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1 **Incidence of mastitis and bacterial findings at clinical mastitis in Swedish primiparous**  
2 **cows – influence of breed and stage of lactation**

3

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18

18 **Abstract**

19

20 Mastitis is a common disease also among primiparous dairy cows. Identification of the  
21 extent and type of problem is important to initiate correct control measures. In Sweden,  
22 unique national production and disease databases are available. The main aim of the study  
23 was to investigate the occurrence of mastitis, measured by the annual incidence of veterinary-  
24 treated clinical mastitis (VTCM) and geometric mean of monthly milk somatic cell count  
25 (SCC) recordings in Swedish primiparous cows in relation to older cows during 2002-2006  
26 with emphasis on breed differences. Other aims were to study differences between  
27 primiparous and older cows in the distribution of bacterial findings at clinical mastitis, and the  
28 occurrence of VTCM and bacterial findings in relation to stage of lactation using data from a  
29 Swedish field study performed 2002-2003. Descriptive statistics and univariable analyses  
30 were used in the investigations. During 2002-2006 approximately 10% of Swedish  
31 primiparous cows experienced VTCM each year, while the geometric mean SCC of Swedish  
32 primiparous cows was approximately 65,000/ml. Both parameters were lower than in older  
33 cows. Primiparous cows of the Swedish Red (SR) breed had better udder health than cows of  
34 the Swedish Holstein (SH) breed. The overall distribution of udder pathogens was similar in  
35 primiparous and older cows. In primiparous cows, most VTCM occurred during the first week  
36 after calving, and *Staphylococcus aureus* and *Streptococcus dysgalactiae* were the most  
37 common udder pathogens during this period. Better control measures directed at these  
38 infections are warranted around calving to reduce the risk of mastitis in primiparous cows.

39

40 **Keywords:**

41 Primiparous cows, mastitis, breed, udder pathogens, stage of lactation.

42

## 43 1. Introduction

44

45 The investments during the upbringing of dairy heifers make the primiparous dairy  
46 cows very valuable. It is therefore essential that they remain healthy to make the investment  
47 worthwhile. Unfortunately, mastitis is a common disease among primiparous cows (Barkema  
48 et al., 1998; Valde et al., 2004; Nyman et al., 2007). Identification of the extent and type of  
49 problem is important to initiate correct control measures against mastitis.

50 When possible, investigating trends in mastitis over time at the national level can give  
51 useful information. In Sweden, approximately 78% of the dairy herds and 86% of the dairy  
52 cows are enrolled in the Swedish official milk-recording scheme (SOMRS; Olsson et al.,  
53 2001). Moreover, only veterinarians are allowed to initiate an antibiotic treatment and every  
54 treatment must be reported to the Swedish animal disease recording system (SADRS;  
55 Emanuelson, 1988). These two systems are linked, which gives unique possibilities for  
56 studies of the dairy cow population.

57 In Sweden, the two main dairy breeds are the Swedish Red (SR) and the Swedish  
58 Holstein (SH), each contributing approximately half of the population (Swedish Dairy  
59 Association, 2006). Breed differences in mastitis incidence have been reported (e.g.  
60 Bendixen, 1988; Elbers et al., 1998), but data on primiparous cows are scarce.

61 To optimise preventive measures for mastitis in primiparous cows, information on risk  
62 periods in relation to calving is important. In previous studies, most cases of clinical mastitis  
63 (CM) in primiparous cows occurred early in lactation (Myllys and Rautala, 1995; Barkema et  
64 al., 1998; Valde et al., 2004; Svensson et al., 2006; Nyman et al., 2007).

65 Knowledge of the spectrum of udder pathogens causing CM in dairy cows is also  
66 important for prevention and control of udder health problems. As primiparous cows often  
67 experience a different environment and management than older cows, especially pre-calving,

68 this may result in a different microbial profile associated with mastitis. The variety of udder  
69 pathogens could also differ depending on stage of lactation.

70 The main aim of this study was to investigate the occurrence of mastitis, measured by  
71 the annual incidence of veterinary-treated clinical mastitis (VTCM), and the geometric mean  
72 of milk somatic cell counts (SCC) at monthly milk recordings, in all Swedish primiparous  
73 cows enrolled in the SOMRS in relation to older cows during the years 2002-2006 with  
74 emphasis on differences between the breeds SR and SH. Other aims were to study differences  
75 in the distribution of bacterial findings at CM between primiparous and older cows, and to  
76 study the occurrence of bacterial findings in relation to stage of lactation using data from a  
77 Swedish field study performed 2002-2003.

