Journal of Dairy Research (2012) **79** 304–309. doi:10.1017/S0022029912000222

Risk factors for intramammary infections and subclinical mastitis in post-partum dairy heifers

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Received 28 February 2011; accepted for publication 1 March 2012; first published online 7 June 2012

The prevalence of intramammary infections (IMI) and subclinical mastitis (SCM) in 436 German Holstein heifers was put in relation with clinical findings of the udder and data regarding individual rearing and housing conditions of the animals. The clinical examination took place on the day of the livestock auction (at approximately 41 d in milk, DIM). On that day, 31% of the heifers had IMI in at least one quarter, and 18% of all quarters were infected. Coagulase-negative staphylococci were the most prevalent bacteria isolated, accounting for 68% of the positive samples. Data were analysed by logistic regression. Criteria such as 'juvenile intersucking', 'teats shorter than 35 mm', 'teats with a diameter <18 mm' and 'udder oedema at the day of the auction' were associated with IMI in heifers during the first 41 DIM. Loose-housing systems during pregnancy (as opposed to tie-stalls), juvenile intersucking, clinical mastitis during the first week after calving, teat diameters <18 mm, and employing organic bedding material in the stables before calving were associated with subclinical mastitis.

Keywords: Heifer, mastitis, risk factor, auction.

Mastitis in dairy heifers has been studied increasingly over the last few years since the economic damage associated with this disease has been proven to be significant, and several studies have found a high prevalence of subclinical and clinical intramammary infections (IMI) in heifers (Aarestrup & Jensen, 1997; Bareille et al. 2000; De Vliegher et al. 2005; Reinecke et al. 2006). Infected heifers may represent an important reservoir of pathogens for the dairy herd (Waage et al. 1999).

Three major pathogenic pathways have been described so far for heifer mastitis: first, calves and heifers sucking on each others' teats can affect the development of the juvenile udder ('juvenile intersucking'). This, in conjunction with the transmission of mastitis pathogens (e.g. *Streptococcus agalactiae*) is prone to lead to heifer mastitis (Schalm, 1942). Second, it has been proven that stable flies can transmit pathogens such as *Staphylococcus aureus* from lactating or dry cows to non-lactating heifers (Nickerson et al. 1995). Third, a high proportion of teat canals already open several months before calving. This opening of the teat canal before calving is an important factor in the aetiology of heifer mastitis (Krömker & Friedrich, 2009). A series of risk factors for both clinical (CM) and subclinical mastitis (SCM) in dairy heifers have already been identified. They comprise factors affecting the entire herd (e.g. season and climate; Fox et al. 1995; Hallberg et al. 1995; De Vliegher et al. 2001) but also those associated specifically with management peripartum e.g. insufficient fly control and poor hygiene in the calving area (Bareille et al. 2000; Reinecke et al. 2006). Finally, animal and teat-related risk factors such as open teat canals before calving or an advanced age at first calving (Nickerson et al. 1995; Bassel et al. 2003; Krömker & Friedrich, 2009) must also be considered.

Several authors demonstrated that the prevalence of IMI increased as the calving date approaches and that it was highest during the last trimester of pregnancy. This suggests that heifers may be most susceptible during that period of gestation. Most likely this circumstance is associated with

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the rapid mammary gland development during that time period (Oliver & Mitchell, 1983; Fox et al. 1995; Aarestrup & Jensen, 1997). An increased age at first calving is a significant risk factor for IMI by *Staph. aureus* and environmental pathogens (Bassel et al. 2003).

Reported IMI prevalence at quarter level ranges from 29 to 86% before calving and from 18 to 55% at calving (Meaney, 1981; Oliver & Mitchell, 1983; Trinidad et al. 1990; Pankey et al. 1991; Roberson et al. 1994; Fox et al. 1995; Myllys, 1995; Aarestrup & Jensen, 1997). These authors detected IMI in heifers, particularly those due to coagulase-negative staphylococci (CNS), *Staph. aureus* and environmental pathogens, in the time close to parturition. IMI at calving increased the risk of CM within the first week after calving. Mastitis prior to calving and mastitis within the first week post partum increased the risk of further cases of CM and culling during the first 45 d of lactation (Edinger et al. 1999). In most cases, the number of infected quarters decreases markedly after parturition. After several weeks, only a few quarters remain infected, but these are responsible for clinical cases during the rest of the first lactation and for repeated mastitis cases in the subsequent lactation (Krömker & Friedrich, 2009).

