7.7	77	9.2
<i>Escherichia coli</i>	168	15.9	13.8-18.3	134	16.0
<i>Klebsiella</i> spp.	44	4.2	3.1-5.6	19	2.2
Other coliform bacteria <sup>1</sup>	9	0.9	0.4-1.7	8	0.8
Other bacteria <sup>2</sup>	16	1.5	0.9-2.5	9	0.9
Contaminated	48	4.5	3.4-6.0	49	5.9
No growth	112	10.6	8.8-12.7	66	7.9

406 <sup>1</sup>*Enterobacter* spp. (4), *Citrobacter freundii* (2), *Serratia* spp. (2), *Proteus mirabilis* (1).

407 <sup>2</sup>*Bacillus* spp. (2), *Corynebacterium* spp.(7), Gram-positive cocci (1), yeast (4), *Pseudomonas*  
 408 *aeruginosa* (1), *Mannheimia haemolytica* (1).

409 <sup>3</sup>Given as  $\beta$ -hemolysing streptococci.

410