78

## 79 **2. Material and Methods**

80

### 81 *2.1. Animals in the national database*

82

83 All cows (340,235 to 380,340 cows/year) included in the SOMRS 2002-2006 were  
84 used when studying occurrence of mastitis in primiparous cows (132,157 to 139,428  
85 cows/year) in relation to older cows, and differences between the breeds SR and SH. Annual  
86 data on VTCM (number of diagnosed cases per 100 cows) and geometric mean milk SCC  
87 emanating from monthly milk recordings were collected (Swedish Dairy Association,  
88 Stockholm). According to the criteria set by the Swedish Dairy Association cases of VTCM  
89 where the same cow was treated again within 2 weeks were not included.

90

### 91 *2.2. Field study on bacteriological findings associated with CM*

92

93 Field practitioners from the whole country were asked to collect a specified number of  
94 milk samples per region and season from cases of CM in 2002-2003. Information on parity  
95 and days in milk was also collected when available. The cows included were geographically  
96 distributed in a manner comparable to the total distribution of cows in Sweden. Only lactating  
97 cows were included in the study. A CM case was defined as a cow with CM not treated earlier  
98 in the lactation and with a composite SCC below 200,000 cells/ml at the previous monthly  
99 milk recording. Quarter milk samples from affected udder quarters were collected aseptically  
100 by the field practitioner. Milk samples (10  $\mu$ l) were directly cultured on 5% bovine blood agar  
101 plates. The agar plates were incubated at 37 °C for 16-24 h, and evaluated in accordance with  
102 the routine of each field veterinarian. All agar plates were sent to the National Veterinary  
103 Institute for bacteriological verification. The growth on the plates was evaluated at the  
104 laboratory and additional tests were performed in accordance with routines at the laboratory.  
105 *Staphylococcus aureus* was determined by means of colony morphology, CAMP-reaction and  
106 coagulase reaction. Other staphylococci were not determined to the species level, but  
107 considered as coagulase negative staphylococci (CNS). For typing of streptococci to the  
108 species level, 12 biochemical reactions (hippurate, esculine, salicine, sorbitol, mannitol,  
109 raffinose, lactose, saccharose, inuline, trehalose, starch and glycerine) and CAMP-reaction  
110 were used. For isolates not confirmed with these methods, Lancefield grouping (Streptex,  
111 Murex Biotech Limited, Dartford, UK) was used. Gram-negative bacteria with typical colony  
112 morphology and positive for p-nitrophenyl- $\beta$ -D-glucopyranosiduronic acid (PGUA) and  
113 indole were considered as *Escherichia coli*. For Gram-negative bacteria that could not easily  
114 be determined as *E. coli*, oxidase reaction and API 20 E (bioMérieux, Craponne, France) or  
115 API 20 NE (bioMérieux, Craponne, France) was used. To confirm growth of  
116 *Arcanobacterium pyogenes* colony morphology, Gram staining and culture in 3% CO<sub>2</sub> was  
117 used. *Bacillus* spp., yeasts and *Corynebacterium* spp. were confirmed by means of colony

118 morphology and Gram staining. The milk samples was considered infected when growth of at  
119 least one colony forming unit (CFU) of *S. aureus*, or at least three CFU of other bacteria were  
120 detected. Milk samples were cultured from a total of 987 udder quarters from 829 dairy cows,  
121 and 1056 bacterial diagnoses were obtained. In 1014 of those information on parity was  
122 available, and 38% (n=385) of those 1014 originated from primiparous cows.

123

### 124 2.3. Statistical analyses

125

126 Descriptive statistics were given for the various studies. Data based on all cows  
127 included in the SOMRS were not analyzed statistically as they represented the whole cow  
128 population. Differences in the distribution of bacterial diagnoses between parities and within  
129 stages of lactation were tested using Chi-square analysis. Significance levels are given in the  
130 results section when appropriate.

131

## 132 3. Results

133

134 During the study period 2002-2006, approximately 10% of all Swedish primiparous  
135 cows enrolled in the SOMRS were veterinary-treated for CM each year, and the geometric  
136 mean SCC of Swedish primiparous cows was approximately 65,000 cells/ml. The annual  
137 incidence of VTCM (Figure 1A) and geometric mean milk SCC (Figure 1B) were lower in  
138 primiparous cows than in older cows. In the same material, primiparous SR cows had lower  
139 incidences of VTCM (Figure 2A) and lower SCC (Figure 2B) than primiparous SH cows.  
140 Similar differences between breeds were observed each year studied.