The objective of this study was to evaluate the distribution of mastitis pathogens in dairy heifers in the timeframe between 4 and 8 weeks after calving and to determine animal and management-related variables associated with IMI and SCM.

Materials and Methods

This cross-sectional study was conducted at three auction places in Lower Saxony (Verden, Uelzen, Osnabrück). The animals traded there were dairy heifers in their first months of lactation and on the basis of milk yield and health status, probably extraordinary within their age cohorts. At these three auction places, more than 95% of German Holstein heifers available in Lower Saxony auctions (approx. 600) are traded at regular monthly intervals. On the days of the auctions between March 2003 and February 2004, a total of 512 German Holstein heifers were randomly selected. On an auction day, only one animal per herd was included in the study to avoid confounding, and so a farm could participate only once in this evaluation. If a farmer offered more than one animal on a given auction day, randomization was achieved by selecting the cow with the lowest auction number. A documented yield of >30 kg of fat- and protein-corrected milk per day were preconditions for an animal to be part of this survey, as animals without this milk yield were not considered marketable by the traders. The number of animals examined on an auction day was determined by using a simple computerized randomization list, based on the auction number. Participation in this trial was optional. All heifers had been evaluated at least once in relation to their production parameters by the local (Lower Saxony) dairy herd improvement association. Data regarding udder health history, previous diseases and treatments of a given animal, as well as management practices and housing and rearing conditions of heifers were obtained by interviewing the owners; a questionnaire covering 14 items supported this interview. Before the auction, a clinical examination took place; it comprised the udder conformation and eventual pathomorphological findings (hyperkeratosis, injuries, skin modifications). This clinical examination was conducted based on the methods described by Rosenberger (1979) and Mein et al. (2001). The data obtained from the interviews and the clinical examination of the heifers were encoded binomially. The list of questions that could be answered either positively or negatively is presented in Tables 3 & 4.

Aseptic duplicate quarter foremilk samples were collected for cyto-microbiological diagnosis from the front right and the hind left quarters owing to the limited time available for individual testing at the auctions. Teat ends were cleaned and disinfected with ethanol (70%) before sampling. Strict foremilk (first jets) was discharged, and then two 10-ml samples of milk were collected aseptically from the udder quarters into sterile vials. Samples were kept at 4 °C until cyto-microbiological examination took place.

Laboratory procedures

For culturing, 10 µl of each milk sample was spread on blood agar plates (5% defibrinated sheep blood, Oxoid, Wesel, Germany). The plates were incubated aerobically at 37 °C and examined after 24 h and 48 h. Colonies were provisionally identified on the basis of Gram stain, morphology and haemolysis patterns, and the numbers of each colony type were recorded. Representative colonies were then subcultured on blood agar plates and incubated aerobically at 37 °C for 24 h to obtain pure cultures. Catalase and coagulase production was tested for Gram-positive cocci. Specific identification of staphylococci was done using the coagulase test. Gram-positive, catalase-negative isolates were tested by CAMP, aesculin-reaction, growth at 45 °C, and commercial micromethods (Oxoid DR0575M Strep Plus Kit, Germany). Some Gram-positive rods were identified with simple procedures (Gram-staining, cell morphology, catalase test), e.g. *Corynebacterium bovis*. Gram-negative rods were subcultured on Violet Red Bile Agar, tested afterwards for oxidase and indole-reaction and additionally cultured in triple sugar iron agar and Simmons citrate agar. This method allowed identification of bacteria at genus or species level in most cases; otherwise, unidentified organisms were recorded as either Gram-negative or Gram-positive.

