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

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Abstract

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

Keywords:

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

1. Introduction

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

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

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

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

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

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

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

2. Material and Methods

2.1. Animals in the national database

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

2.2. Field study on bacteriological findings associated with CM

Field practitioners from the whole country were asked to collect a specified number of milk samples per region and season from cases of CM in 2002-2003. Information on parity and days in milk was also collected when available. The cows included were geographically distributed in a manner comparable to the total distribution of cows in Sweden. Only lactating cows were included in the study. A CM case was defined as a cow with CM not treated earlier in the lactation and with a composite SCC below 200,000 cells/ml at the previous monthly milk recording. Quarter milk samples from affected udder quarters were collected aseptically by the field practitioner. Milk samples (10 µl) were directly cultured on 5% bovine blood agar plates. The agar plates were incubated at 37 ºC for 16-24 h, and evaluated in accordance with the routine of each field veterinarian. All agar plates were sent to the National Veterinary Institute for bacteriological verification. The growth on the plates was evaluated at the laboratory and additional tests were performed in accordance with routines at the laboratory. *Staphylococcus aureus* was determined by means of colony morphology, CAMP-reaction and coagulase reaction. Other staphylococci were not determined to the species level, but considered as coagulase negative staphylococci (CNS). For typing of streptococci to the species level, 12 biochemical reactions (hippurate, esculine, salicine, sorbitol, mannitol, raffinose, lactose, saccharose, inuline, trehalose, starch and glycerine) and CAMP-reaction were used. For isolates not confirmed with these methods, Lancefield grouping (Streptex, Murex Biotech Limited, Dartford, UK) was used. Gram-negative bacteria with typical colony morphology and positive for p-nitrophenyl-β-D-glucupyranosiduronic acid (PGUA) and indole were considered as *Escherichia coli*. For Gram-negative bacteria that could not easily be determined as *E. coli*, oxidase reaction and API 20 E (bioMérieux, Craponne, France) or API 20 NE (bioMérieux, Craponne, France) was used. To confirm growth of *Arcanobacterium pyogenes* colony morphology, Gram staining and culture in 3% CO_2 was used. *Bacillu*s spp., yeasts and *Corynebacterium* spp. were confirmed by means of colony

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

2.3. Statistical analyses

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

3. Results

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

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

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

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

4. Discussion

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

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

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

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

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

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

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

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

5. Conclusions

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

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

Conflict of interest

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

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339

Figure captions

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

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

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

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

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

Figure 6. Proportional distribution of findings of A) *Staphylococcus aureus* (n=57), 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.