141 According to the field study, 66% of all VTCM in primiparous cows occurred just  
142 before calving or during the first month of lactation, and a majority of those were found



143 during the first week after calving (Figure 3). The distribution of cases over time differed  
144 between primiparous and older cows. In primiparous cows 65% of the cases occurred -7 to 30  
145 days after calving, while 15 and 20% occurred 31-120 days and >120 days after calving,  
146 respectively. Corresponding figures for older cows were 36, 35 and 28%, respectively. The  
147 distribution of cases differed ( $P<0.01$ ) between primiparous and older cows at the first two  
148 stages of lactation.

149 The most common udder pathogen isolated in cases of CM in primiparous cows was  
150 *S. aureus* followed by *Streptococcus dysgalactiae* and *E. coli*. The overall distribution of  
151 udder pathogens did not differ significantly ( $P>0.05$ ) between primiparous and older cows  
152 (Figure 4). The proportions of the most common bacterial diagnoses in primiparous cows in  
153 different stages of lactation (days -7-30, days 31-120, days >120) are given in Figure 5. A  
154 larger ( $P<0.05$ ) proportion of *S. aureus* CM was observed in the first month after calving  
155 compared to later stages of lactation. In contrast, a lower ( $P<0.01$ ) proportion of *E. coli* CM  
156 was found in early lactation compared to in later stages of lactation. Such a difference  
157 ( $P<0.01$ ) in *E. coli* CM was also observed in older cows when comparing the first month after  
158 calving and 31-120 days after calving (data not shown). The distribution of cases of CM in  
159 primiparous cows with cultures of *S. aureus*, CNS, *A. pyogenes*, *Str. uberis*, *Str. dysgalactiae*,  
160 and *E. coli* per day during the first 30 days after calving is presented in Figure 6. Most of  
161 these CM cases occurred during the first week after calving and *S. aureus* and *Str.*  
162 *dysgalactiae* were the most common pathogens cultured during this period. *A. pyogenes* CM  
163 was mainly found during the first week after calving.

164

#### 165 4. Discussion

166

167 Annually, primiparous cows had fewer cases of VTCM than older cows, which is in  
168 line with a previous Nordic study based on national data from 1997 (Valde et al., 2004).  
169 Although not fully comparable due to different study designs, similar effects of parity have  
170 been found in other studies on CM (e.g. Bendixen et al., 1988; Emanuelson et al., 1993;  
171 Barkema et al., 1998). The SCC was also lower in primiparous cows than in multiparous  
172 cows, which also is consistent with the literature (e.g. Brolund, 1985; Reneau, 1986). The  
173 higher SCC in older cows is mainly due to an increased presence of udder infections (Laevens  
174 et al., 1997; Schepers et al., 1997).

175 Primiparous cows may have a large impact on herd udder health as they represent a  
176 large proportion of the total number of cows. In 2006, primiparous cows constituted  
177 approximately 39% of all Swedish dairy cows (Swedish Dairy Association, 2006). In line  
178 with this, and using herd-level data, Nyman et al. (2007) found a significant association  
179 between a high incidence of VTCM and increasing proportion of primiparous cows in the  
180 herd.

181 The results from the national database as presented here clearly indicated a difference  
182 in primiparous cows between the SR and SH breeds both in clinical (VTCM) and subclinical  
183 (SCC) mastitis, with the SR breed having better udder health. A lower incidence of VTCM in  
184 the SR breed compared to SH breed was also found in earlier studies including Swedish cows  
185 of all parities (Bendixen et al., 1988; Emanuelson et al., 1993). In addition, Nyman et al.  
186 (2007) found that herds comprised mainly of SR cows had fewer VTCM than herds  
187 comprised mainly of SH cows. Moreover, Myllys and Rautala (1995) found a lower incidence  
188 of CM in Ayrshires compared to Friesians in a Finnish study. The differences between SR and  
189 SH cows may be due to the inherent mastitis resistance of each breed and a more efficient  
190 immune defense in the SR cows. Other possible factors of importance are differences in milk  
191 production, SH producing more milk than SR, and differences in management systems in SR-

192 and SH-herds. In Sweden, most cows are housed in tie-stalls, but the proportion of free-stall  
193 housing increases, as does herd size. National data on VTCM and SCC for each management  
194 system was, however, not available at the time of study. In recent Swedish field studies,  
195 however, breed differences in udder health, in favour of the SR breed, have been found in  
196 herds with tie--stalls, and average herd size and milk production (Nyman et al., 2007), as well  
197 as in large herds with high milk production and free-stall housing systems (Nyman et al.,  
198 unpublished data).