Culture status of milk samples was defined according to the procedures recommended by the German Veterinary Association (GVA, 2002). A milk sample was defined as being contaminated if >3 bacterial species were isolated. The somatic cell count (SCC) of every milk sample was determined by fluorescence (Fossomatic 360, Foss Electric, Hillerød, Denmark).

https://doi.org/10.1017/S0022029912000222 Published online by Cambridge University Press

Definition of udder health

Udder health categorization as recommended by IDF and cited by GVA (2002; i.e. the presence or absence of pathogenic bacteria and a threshold of 100 000 somatic cells/ml quarter foremilk sample) started on quarter level. Based on this, the corresponding cows were categorized accordingly. IMI was recorded if in duplicate samples >500 CFU/ml of the same bacterial species were cultured, and 1–3 bacterial species could be isolated. Quarters with only one bacteriological positive sample were considered to be non-infected. We defined a quarter as subclinically mastitic (SCM) when its SCC ranged >100 000/ml (foremilk samples in duplicate) and it did not display any signs of CM. Following IDF definitions, bacteriological findings and SCC were analysed separately, because on one hand, finding pathogens in the milk of an udder quarter relates only to a (latent) infection. On the other hand, increased cell counts are a sign of an inflammatory reaction that may have been produced by a pathogen that is no longer detected.

Statistical analysis

Data were gathered and analysed using the programs Excel, Access 2000 (Microsoft Corporation), and SPSS (SPSS 13.0, Chicago IL, USA). The udder quarter was the statistical unit. The associations between IMI and SCM respectively and morphological, pathomorphological or management variables (covariates, factors) were analysed using logistic regression procedures (Urban, 1993). Binary dichotomous dependent variables comprised 'IMI/no IMI' and 'SCM/no SCM' in one quarter. Independent explanatory variables that were graded at more than two levels in the questionnaire or the clinical protocol were dichotomized and coded as 0 or 1. The relationships between animal history, management and clinical examination, and IMI or SCM were tested using Student's t test for continuous measurements and the χ^2-test (likelihood ratio statistic) for proportions in a first step. However, predictors that strongly correlated with each other ($r>0{\cdot}70$) were not included in the same model to avoid multicollinearity. As a second step, variables that were associated with the outcome variables at $P<0{\cdot}10$ in Student's t test for continuous measurements and the χ^2-test for proportions were included in binary logistic regressions with IMI and SCM as the binary outcome. A forward stepwise process was used for final model selection, applying a P value $<0{\cdot}05$ for inclusion. We used likelihood-ratio tests for significance test to include predictors. Goodness of fit of models was assessed by the Hosmer–Lemeshow goodness of fit statistics (Hosmer & Lemeshow, 2000). The predictive power of a model was measured by a rescaled pseudo R^2 with the maximum of 1 (Nagelkerke, 1991).

Odds ratios (OR) with 95% confidence intervals (95% CI) were calculated. Statistical significance was assumed at $P \leqslant 0{\cdot}05$.

Table 1. Culture results by organism

Organism	Front quarters (%)	Rear quarters (%)	Total (%)
No growth	361 (83)	353 (81)	714 (82)
CNS	52 (12)	56 (13)	108 (12)
Stretococcus uberis	6 (1)	13 (3)	19 (2)
Mixed (with CNS)	6 (1)	5 (1)	11 (1)
Staphylococcus aureus	4 (1)	2 (0)	6 (1)
Corynebacterium bovis	4 (1)	3 (1)	7 (1)
Other	3 (1)	4 (1)	7 (1)
Total	436	436	872

Results

The animals used for this survey originated from dairy herds in Lower Saxony and were 2·8 years old (mean value) on the date of the auctions. On average they had been in milk for 40·7 ± 12·4 d. The material available for analysis included 436 heifers with complete data sets (milk samples, clinical examinations and questionnaire-based interviews); in the other cases, data sets were incomplete as data were raised during the ongoing auctions, and these animals were brought to auction before data raising could be completed. 31% of heifers ($n=136$) had IMI on the day of the auction in at least one quarter. 18% ($n=158$) of quarters were infected. CNS were the most prevalent bacteria isolated, accounting for 68% of positive samples. After CNS, environmental streptococci were most frequent, followed by mixed infections (CNS and streptococci) and *Staph. aureus* (Table 1). SCM on one or two quarters at the auction was diagnosed in 16% of heifers ($n=68$).