367 **Figures**

368 Figure 1

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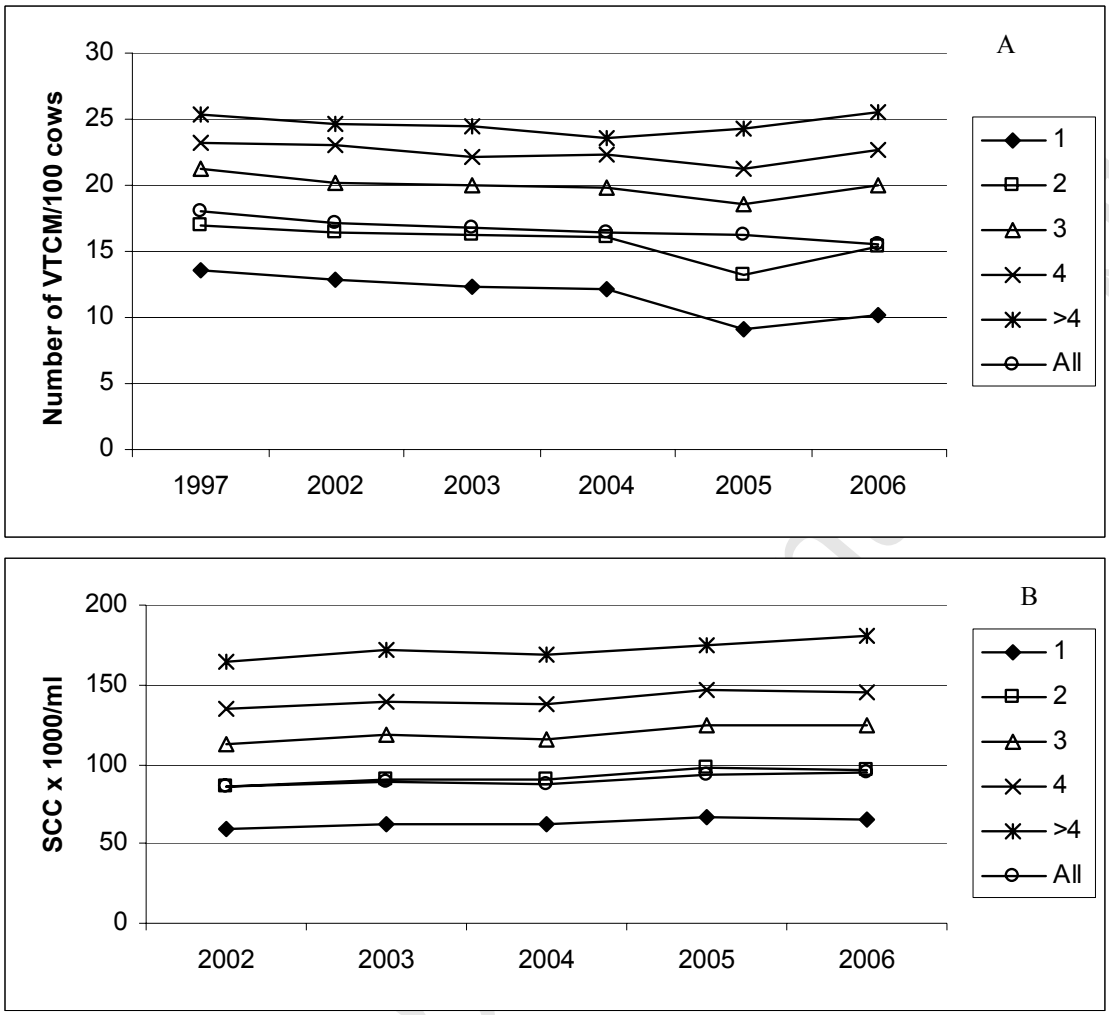

369 Figure 2

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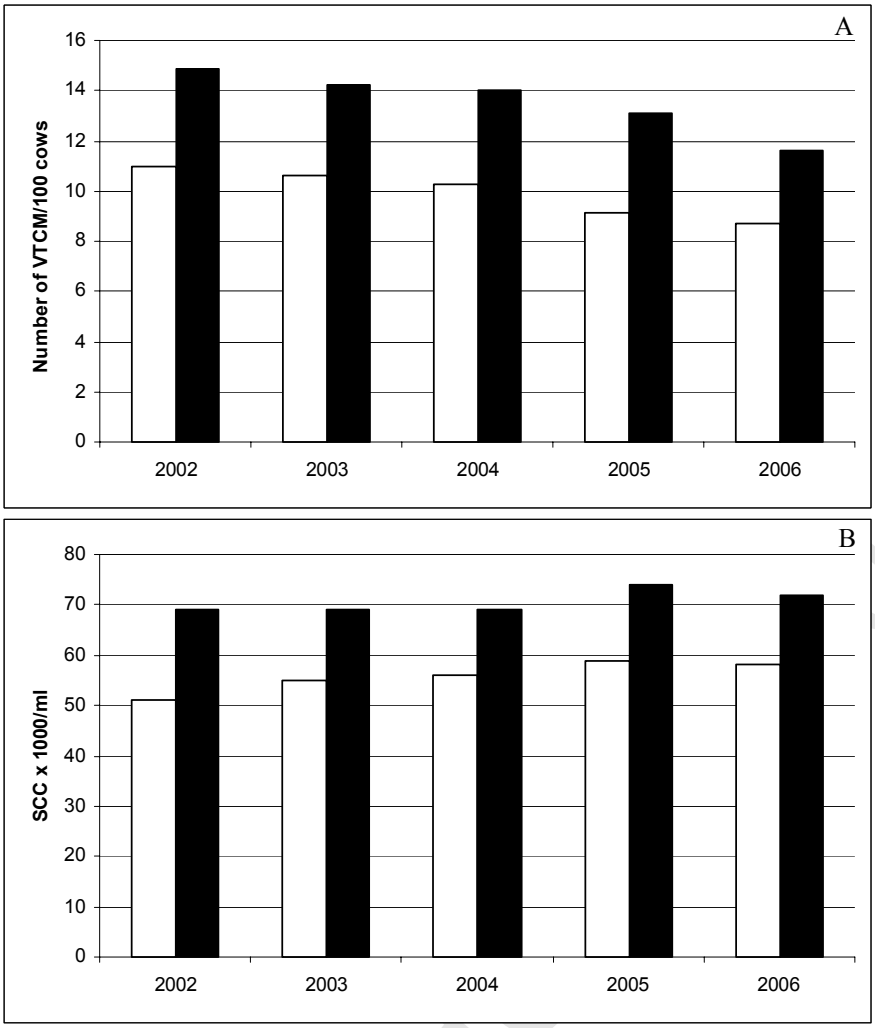