199         The results from the field study indicated that the majority of VTCM in primiparous  
200 cows occurred during the first month of lactation, with emphasis on the first week after  
201 calving. This was in line with two previous Swedish field studies on primiparous cows  
202 (Svensson et al., 2006; Nyman et al., 2007) as illustrated in Figure 3. The results are also  
203 supported by earlier studies (Myllys and Rautala, 1995; Barkema et al., 1998; Valde et al.,  
204 2004). During this period the incidence of VTCM was higher in primiparous cows than in  
205 older cows, which has also been reported previously (Barkema et al., 1998; Valde et al., 2004;  
206 Nyman et al., 2007). A high incidence of VTCM in early lactation indicates increased risk for  
207 udder infection, possibly due to impaired immune function in late pregnancy/early lactation.  
208 We have also found that a high overall incidence of mastitis in the herd, and reproductive  
209 disorders (i.e., retained placenta, endometritis, pyometra, dystocia, or twin birth) were  
210 important risk factors for early lactation VTCM in primiparous cows (Svensson et al., 2006).  
211 Further studies on factors affecting udder health of Swedish primiparous cows in early  
212 lactation are ongoing.

213         The range of udder pathogens isolated in cases of CM did not differ between  
214 primiparous and older cows despite likely differences in environment and management of  
215 these groups. The distribution of udder pathogens in primiparous cows differed, however,  
216 depending on stage of lactation, with a higher proportion of *S. aureus* being cultured during

217 the first month after calving than later in lactation. Overall, *S. aureus* and *Str. dysgalactiae*  
218 were the most common udder pathogens in primiparous cows, followed by *E. coli* and *Str.*  
219 *uberis*. The same relationship among pathogens was found during the first week after calving,  
220 when most cases of mastitis occurred. This result differs from a previous Swedish study on  
221 clinical mastitis in Swedish heifers where *Str. dysgalactiae* was the pre-dominant organism  
222 cultured and *S. aureus* was less common (Jonsson et al., 1991). Moreover, in the present  
223 study, *S. aureus* was the most common finding during weeks 2-4 after calving. In a  
224 Norwegian study, *S. aureus* was the most common finding in clinical mastitis in primiparous  
225 cows (Waage et al., 1999), while *E. coli* and *Str. uberis* dominated in a recent Estonian study  
226 (Kalmus et al., 2006). The high incidence of CM during the first week after calving indicates  
227 that udder infections occurred mainly before or at calving. Udder colonizations and infections  
228 with *S. aureus* have been reported in heifers already before first breeding or early in  
229 pregnancy (Trinidad et al., 1990). In a Danish study (Aarestrup and Jensen, 1997), however,  
230 infections with *S. aureus* rarely occurred in heifers before parturition, but their numbers  
231 increased during the first week after calving. In the same study, *Str. dysgalactiae* infections  
232 were mainly found one week before and one week after calving. A number of risk factors for  
233 *S. aureus* infections in heifers have been reported such as colonization on teat skin or in the  
234 inguinal area, transmission with flies, keeping young stock with older cows, and milking fresh  
235 heifers after older cows (Sears and McCarthy, 2003). Little is known, however, about risk  
236 factors for udder infections with *Str. dysgalactiae*. Barkema et al. (1999), however, found a  
237 strong correlation between occurrence of clinical mastitis caused by *S. aureus* and *Str.*  
238 *dysgalactiae*, and between risk factors for clinical mastitis caused by these two pathogens  
239 indicating similar epidemiological patterns. More research is needed on effective control  
240 measures for *Str. dysgalactiae* mastitis.

