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Effect of subclinical intramammary infection on somatic cell counts, NAGase activity and gross composition of goats' milk

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The study was aimed at identifying the pathogens causing subclinical udder infections in representative Israeli dairy goat herds and determining their effect on milk quality. Five hundred goats in ten flocks of various breeds and crossbreeds were surveyed. Of the 500 goats, 13·4% were in their first lactation, 36·4% were in their second lactation and 50·2% were in their third or higher lactation. Percentages of udder halves with subclinical intramammary infection in the flocks ranged from 35 to 71%. The effect of the bacteriological infection on somatic cells count (SCC) was significant ($P<0·001$). Various species of coagulase-negative staphylococci (CNS), mainly *Staphylococcus caprae* and *Staphylococcus epidermidis*, were the main pathogens in infected udder halves. Lactation number did not significantly influence either infection rate of udder halves or SCC, although the percentage of udder halves with no bacteriological findings was higher at the first lactation than at the third lactation. Milk composition (fat, protein and lactose) varied among flocks, with lower mean total protein in uninfected halves than in infected ones and higher lactose in uninfected than infected halves.

Keywords: Subclinical mastitis, milk composition, goat.

Intramammary infection (IMI) raises milk somatic cell count (SCC) and reduces milk yield and milk quality in dairy cows (Harmon, 1994) and sheep (Watson & Buswell, 1984), but in goats this interrelationship is less clear (Haenlein & Hinckley, 1995; Sanchez et al. 2002). In goats, involution tends to be spontaneous and is associated with an increase of SCC independently of IMI, which confounds the interrelationship between IMI and milk yield (Wilson et al. 1995; Zeng & Escobar, 1996; Foschino et al. 2002).

In goats, possible limitations of bacteriological analyses as gold standard in mastitis diagnosis cannot be met by the indirect tests proposed, such as SCC, California mastitis test (CMT) or N-acetyl-β-D-glucosaminidase (NAGase) activity. The validity of these tests in goat milk is not clear and further studies are needed for different goat breeds and systems of production. In cows, SCC is an indicator of normal or abnormal milk while, for goat milk, it is inappropriate because of the presence of many cytoplasmic particles resulting from apocrine milk secretion (Maisi, 1990; Atherton, 1992). However, Fossomatic calibrated with goat milk, CMT or even Coulter Counter, after applying an appropriate correction factor, could be used as indirect tests (Heanlein, 2002). In dairy cows (Chaffer et al. 1999; Leitner et al. 2001) and dairy sheep (Leitner et al. 2003) the changes accruing throughout lactation in the bacteriological status of a mammary gland (quarter or half) are minimal with a high correlation between the pathogens identified, SCC and NAGase activity. These results indicate that testing cows and sheep once in mid lactation gives a good indication of bacteriological status of the udder. In goats, indirect tests are questionable if carried out shortly after kidding and towards the end of lactation.

The present study was aimed at identifying the pathogens causing subclinical udder infections in representative Israeli dairy goat herds asnd determining their influence on milk quality. This was done by single sampling of milk of individual udder halves between 25 and 130 d *post partum*.

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Materials and Methods

Animals

Ten Israeli dairy goat herds representing various breeds (Saanen, Shami) and crossbreeds (Shami × Anglo Nubian, Saanen × Anglo Nubian), from different locations, size of herd (150–800 goats) and lactation number (1–9) were surveyed. From each herd, 50 goats were randomly chosen from the pool of goats that were at least 25 d and not more than 130 d after kidding. Annual lactation milk yield of these herds ranged from 350 to 1200 l/goat and the daily yield ranged from 0·5 to 6 l/d. Under Israeli goat herd management, the kids are removed immediately after birth and goats are milked with milking machine twice a day with teat post-dipping only. At drying-off, no treatment is applied. Food is usually offered in mangers in free stall barns.

Milk sampling

In each goat, udder halves were sampled and tested for udder bacterial condition, SCC, NAGase activity and the concentrations of fat, protein and lactose in milk were measured. Udder halves were sampled during the morning or evening milking. Teats were cleaned and disinfected before sampling with non-woven towelets moistened with chlorhexidine, cetrimide and ethanol (MediWipes, Al-Baad, Massuot Itzhak, Israel). The first few squirts of milk were discarded and ~5 ml was then taken into a sterile tube for bacteriological testing and NAGase activity. Samples were taken aseptically according to International Dairy Federation (IDF) procedures (1985) and kept at 4–8 °C until tested at the laboratory 1–5 h later. SCC and milk composition were determined according to the revised protocol of the A2B sub-group of mastitis experts (IDF, 1991). Foremilk (50 ml) was taken from each half to determine SCC with a Fossomatic 360 and milk composition with a Milkoscan 6000 (Foss Electric, Hillerød, Denmark), both calibrated with goat milk, at the Milk Control Laboratory, Israel Cattle Breeders Association, Caesarea, Israel.

Bacteriological procedure

Bacteriological examination was according to accepted standards (Hogan et al. 1999): 0·01 ml of each milk sample was spread onto blood-agar plates (Bacto-Agar, Difco Laboratory) containing 5% of washed sheep red blood cells and on MacConkey plates. All plates were incubated at 37 °C and examined for growth at 18 and 42 h. Colonies suspected of being staphylococci were tested for coagulase (tube test, Anilab, Rehovot, Israel). Strain identification was carried out with the API STAPH-IDENT, 32 Staph kit (bioMerieux S.A., 69280 Marcy-l'Etoile, France). When the percentage of micrococci-like bacteria that matched the test strain was >90%, the strain was regarded as specific. If the percentage was <90%, the strain was registered as unidentified coagulase-negative staphylococci (CNS).