370 Figure 3

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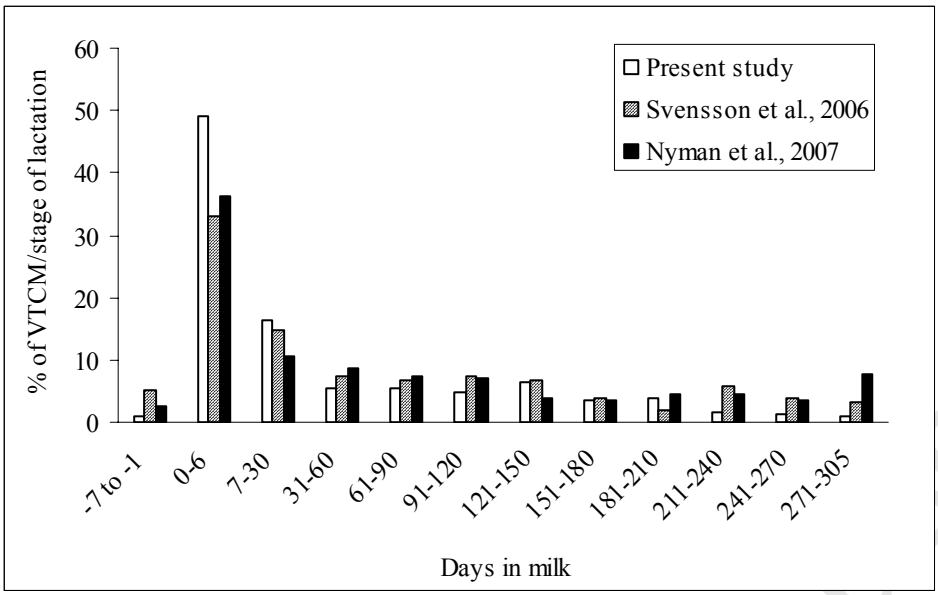