241 Studies on CM are difficult to compare due to differences in study design and  
242 definition of disease. In the present material CM was defined as VTCM, i.e. clinical cases  
243 treated by a veterinarian, which is different from actual registration of cases of CM in the  
244 herds. Moreover, in the study on bacteriological findings other inclusion criteria were also  
245 used to select acute cases of CM. The reason for using VTCM and not CM was that records of  
246 VTCM are easily available through the national database. Both VTCM and CM depend on the  
247 actual observation of a case of mastitis. Thus, both the nature of the case and the awareness of  
248 the farmer/worker can influence the number of cases being observed and reported.  
249 Management decisions also have a strong influence on the incidence of VTCM (Nyman et al.,  
250 2007). According to a recent study on validity of the database, the farmers contacted a  
251 veterinarian for treatment of CM in 78% of all cases observed at the farm (Mörk et al.,  
252 unpublished). Based on interviews of veterinary practitioners and farmers it is estimated,  
253 however, that the willingness of farmers to call a veterinarian if a primiparous cow  
254 experiences CM is higher than if an older cow experiences CM (Persson Waller, unpublished  
255 data). Thus, the VTCM data likely include the majority of cases of CM in primiparous cows.  
256 The overall incidence of VTCM for primiparous cows was within the range previously  
257 reported for primiparous cows from the Finnish official milk- and health-recording programs,  
258 but lower than the corresponding figures from Norway and Denmark (Valde et al., 2004).

259

## 260 **5. Conclusions**

261

262 Approximately 10% of Swedish primiparous cows are veterinary-treated for clinical  
263 mastitis each year, and the geometric mean SCC of primiparous cows is approximately  
264 65,000/ml. Both numbers are clearly lower than in older cows. Primiparous SR cows have  
265 fewer VTCM and lower SCC than SH cows. In primiparous cows, a large proportion of cases

266 of CM occurred during the first week after calving, and *S. aureus* and *Str. dysgalactiae* were  
267 the most common udder pathogens during this period. Better control measures are warranted  
268 just before and after calving to reduce the risk of mastitis in primiparous cows.

269

#### 270 **Conflict of interest**

271

272 None of the authors (K. Persson Waller, B. Bengtsson, A. Lindberg, A. Nyman, H.  
273 Ericsson Unnerstad) has a financial or personal relationship with other people or  
274 organizations that could inappropriately influence or bias the paper entitled “Incidence of  
275 mastitis and bacterial findings at clinical mastitis in Swedish primiparous cows – influence of  
276 breed and stage of lactation”.

277

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279

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282

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- 339



339 **Figure captions**

340

341 Figure 1. A) Incidence of veterinary-treated clinical mastitis (VTCM; cases per 100 cows),  
342 and B) geometric mean milk SCC, for lactation numbers 1, 2, 3, 4, >4, and all cows,  
343 respectively, in all dairy cows enrolled in the Swedish milk recording scheme 2002-2006.

344

345 Figure 2: A) Incidence of veterinary-treated clinical mastitis (VTCM; cases per 100 cows),  
346 and B) geometric mean milk SCC, in all primiparous cows of the breeds SR (□) and SH (■)  
347 enrolled in the Swedish milk recording scheme 2002-2006.

348

349 Figure 3. Proportions of cases of veterinary-treated clinical mastitis (VTCM) in primiparous  
350 cows in different stages of lactation (up to 305 days in milk) in a field study (n=364 cases)  
351 performed 2002-2003 compared to two Swedish field studies performed 1997-2001  
352 (Svensson et al., 2006 (n=256 cases); Nyman et al., 2007 (n=360 cases)).

353

354 Figure 4. Proportions (%) of bacterial findings in milk samples from cases of clinical mastitis  
355 in primiparous (n=385) and older (n=629) cows.

356

357 Figure 5. Distribution of the most common (>5% of the cases) bacterial findings in milk  
358 samples from cases of clinical mastitis in primiparous cows within different stages of  
359 lactation (-7-30, 31-120, and >120 days in milk). \* Significantly ( $P<0.05$ ) different from the  
360 other stages of lactation within bacterial species.

361

362 Figure 6. Proportional distribution of findings of A) *Staphylococcus aureus* (n=57),  
363 coagulase-negative staphylococci (CNS; n=18), and *Arcanobacterium pyogenes* (n=21), and

