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Subclinical mastitis in dairy cows in Swiss organic and conventional production systems

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The objective was to compare the prevalence of subclinical mastitis (SM) and of udder pathogens in 60 Swiss organic (OP) and 60 conventional production systems (CP). Cows (n=970) were studied for SM prevalence and udder pathogens at median 31 d and 102 d *post partum*. Cows showing a ⩾1+ positive California Mastitis Test (CMT) in at least one quarter were considered to have SM. Cow-level prevalences of SM for visits at 31 d and 102 d *post partum* (39% and 40% in OP and 34% and 35% in CP) were similar, but quarter-level prevalences of SM were higher (P<0·02) in OP than CP (15% and 18% in OP and 12% and 15% in CP). Median somatic cell counts in milk at 31 d *post partum* were higher (P<0·05) in OP than CP cows (43 000 and 28 000 cells/ml, respectively), but were similar at 102 d *post partum* in OP and CP cows (45 000 and 38 000 cells/ml, respectively). In milk samples from quarters showing a CMT reaction ⩾2+ the prevalences of coagulase negative staphylococci were lower (P<0·05) at 102 d *post partum*, whereas prevalences of non-agalactiae streptococci were higher (P<0·05) in OP than in CP cows at 31 d and 102 d *post partum*. In conclusion, under Swiss conditions, subclinical mastitis is a greater problem in organic than in conventional production systems, but differences are not marked.

Keywords: Dairy cow, subclinical mastitis, organic farming.

Subclinical mastitis (SM) accounts for high economic losses in dairy farms (Batra, 1986; Tyler et al. 1989). In Swiss dairy herds up to 13% of all cullings are due to mastitis (Aeberhard et al. 1997). Mastitis-related losses are due to decreased milk yield, increased costs for udder treatment, milk withholding times and discarded milk, increased cow replacement as well as penalties for exceeding the legal limits stipulated by milk quality payment (Dohoo & Martin, 1984; Fetrow et al. 1991). Changes in milk composition may affect milk processing (Urech et al. 1999). Decreased milk production due to mastitis (Dohoo et al. 1984; Fetrow et al. 1991; Hortet & Seegers, 1998; Bennedsgaard et al. 2003a) is most important since it accounts for up to 75% of the total losses, with each infected quarter accounting for 10–26% of the decrease (Fetrow et al. 1991).

Most (90–95%) conventionally producing Swiss dairy farms (CP) follow the guidelines of integrated production. The general goals are not only economic success, but also sustainability of production, protection of soil, water, air, landscape and nature. In these dairies, treatments for clinical and subclinical mastitis as well as mastitis prevention with dry cow therapy consist mostly of antibiotics. In organic production systems (OP) therapeutic interventions should be based on alternative methods. The prophylactic use of allopathic drugs or antibiotics is forbidden.

There is evidence, although not consistent, that SM and high somatic cell counts (SCC) are a frequent problem in OP (Krutzinna et al. 1997; Weller et al. 1998; Busato et al. 2000; Fehlings & Deneke, 2000; Hovi & Roderick, 2000; Zwald et al. 2004). Impaired udder health as a cause of cow replacements was significantly (2·3-times) more important in OP than in CP (Roesch et al. 2006b). Consequences of SM in OP may be more important than

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in CP because of prolonged milk withholding times after treatment and problems with use for human consumption of animals that were treated with antibiotics. Causes for possibly high prevalences of SM in OP may be the restrictions in the use of antibiotics for prophylaxis and treatment of udder infections (Krutzinna et al. 1997; Weller & Davies, 1998; Busato et al. 2000; Hertzberg et al. 2003; Zwald et al. 2004). However, the prevalence of SM is known to be influenced by many additional factors (Elbers et al. 1998; Bielfeldt et al. 2004) such as husbandry, management, genetics, nutrition and associated metabolic and endocrine changes.