The distribution of clinical examination variables and management variables across the categories IMI and SCM is presented in Tables 2–4. Some variables were correlated with IMI and SCM. Short front teats and a higher calving age were associated with IMI and SCM. Increased IMI were particularly observed in teats shorter than 35 mm and with diameters <18 mm. The thresholds of 35 mm in teat length and 18 mm in teat diameter were chosen because they represented the quartile of German heifers with shortest and thinnest teats (Krömker & Grabowski, 2002). Chronical ring formation and necrotic dermatitis ('foul udder') were more frequent in heifers with IMI on the day of the auction. Heifers with SCM at the auctions had been kept in freestall barns and on organic bedding material during gestation more often than non-infected heifers. Furthermore these heifers had had more cases of CM during the first 5 d after calving or during the entire time before the auction. When known cases of juvenile intersucking in the breeding group had occurred, the animals subjected to this condition were more likely to develop IMI and SCM.

Table 5 provides the final logistic regression models for IMI and SCM as dependent variables. The rescaled R^2 of the final models were 0·248 and 0·277 for IMI and SCM, respectively. The goodness of fit statistic did not give any

https://doi.org/10.1017/S0022029912000222 Published online by Cambridge University Press

Table 2. Continuous variables

	IMI		SCM		
Variable (mean ± SD)	Yes (n = 136)	No (n = 300)	Yes (n = 68)	No (n = 368)	Total (n = 436)
Length front teats, mm	47·6 ± 9·7†	49·6 ± 8·7	45·5 ± 10·7‡	49·6 ± 8·6	49·0 ± 9·1
Diameter front teats, mm	21·9 ± 6·0	22·4 ± 4·5	22·0 ± 7·9	22·2 ± 4·3	22·2 ± 5·0
Length rear teats, mm	40·3 ± 8·1	40·9 ± 7·8	39·3 ± 9·3	41·0 ± 7·6	40·7 ± 7·9
Diameter rear teats, mm	20·3 ± 4·2	21·7 ± 4·1	20·2 ± 4·9	21·5 ± 4·0	21·3 ± 4·2
First calving age, d	951 ± 125†	892 ± 101	932 ± 111	906 ± 112	910 ± 112

† = Different (P < 0·05) from animals without IMI (intramammary infections)
‡ = Different (P < 0·05) from animals without SCM (subclinical mastitis)

Table 3. Distribution of clinical examination variables in animals (% animals)

	IMI		SCM		
Variable description	Yes (n = 136)	No (n = 300)	Yes (n = 68)	No (n = 368)	Total (n = 436)
Udder shape (not normal)†,¶	39·7	44·7	38·2	44·0	43·1
Teat shape (not normal)	32·4‡	42·7	41·2	39·1	39·4
Teat tip shape (not normal)	48·5	50·3	48·5	50·0	49·8
Teat alignment (oblique)	20·6	18·7	20·6	19·0	19·3
Udder oedema (present)	27·9	20·0	26·5	21·7	22·5
Necrotic dermatitis (present)	7·4‡	2·7	5·9	3·8	4·1
Cricoid ring impairment (present)	4·4‡	1·3	2·9	2·2	2·3
Hyperkeratosis (> white ring)	4·4‡	8·7	0	8·7	7·3
Teat length < 35 mm	11·8‡	4·7	20·6	4·3	6·9
Teat diameter < 18 mm	17·6‡	9·3	17·6§	9·3	11·9

† = Percentage of animals
‡ = Different (P < 0·05) from animals without IMI (intramammary infections)
§ = Different (P < 0·05) from animals without SCM (subclinical mastitis)
¶ = Clinical examination based on the methods described in Rosenberger (1979) and Mein et al. (2001)

reason to doubt the validity of the models (IMI: P = 0·979; SCM: P = 0·292). Significant risk factors for IMI identified by the final logistic regression model were 'juvenile intersucking', 'teats shorter than 35 mm', 'teat diameter < 18 mm', 'udder oedema (at the time of examination)'.

Significant risk factors for SCM identified by the final logistic regression model were 'freestall pens during gestation', 'juvenile intersucking', 'CM during the 1st week after calving', 'teat diameter < 18 mm', 'organic bedding material in the stable before calving'.