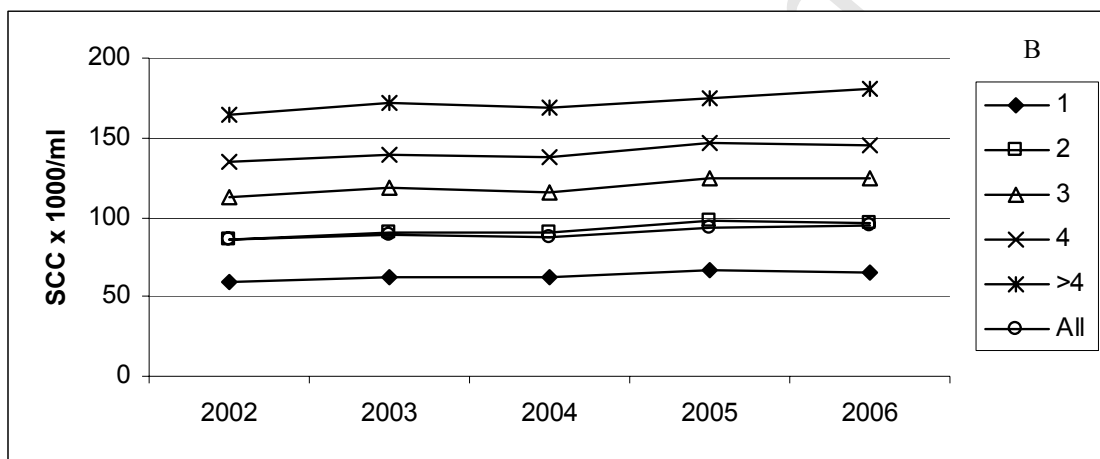
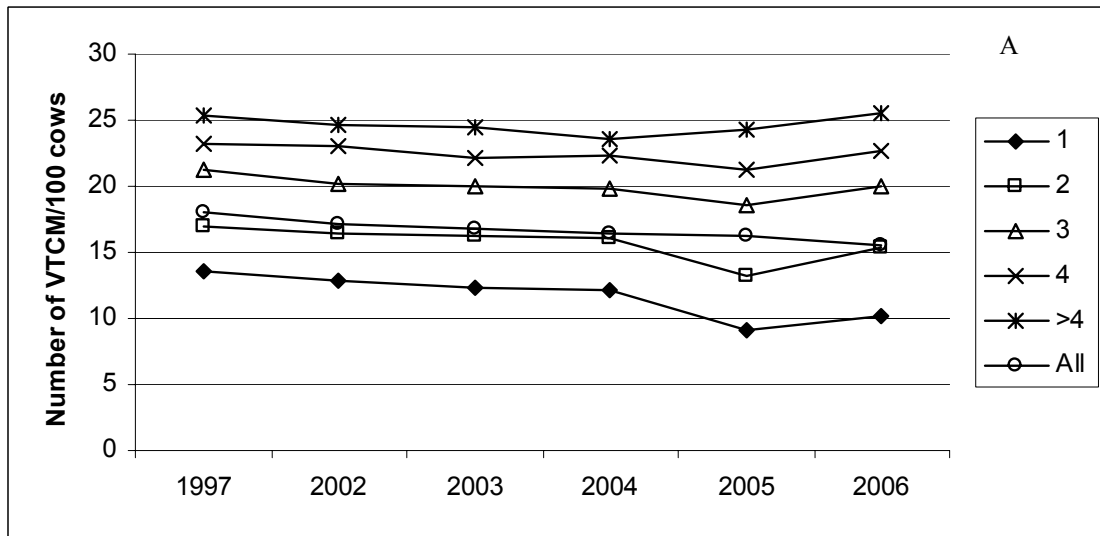
364 B) *Streptococcus uberis* (n=30), *Streptococcus dysgalactiae* (n=49) and *Escherichia coli*  
365 (n=25) during the first 30 days after calving in cases of clinical mastitis (CM) in primiparous  
366 cows. No cases occurred before calving.