371 Figure 4.

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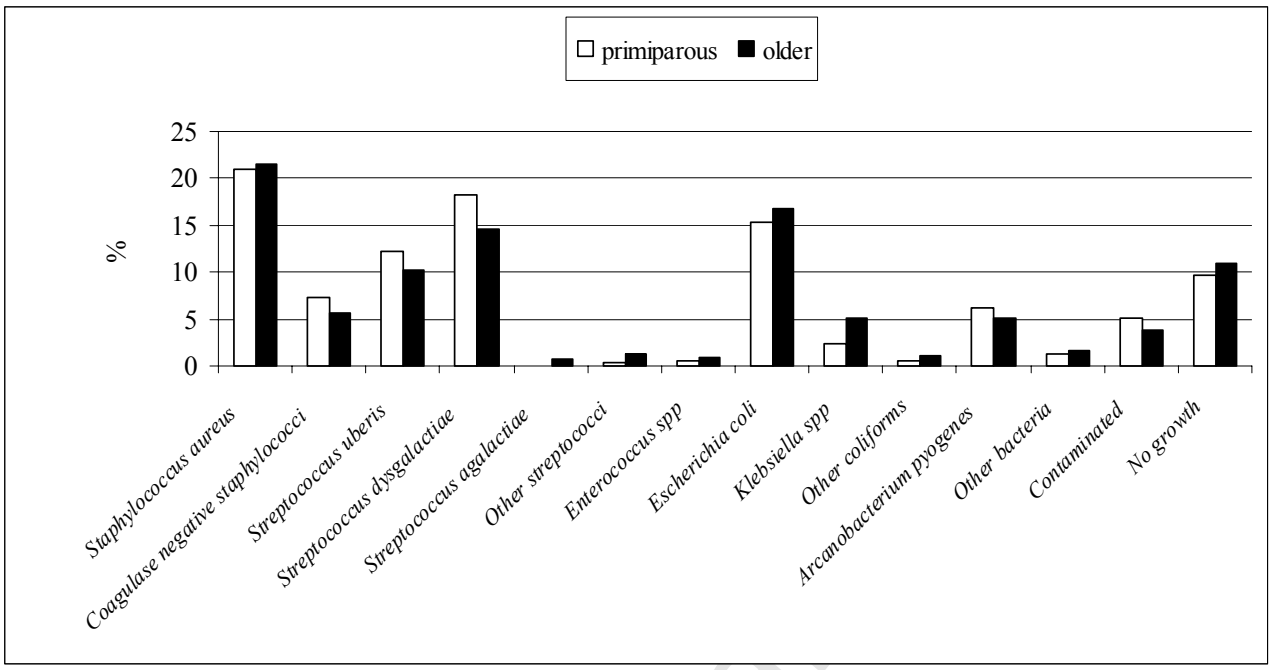

373 Figure 5

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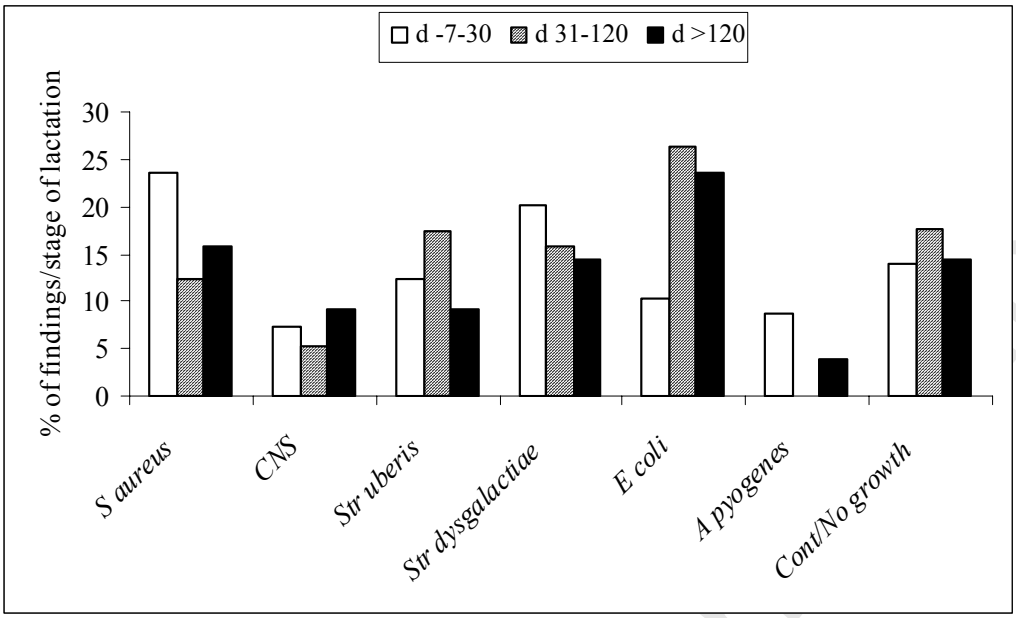

375 Figure 6

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