Discussion

As stated before (Krömker & Friedrich, 2009) quarters with IMI during the first weeks after calving are prone to develop CM both afterwards and repeatedly. This is why the present study focused on the association among these variables (morphological, pathomorphological and management variables) and the occurrence of IMI and SCM during early lactation, exemplified by choosing the day of the auction. As a second goal, it was intended to define criteria to easily identify animals prone to develop IMI and SCM on the day of the auction.

This paper is based on a selection of animals from a single age cohort. Heifers destined to be sold during an auction have to be clinically healthy and belong, in terms of their milk yield, to the top flight of their age cohort. We accepted this selection bias deliberately in order to examine the conditions for better heifers in better-managed farms. All farmers who brought animals to the auction gladly participated in this survey. The data obtained were not passed on to potential buyers. Only two farmers refused to participate, so that the effect by omitting non-responders was irrelevant. Therefore we assume that our results may be transferable to other high-yielding animals. Since the interviewer himself may also affect the answers (Schukken et al. 1989), all questionnaires in this study were conducted by one and the same person.

By selecting one front and one rear quarter, the different probabilities of getting infected are in fact considered. The observed array of mastitis pathogens corresponds largely to bacteriological distributions reported by Oliver & Mitchell (1983), Trinidad et al. (1990), Pankey et al. (1991), Roberson et al. (1994), Fox et al. (1995), Myllys (1995) and Aarestrup & Jensen (1997).

https://doi.org/10.1017/S0022029912000222 Published online by Cambridge University Press

Table 4. Distribution of management practice and animal variables in the animals' history (% animals)

	IMI		SCM		
Variable description	Yes ($n=136$)	No ($n=300$)	Yes ($n=68$)	No ($n=368$)	Total ($n=436$)
Pasturing (1st half of gestation)†	47·1	41·3	44·1	42·9	43·1
Pasturing (2nd half of gestation)	25·0	30·0	23·5	29·3	28·4
Keeping indoors (entire gestation)	85·3	79·3	94·1§	78·8	81·2
Cubicles with organic bedding material	51·5	45·3	58·8§	45·1	47·2
Warts on teat and/or udder before calving	1·5	4·0	2·9	3·3	3·2
Known cases of juvenile intersucking in breeding group	7·4‡	1·3	8·8§	2·2	3·2
Udder and/or teat wounds	0	1·3	0	1·1	0·9
Milk leakage pre-calving	4·4	4·0	2·9	4·3	4·1
CM¶ case in the first week of lactation	5·9	6·0	11·8§	4·9	5·0
CM >1st week of lactation	7·4	4·0	11·8§	3·8	5·0
Blocked milk secretion during the first milkings	7·4	5·3	8·8	5·4	6·0
Oxytocin administration necessary for the first milkings	4·4	6·0	5·9	5·4	5·5
Frightful or insubordinate	13·2	12·0	17·6	11·4	12·4
Dry cow treatment pre-calving	4·4	6·0	2·9	6·0	5·5

† Percentage of animals
‡ Different ($P<0·05$) from animals without IMI (intramammary infections)
§ Different ($P<0·05$) from animals without SCM (subclinical mastitis)
¶ Clinical mastitis

Table 5. Final logistic regression models for the probability of a quarter ($n=872$) to acquire an intramammary infection or a subclinical mastitis during the first 41 d of lactation

Variable	β	SE (β)	*P*	Odds ratio	95% CI (OR)
IMI					
Intercept	1·775	1·218	0·145	5·90	
Teat diameter <18 mm	0·895	0·330	0·007	2·45	1·28–4·67
Juvenile intersucking	1·912	0·625	0·002	6·77	1·99–23·04
Teat length <35 mm	1·034	0·424	0·015	2·81	1·23–6·46
Udder oedema	2·980	0·577	0·0269	1·78	1·07–2·96
SCMM					
Intercept	0·177	1·286	0·891		
Teat diameter <18 mm	1·093	0·374	0·003	2·98	1·43–6·21
Juvenile intersucking	1·462	0·573	0·011	4·31	1·40–13·26
Organic bedding *ap*	0·615	0·282	0·029	1·85	1·06–3·22
Indoor housing	1·582	0·544	0·004	4·86	1·68–14·11
CM† in 1st week of lactation	1·166	0·490	0·017	3·21	1·29–8·38