We performed an epidemiological investigation with the goal of assessing the prevalence of SM, and of udder pathogens involved, in Swiss dairy cows held on OP and CP. The present study extends our work on management, nutrition, performance and health status of cows held in OP and CP and adds to studies on antibiotic resistance of mammary pathogens isolated from the same cows in the same OP and CP (Kuhnert et al. 2005; Roesch et al. 2005, 2006a, b).

Material and Methods

Data collection and selection of farms and cows

All study farms were located in the canton of Bern, Switzerland. Logistic limitations allowed the inclusion of approximately 120 farms and 1000 cows. Selection of farms and animals was as described in detail elsewhere (Roesch et al. 2005, 2006a, b). In short, 60 certified OP with at least 3 years of organic production were randomly selected out of a pool of OP farms within the canton of Bern, Switzerland. Then, 60 CP, again from a pool of farms, were selected on the basis of their geographic proximity (ZIP code), to the same agricultural zone (altitude above sea level) and farm size (number of cows) as OP with the aim to find matching pairs of OP and CP. On each farm, 5–13 dairy cows (depending on herd size) were randomly selected. The plan was to study cows 3–7 weeks *post partum* (visit 1) and around 100 d *post partum* (visit 2). In total, 483 and 487 cows held on OP and CP, respectively, were tested for SM at 21–43 d (median 31 d) *post partum* (visit 1) and 419 and 421 cows on OP and CP, respectively, were tested at 90–120 d (median 102 d) *post partum* (visit 2). To collect data from all the cows, 3–15 visits per farm were necessary. Farm visits, performed by two persons, started in June 2002 and were completed in May 2003. Most cows at the first visit were studied in winter (December–February: $n=388$), followed by autumn (September–November: $n=294$), spring (March–May: $n=214$) and summer (June–August: $n=74$), but in the four seasons the number of studied cows in OP and CP was similar.

The California Mastitis Test (CMT) was performed by two persons during the first and second visit. After udder sanitation, appraisal and discarding of foremilk, the CMT was performed at a quarter level. CMT results were classified as 0+ (negative), $\geqslant$1+ (traces), 2+ (gel), and 3+ (clumps, highly viscous). Quarters with CMT 1+ were considered subclinically inflamed. From all quarters diagnosed as CMT $\geqslant$2+, milk samples were collected aseptically for bacteriological cultures. The samples were immediately cooled on ice and then submitted for bacteriological cultures. Current milk production data (milk yield and concentrations of fat, protein, lactose, and urea) and the individual test-day SCC from 958 cows were made available within 30 d after the visit from the Swiss Simmental and Red and White Cattle Breeding Association, from the Swiss Brown Cattle Breeder Federation, and from the Swiss Holstein Breeding Association. We used the data of the official milk measurement that was closest in time to the day of our cow visit.

Laboratory procedures

Standard procedures according to the guidelines of the National Mastitis Council (NMC 1999) were applied for bacteriological examinations. Isolates from cultures on agar plates (incubated at 37 °C for up to 48 h) not showing more than two different colony types were further identified by means of Gram staining, colony morphology, haemolysis, catalase activity, CAMP test, hydrolysis of aesculin, and coagulase production. The number of colonies per plate was classified as follows: 1, 2, 3 or 4 colonies; ±, 5–10 colonies; +, 11–25 colonies; ++, 26–100 colonies; +++, >100 colonies (plate fully covered with colonies). In the case of *Staphylococcus aureus*, one characterized colony per plate was accepted to speak for an infection in the CMT 2+ positive samples. In the case of coagulase-negative staphylococci, (non-agalactiae) streptococci, *Corynebacterium bovis* and *Escherichia coli*, $\geqslant$5 and more colonies were needed to speak for an infection of CMT 2+ samples. Samples of cows with clinical mastitis and of cows that actually underwent an antimicrobial treatment were excluded.