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367 **Figures**

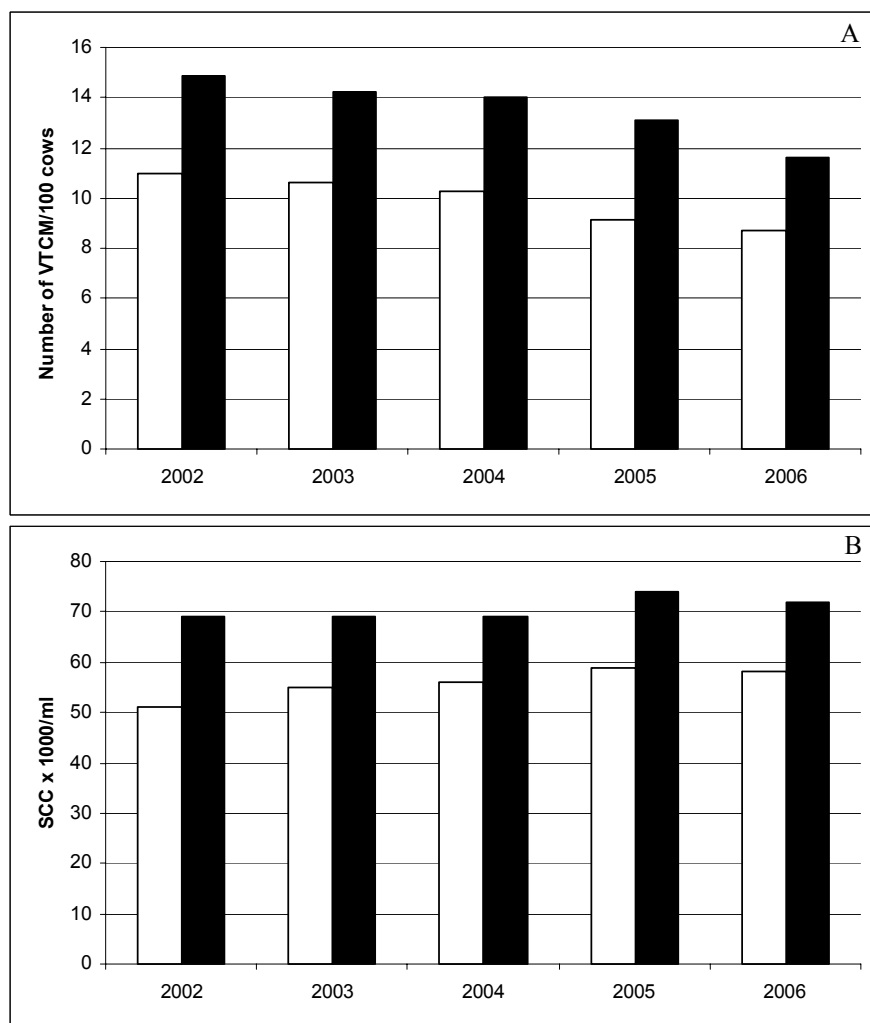
368 Figure 1

369



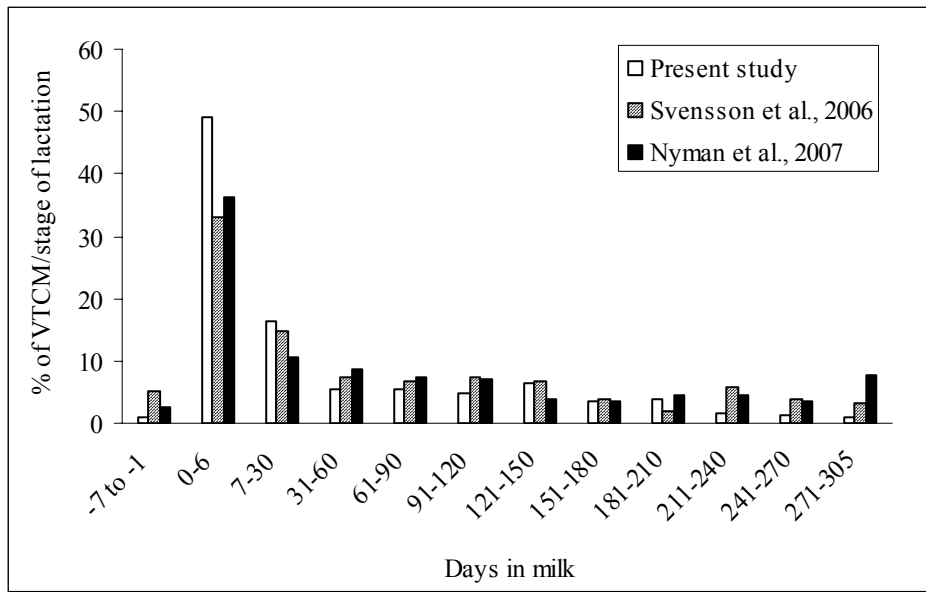
369 Figure 2

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370 Figure 3

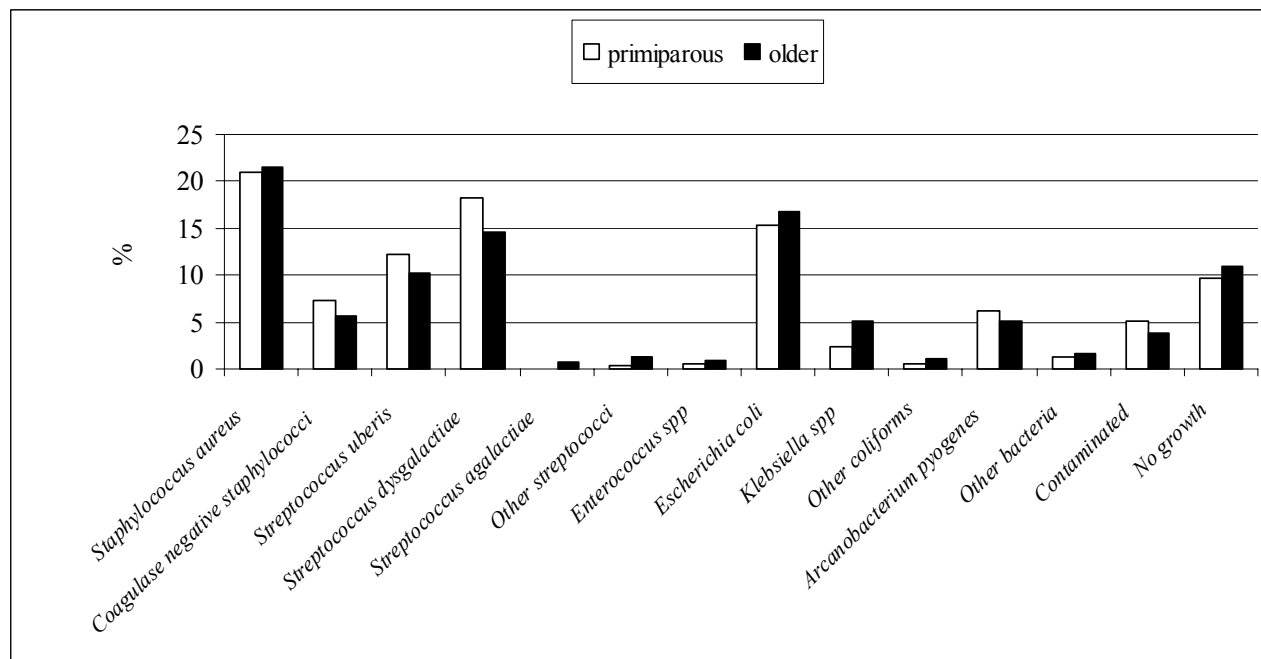