† Clinical mastitis

In theory, variables identified as risk factors for the development of IMI and SCM relate to these diseases mostly owing to their connection with an opening of the teat canal before calving. Very thin and short teats, owing to their short teat canals, teats from animals with a history of juvenile intersucking, and teats of animals with marked udder oedema are theoretically more prone to present open teat canals before parturition. The open teat canals are directly linked to an elevated risk of infection (Krömker & Friedrich, 2009). However, very thin and very short teats are more easily subjected to milking technopathies, and so corresponding quarters might have become infected after calving, possibly owing to a massive impairment of the teat condition (Wendt et al. 2007). So, these results suggest that the onset and the occurrence of heifer mastitis could be reduced if breeding programmes focusing on less short and less thin teats were applied.

Regarding SCM in particular, management conditions pre-calving also seem to play an important role. Keeping the animals in freestall barns apparently increases the bacterial counts and encourages the development of SCM, especially when organic bedding material is used (Zadoks et al. 2001; Magnusson et al. 2008). SCM is a disease, which is the last step of an IMI. Therefore the pre-calving management conditions are able to promote an infection which can be identified as SCM in their first months of lactation.

As a matter of principle, any observational study is only capable of showing associations between variables rather than predicting causal relations. Yet, if causal mechanisms for the development of an infection are known, or if the

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relations encountered are biologically sound, these results may allow hypotheses on causal relations.

The results suggest that the risk of purchasing a heifer with IMI or SCM can be reduced if animals with very short and very thin teats and animals presenting an udder oedema on the day of the auction are excluded from the buyer's selection.

Conclusions

The survey showed that the prevalence for IMI and SCM in heifers on the day of the auction was 31% and 16%, respectively, CNS being the most prevalent bacteria isolated. Juvenile intersucking, teats shorter than 35 mm, teats with diameters <18 mm and an udder oedema at 41 d after calving were identified as risk factors for IMI in heifers during the first two months after calving. Freestall barns during gestation as opposed to tied-stalls, juvenile intersucking, CM during the first week of lactation, teat diameters <18 mm, and organic bedding material in the stable before calving were identified as risk factors for SCM.

The authors thank the farmers who provided information on their heifers. We also gratefully acknowledge the support of Masterrind (Verden, Germany) and OHG-Genetic (Osnabrück, Germany). This project was partially funded by the chamber of agriculture of Lower Saxony.

References

Aarestrup FM & Jensen NE 1997 Prevalence and duration of intramammary infection in Danish heifers during the prepartum period. *Journal of Dairy Science* **80** 307–312

Bareille N, Seegers H, Kiebre-Toe MB, Beaudeau F & Fourichon C 2000 Risk factors for elevated milk somatic cell counts during early lactation in dairy heifers. In Proceedings of the 10th International Congress on Animal Hygiene, Maastricht, The Netherlands, pp. 509–514

Bassel L, Kelton D, Godkin A, Leslie K & Lissemore K 2003 Risk factors for intramammary infection at first calving in Ontario dairy heifers. In Proceedings of the 36th Annual Convention of American Association of Bovine Practice, Columbus, OH, USA, pp. 177–178

De Vliegher S, Barkema HW, Stryhn H, Opsomer G & de Kruif A 2005 Impact of early lactation somatic cell count in heifers on milk yield over the first lactation. *Journal of Dairy Science* **88** 938–947

De Vliegher S, Laevens H, Opsomer G, de Mûelenaere E & de Kruif A 2001 Somatic cell counts in dairy heifers during early lactation. *Flemish Veterinary Journal* **70** 212–215

Edinger D, Tenhagen BA, Heuwieser W, Kalbe P, Klunder G & Baumgärtner B 1999 Effect of puerperal mastitis in primiparous cows on milk production, cell count and culling. *German Veterinary Journal* **106** 470–474

Fox LK, Chester ST, Hallberg JW, Nickerson SC, Pankey JW & Weaver LD 1995 Survey of intramammary infections in dairy heifers at breeding age and first parturition. *Journal of Dairy Science* **78** 1619–1628

German Veterinary Association (GVA) 2002 *Guidelines for Mastitis Control in Dairy Herds*. Gießen, Germany: German Veterinary Association