At laboratories of the breeder organizations SCC were determined by a fluoro-opto-electronic method in accordance with the FIL-IDF standard 148A (IDF 1995) using a Combifoss 4000S (Foss Electric, Hillerød, Denmark).

Data analyses

Descriptive statistics, basic univariate data analyses, and linear-type models were performed according to Hosmer & Lemeshow (2000) and Dohoo et al. (2003) using MS Excel, NCSS 2004 (www.ncss.com) and STATA v7 (www.stata.com). Medians (50 percentiles) and geometric means (based on log transformation) with respective confidence intervals were calculated using the standard functions available in NCSS 2004.

Comparisons between OP and CP farms. Numbers of cows studied and their lactation numbers were calculated

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Table 1. Prevalence of subclinical mastitis (SM) based on California Mastitis Test (CMT) reactions at the farm, cow and quarter level. Data were obtained from 60 dairy farms with organic production (OP) and on 60 dairy farms with (conventional) integrated production (CP)

	Visit 1 (median 31 d *post partum*)					Visit 2 (median 102 d *post partum*)				
	OP farms		CP farms			OP farms		CP farms		
Level	SM pos/ Total	Prevalence (%)	SM pos/ Total	Prevalence (%)	*P*-Value	SM pos/ Total	Prevalence (%)	SM pos/ Total	Prevalence (%)	*P*-Value
Farms	56/60	93·3	56/60	93·3	1·0‡	55/60	91·7	57/60	95	0·71‡
Cows†	186/472	39·4	164/480	34·2	0·11‡	165/416	39·7	146/415	35·2	0·20‡
Quarters	288/1879	15·3	239/1919	12·5	0·01‡	293/1654	17·7	244/1658	14·7	0·02‡

† At least one quarter CMT test positive
‡ Comparison of OP and CP proportion (prevalence) using Fishers Exact Test (2-sided)

as previously described (Roesch et al. 2005). Crude numbers of cows and quarters with SM and the frequency of bacteriological findings at cow-level and farm-level were compared between farm types using cross tabulation and Fishers Exact Test. Associations between farm type (OP and CP) and categorical (binary, nominal and ordinal) variables were assessed in an univariable matched logistic regression (LR) routine (clogit). All continuously (interval) measured variables were first ranked by ascending values, and a repeated measures ANOVA on these ranked values with farm type as factor A and the matching (farm pairs) as repetition factor was performed. It was impossible to correct for both matching and clustering of cows within farms in the same analysis. Therefore, the association between farm type (OP and CP) and individual cow-level variables was assessed using an univariable logistic regression model with correction for farm-level clustering [STATA logistic, r cl(farm)]. For this approach all interval-measured variables such as SCC were categorized into four levels based on quartiles and subsequently analysed as categorical variables. As an example, a significantly larger proportion of OP cows in the highest SCC quartile category (when compared to CP cows) and a lower proportion in the lowest SCC quartile would indicate a higher SCC in OP cows.

California mastitis test and somatic cell counts. The association between the number of quarters per cow positive in the CMT (CMT $\geqslant$1+) and the composite sample SCC measurement closest (in time) to the CMT was evaluated graphically (geometric SCC means over the number of positive quarters) and by simple linear regression.

Results

Breed compositions and lactation numbers

There were more Simmental × Red Holstein crossbreed cows on OP than on CP (266 and 239, respectively), whereas the number of Holstein Friesian/Red Holstein crossbreeds was lower on OP than on CP (95 and 127, respectively). The number of Simmental (91 and 94, respectively) and other breeds (Brown cattle, Montbéliard, Jersey; 31 and 27, respectively) did not differ between OP and CP. At the first visits ages of cows (medians: 5·3 and 5·2 years, respectively; ranges: 3·1–13·4 and 2·6–16·6 years, respectively) and lactation numbers of cows (both medians: 4; ranges: 2–12 and 2–14, respectively) in OP and CP were similar.