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371 Figure 4.

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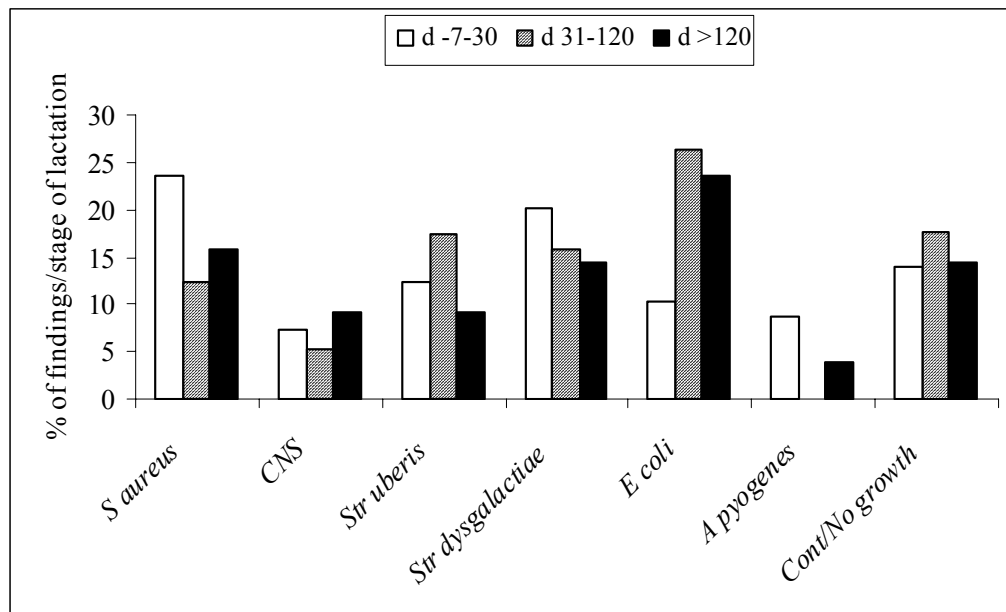


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373 Figure 5

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375 Figure 6

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