Hallberg JW, Dame KJ, Chester ST & Miller CC 1995 The visual appearance and somatic cell count mammary secretions collected from primigravid heifers during gestation and early postpartum. *Journal of Dairy Science* **78** 1629–1636

Hosmer DW & Lemeshow S 2000 *Applied Logistic Regression* (2nd edition). New York, USA: Wiley & Sons

Krömker V & Friedrich J 2009 Teat canal closure in non-lactating heifers and its association with udder health in the consecutive lactation. *Veterinary Microbiology* **134** 100–105

Krömker V & Grabowski NT 2002 Risk factor analysis for mastitis caused by environmental pathogens in the environment of dairy herds. In Abstract XXII World Buiatric Congress, Hannover

Magnusson M, Herlin AH & Ventorp M 2008 Short communication: effect of alley floor cleanliness on free-stall and udder hygiene. *Journal of Dairy Science* **91** 3927–3930

Meaney WJ 1981 Mastitis levels in spring calving heifers. *Irish Veterinary Journal* **35** 205–209

Mein GA, Neijenhuis F, Morgan WF, Reinemann DJ, Hillerton JE, Baines JR, Ohnstad I, Rasmussen MD, Timms L, Britt JS, Farnsworth R, Cook N & Hemling T 2001 Evaluation of bovine teat condition in commercial dairy herds: 1. Non-infectious factors. In Proceedings of the Second International Symposium on Mastitis and Milk Quality, Vancouver, Canada, pp. 347–366

Myllys V 1995 Staphylococci in heifer mastitis before and after parturition. *Journal of Dairy Research* **62** 51–60

Nagelkerke NJD 1991 A note on the general definition of the coefficient of determination. *Biometrica* **78** 691–692

Nickerson SC, Owens WE & Boddie RL 1995 Mastitis in dairy heifers: initial studies on prevalence and control. *Journal of Dairy Science* **78** 1607–1618

Oliver SP & Mitchell BA 1983 Intramammary infections in primigravid heifers during early lactation. *Journal of Dairy Science* **75** 406–414

Pankey JW, Drechsler PA & Wildman EE 1991 Mastitis prevalence in primigravid heifers at parturition. *Journal of Dairy Science* **74** 1550–1552

Reinecke A, Hansen I, Tenhagen BA & Heuwieser W 2006 [Causes and consequences of clinical mastitis during first lactation. An epidemiological investigation on 15 dairy farms in Brandenburg and Saxony-Anhalt]. In Proceedings of the Berlin-Brandenburgischer Rindertag 2006, Berlin, Germany, 103–105

Roberson JR, , Fox LK, Hancock DD, Gay JM & Besser TE 1994 Ecology of *Staphylococcus aureus* isolated from various sites on dairy farms. *Journal of Dairy Science* **77** 3354–3364

Rosenberger G 1979 *Clinical Examination of Cattle*. Berlin, Hamburg, Germany: Paul Parey

Schalm OW 1942 *Streptococcus agalactiae* in the udder of heifers at parturition traced to sucking among calves. *Cornell Veterinarian Journal* **32** 39–60

Schukken YH, van de Geer D, Grommers FJ & Brand A 1989 Assessing the repeatability of questionnaire data from dairy farms. *Preventive Veterinary Medicine* **7** 31–38

Trinidad P, Nickerson SC & Alley TK 1990 Prevalence of intramammary infection and teat canal colonization in unbred and primigravid dairy heifers. *Journal of Dairy Science* **73** 107–114

Urban D 1993 *Logit-Analyse: Statistische Verfahren zur Analyse von Modellen mit qualitativen Response-Variablen*. Stuttgart, Germany: Fischer

Waage S, Mørk T, Røros A, Aasland D, Hunshamar A & Ødegaard SA 1999 Bacteria associated with clinical mastitis in dairy heifers. *Journal of Dairy Science* **82** 712–719

Wendt K, Köhler S, Sass D, Spaskovski S & Piltz E 2007 Influencing factors on the haematogene micro-circulation at the teat of milking cows. *Züchtungskunde* **79** 119–127

Zadoks RN, Allore HG, Barkema HW, Sampimon OC, Gröhn YT & Schukken YH 2001 Analysis of an outbreak of *Streptococcus uberis* mastitis. *Journal of Dairy Science* **84** 590–599