Milk yield and milk composition

At 31 d *post partum*, median ECM yields were lower ($P<0·05$) in OP than CP (25·7 and 28·5 kg/d, respectively). Median milk contents (per l) in OP and CP were 30·0 and 30·3 g protein, 38·4 and 38·7 g fat, 49·2 and 49·4 g lactose, and 200 and 220 mg urea, respectively.

Prevalence of subclinical mastitis and somatic cell counts

As shown in Table 1, at visits 1 and 2, >90% of the farms had at least one cow with a CMT-positive quarter, but the overall prevalence of OP and CP cows with at least one CMT positive quarter was not significantly different. However, there were more CMT positive quarters in OP than CP at the first and second visit ($P<0·01$ and $P<0·02$, respectively).

SCC (cells/ml) of milk was significantly higher ($P<0·05$) in OP than CP at the first visit (medians: $43·5$ and 28×10^3, respectively; ranges, 2·5–97·5 percentiles: 7–1397 and 6–1099, respectively; geometrical means: $52·7\times10^3$ and $38·0\times10^3$ respectively), but not at the second visit (medians: $45·0\times10^3$ and $38·0\times10^3$, respectively; ranges, 2·5–97·5 percentiles: 8–1523 and 7–1049, respectively; geometrical means: $55·3\times10^3$ and $47·7\times10^3$, respectively). From the first to the second visit in 48% and 53% of OP and CP cows all 4 quarters stayed CMT negative, while 26% and 22% remained inflamed (CMT positive), and 12% (both production types) became cured (all quarters CMT negative). New cases of SM (at least one

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Table 2. Cow-level bacteriological findings in quarter milk samples from cows with subclinical mastitis. Data were obtained from 60 dairy farms with organic production (OP) and on 60 dairy farms with (conventional) integrated production (CP). Frequencies (count data) and percent are presented. Samples of cows with clinical mastitis and with current antibiotic treatment were excluded

	Visit 1 (median 31 d *post partum*)					Visit 2 (median 102 d *post partum*)				
	OP cows		CP cows			OP cows		CP cows		
Categorized variable	Positive (n=200)	%	Positive (n=142)	%	P-value†	Positive (n=193)	%	Positive (n=142)	%	P-value†
Bacterial growth	144	72·0	107	75·4	0·54	146	75·6	103	72·5	0·53
Staphylococcus aureus	50	25·0	41	28·9	0·46	40	20·7	32	22·5	0·69
Coagulase-negative staphylococci	41	20·5	40	28·2	0·12	36	18·7	41	28·9	0·04
Non-agalactiae streptococci	56	28·0	25	17·6	0·03	46	23·8	21	14·8	0·05
Corynebacterium bovis	10	5·0	14	9·9	0·09	21	10·9	17	12·0	0·86
Escherichia coli	2	1·0	0	0	0·51	0	0	0	0	1
Others‡	6	3·0	4	2·8	1	12	6·2	5	3·5	0·32

† Fishers Exact Test (2-sided)
‡ *Arcanobacterium pyogenes*, yeasts, mixed flora, coliforms other than *Escherichia coli*

Table 3. Descriptive statistics of the farm-level variable pathogens of subclinically inflamed mammary glands. Data were obtained from cows 60 dairy farms with organic production (OP) and on 60 dairy farms with (conventional) integrated production (CP). Frequencies (count data) and percent are presented. Quarter samples of the combined second and the third visit (31 and 102 d *post partum*) were considered. A farm was considered positive when at least one quarter sample of one cow was positive for the pathogen

	OP farms		CP farms		
Status	Positive	%	Positive	%	P-value†
No isolation	9	15·0	8	13·3	1
Staphylococcus aureus	29	48·3	33	55·0	0·58
Coagulase-negative staphylococci	33	55·0	39	65·0	0·35
Non-agalactiae streptococci	42	70·0	29	48·3	0·03
Corynebacterium bovis	20	33·3	18	30·0	0·85
Escherichia coli	2	3·3	0	0	0·50
Others‡	14	23·3	6	10·0	0·09

† Fishers Exact Test (2-sided)
‡ *Arcanobacterium pyogenes*, yeasts, mixed flora, coliforms other than *Escherichia coli*

quarter CMT-positive) developed in 14% of both OP and CP cows.

SCC of the composite milk of all 4 quarters increased linearly ($b_1 = 63 \times 10^3$ cells/ml; P=0·003; r^2=0·95) with increasing numbers of CMT-positive quarters. At visit 1, the geometrical mean of SCC of healthy cows (all four quarters CMT negative) was 26×10^3 cells/ml and cows with 1, 2, 3 or all 4 quarters with CMT-positive reactions had geometrical means of 81, 190, 215 and 273×10^3 cells/ml, respectively, in the composite sample. In cows with at least one quarter showing a CMT reaction $\geqslant$2+, the geometrical mean SCC in composite milk samples was 190×10^3 cells/ml and ranged up to 566×10^3 cells/ml.

Bacteriological findings in milk samples

In CMT 2+ samples, 5–10 (±) and more bacterial cultures did grow per plate in most cases. For *Staph. aureus* one colony/plate was found in only one case and for coagulase-negative staphylococci one colony/plate was only found in two cases and two colonies/plate were found in only two cases.

In quarter milk samples, bacteria could be isolated in >70% of all cows with at least one quarter showing a CMT $\geqslant$2+ reaction (Table 2). With the exception of a higher prevalence of non-agalactiae (other) streptococci in OP than CP at visits 1 and 2, and a lower prevalence of coagulase-negative staphylococci in OP than CP at visit 2, there were no significant differences in the bacterial prevalences of OP and CP cows. *Streptococcus agalactiae* was never isolated. At the farm level (Table 3), non-agalactiae (other) streptococci were also more frequent in OP than CP (70·0% and 48·3%, respectively; P=0·03).

Discussion

General aspects

Dairy cows in the periparturient period are metabolically stressed and the immune responses are impeded, resulting

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in a high incidence of clinical and subclinical mastitis (Kehrli et al. 1989; Burvenich et al. 2003). It was therefore planned to study cows at 3–7 weeks *post partum* (visit 1). Because the prevalence of SM and thus elevated SCC increases during lactation (Schepers et al. 1997; Busato et al. 2000), another visit was performed around 100 d *post partum* (visit 2).

In this study SM was diagnosed by means of CMT at the quarter level, which is a reliable indirect method to estimate SCC of individual quarters (Schalm et al. 1971). Because of right-skewed SCC data, medians and geometric means were used. SCC in milk from uninfected udders was lower than in milk from infected udders, as expected and was in the range of geometric mean SCC (49 400–68 000 cells/ml) found by Laevens et al. (1997) and Djabri et al. (2002). In our study, composite milk samples of cows with at least one quarter showing a CMT reaction 1+ had geometric mean SCC of 81 000 cells/ml. Because there was a close association between SCC and the number CMT-positive quarters and because SCC increased if CMT was >1+, the use of CMT to diagnose SM is acceptable. SCC in milk samples from cows held in both OP and CP were low, indicating an overall high standard of udder health.

Prevalence of subclinical mastitis

Prevalence of SM in OP was in accordance with previous studies (Krutzinna et al. 1997; Weller & Davies, 1998; Busato et al. 2000; Hertzberg et al. 2003), but prevalence of CMT-positive samples in OP was lower than reported by Busato et al. (2000) both at the cow-level and quarter level. This might be because most cows were tested in winter, when SCC is generally lower than in summer (Schukken et al. 1990). There might also have been an improvement of udder health in OP farms during the last few years. It has to be noted, however, that controversial experiences have been reported after conversion from CP to OP (Bennedsgaard et al. 2003b). Augstburger et al. (1988) reported a prevalence of SM of only 22·1, 27·9 and 32·7% at the herd level for conventional, organic and organic-dynamic producing farms, respectively. This differs from our results because in this study only cows with milk samples showing CMT reaction $\geqslant$2+ were considered to have SM. In the present study there were no significant differences in the prevalence of CMT-positive samples between OP and CP at the farm-level and cow-level, but there were more CMT-positive quarters in OP than CP.

SCC from both OP and CP were at a level indicative of generally high quality milk. However, SCC in cows with SM was higher (significantly at the first visit) in OP than CP. The higher mean SCC in OP cows was due to a higher number of cows having high SCC and not to a generally increased SCC. The higher incidence of SM at the quarter level in OP than CP was in agreement with studies of Zwald et al. (2004). SCC data, together with the CMT analyses at the quarter level, indicate that cows in OP have apparently – and mainly in early lactation – more problems in maintaining good udder health than cows in CP. The more frequent SM problem in OP than CP was considered to be one of the factors leading to lower milk yields in OP than CP (Roesch et al. 2005).

Bacteriological findings

The criteria to perform bacteriological analyses only in milk from quarters with at least CMT reactions $\geqslant$2+ was justified because in cows with at least one quarter showing a CMT reaction $\geqslant$2+, the geometric mean SCC in composite milk samples was 190 000 cells/ml and therefore the SCC of the affected quarter milk sample would have been well above 200 000 cells/ml, which is generally considered clearly abnormal (Hamann, 2004). Therefore, only medium to severely affected quarters were analysed microbiologically.

About 25% of the analysed milk samples were bacteriologically negative. This was in accordance with textbook values of 15–40% (Radostits et al. 2000). Although the overall SCC was low, the number of bacteriologically positive milk samples was relatively high. It must be stressed, however, that only CMT $\geqslant$2+ samples (whose mean SCC was 190 000 cells/ml and ranged up to 566 000 cells/ml) were bacteriologically analysed. In the present study, with the exception of a higher prevalences of non-agalactiae (other) streptococci in OP than CP at visits 1 and 2, and a lower prevalence of coagulase-negative staphylococci in OP than CP at visit 2, there were no significant differences in the bacterial prevalences between OP and CP.

Causes of the higher prevalences of coagulase-negative staphylococci in CP than OP at the second visit are not obvious. The prevalence of *Staphylococcus aureus* in the actual study (25%) was higher than in a previous study in Swiss OP (16·0% for 7–100 d *post partum*) (Busato et al. 2000), but was lower than the 39·8% described for cows held in Swiss CP (Schällibaum, 2001). Post-milking teat dipping is regarded as the single most effective practice for the prevention of new intramammary infections (Pankey, 1984). Compared with other pathogens, such as non-aureus staphylococci and streptococci, the dry cow therapy is not very successful in eliminating intramammary infections caused by *Staph. aureus* (Dingwell et al. 2003). Despite the more frequent use of antibiotic dry cow therapy and the more frequent use of germicidal teat dipping after milking in CP than OP, the prevalence of *Staph. aureus* was slightly higher in CP than OP. From the first to the second visit, there was a decrease in the prevalence of *Staph. aureus*, similar to that described by Busato et al. (2000).

The significantly higher prevalence of streptococci other than *Streptococcus agalactiae* in OP than CP was possibly due in part to the reduced use of antibiotic dry cow therapy in OP compared with CP (Roesch et al. 2006a).

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The dry period contributes markedly to the prevalence of mastitis due to environmental pathogens (Smith et al. 1985). The significantly less frequently performed post-milking teat dipping in OP than CP seemed not to be a risk factor for environmental streptococcal mastitis, which was also found by Oliver & Mitchell (1983). *Str. agalactiae* could not be found in any sample in OP and CP, similar to our previous study in OP (Busato et al. 2000), *i.e.* it seems to be a lesser problem than was previously the case.

In conclusion, under Swiss conditions, subclinical mastitis is a greater problem in organic than in conventional production systems, but differences are not marked.

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