Table 1. Distribution of the 500 various breeds and crossbreeds of dairy goatsused in the experiment, according to flock, lactation number and average (±SD) days in milk (DIM)

	Lactation			
Flock	1	2	>3	DIM†
1	2	36	12	95±50
2	2	8	40	31±5
3	2	24	24	—
4	6	18	26	—
5	6	13	31	—
6	2	26	22	104±114
7	12	13	25	77±17
8	3	9	38	—
9	15	20	15	50±2
10	17	15	18	105±92
Total	67	182	251	

† Four goat herds did not record DIM

Table 2. Mean and SE of NAGase activity and somatic cell count (SCC) by Fossomatic 360 and the effects of udder bacteriological status and flock, lactation number and days in milk for six goat herds

Status	NAGase	SCC × 10^{-3}	log SCC
Uninfected (n=327)	15·6±0·8	338±29·0	5·12+0·07
Infected (n=273)	59·2±5·3	1922±109·8	6·83+0·08
Statistical significance of effects of:			
Bacteriological status	<0·001	<0·001	<0·001
Flock	<0·01	<0·01	<0·01
Lactation	NS	NS	NS
Days in milk	NS	NS	NS

Gram-negative colonies were identified with the API 20 E or API NE kit (bioMerieux S.A., 69280 Marcy-l'Etoile, France).

NAGase test

Concentrations of NAGase were determined fluorimetrically according to the ADL MILK NAGase test kit (Applied Diagnostics Corporation, Helsinki, Finland) with a computerized microplate setting. A value of 100 corresponds to the release of substrate-derived product at 5 μmol l^{-1} min^{-1} at 25 °C.

Statistical analysis

Results were analysed with the SAS General Linear Model procedure (SAS/STAT® User's Guide, 1990). No differences were found in the arithmetic means of IMI parameter and milk composition between all the herds and the six goat herds that had a complete dataset. Therefore, statistical analysis was done only on the six goat herds that had recorded days in milk (DIM). Dependent variables

Table 3. Distribution of 500 dairy goat udders (1000 halves) from 10 different flocks, according to udder bacteriology infection and SCC measured with Fossomatic 360

Bacteria	Number of udder halves infected	Number of flocks	SCC $\times 10^{-3} \pm$ SE	Log SCC $\pm$ SE
Staph. aureus	38	8	3593 ± 259	6·49 ± 0·04
CNS:				
Staph. caprae	167	10	1426 ± 205	5·93 ± 0·04
Staph. chromogenes	41	8	1744 ± 236	6·06 ± 0·07
Staph. epidermidis	95	10	1529 ± 208	6·02 ± 0·04
Staph. simulans	66	10	1996 ± 182	6·16 ± 0·05
Staph. xylosos	13	4	791 ± 192	5·8 ± 0·08
Non-identified CNS	53	8	1189 ± 205	5·63 ± 0·1
Total CNS	435	10	1676 ± 74	5·99 ± 0·02
Other & not identified	39	9		
Total uninfected†	488 (48%)		288 ± 21·1	5·19 ± 0·02
Total infected	512 (52%)		1626 ± 70·8	6·00 ± 0·02

† Uninfected halves, no bacterial growth on blood-agar and MacConkey plates

were: SCC and Log SCC, NAGase, fat, protein and lactose. The independent variables (bacteriological status, flock, lactation number and DIM) were examined according to the model:

$$Y_{ijk} = \mu + B_i + H_j + L_k + DIM_{ijk} + e_{ijk}$$

where: Y = dependent variable; μ = overall mean; B = bacteriological status I = 1,2; H = flock, J = 1,6; L = lactation number, k = 1,2,3+; DIM is a continuous variable; e = error.

No significant difference was found between morning and evening milk sampling and because most herds (8 out of 10) were sampled in the morning, this variable was excluded from the model. No significant difference was found between analyses based on SCC and those based on log SCC.

Results

Table 1 shows the distribution of the 500 surveyed goats according to flock, lactation number and average DIM. Of the 500 goats, 13·4% (67/500) were in their first lactation, 36·4% (182/500) were in their second lactation and 50·2% (251/500) were in their third or higher lactation. The percentage of bacteriologically infected udder halves in the flocks ranged from 35 to 71%. Although the percentage of uninfected udder halves was numerically higher for first and second lactations than for third lactation (54, 56 and 31%, respectively) the difference was not statistically significant. The correlation between SCC and NAGase activity was low ($R < 0·7$). No interaction was found between bacteriological status and flock in its effect on SCC or NAGase activity. Bacteriological status (infected or uninfected half) significantly affected both NAGase and SCC ($P < 0·001$). Flock effect was significant for NAGase, SCC and log SCC ($P < 0·01$) (Table 2). Effects of lactation number and DIM on SCC were not significant.

No bacterial growth was detected in the milk of 48% of halves (488/1000) and those were classified as uninfected. *Staphylococcus aureus* was detected in eight flocks, in only one to six goats per flock, and caused marked increases in SCC, to $>3 \times 10^6$ cells/ml (Table 3). Various species of CNS formed the main pathogenic group in infected udders and for the majority of the CNS species; infection of one half did not affect the other. *Staphylococcus caprae* and *Staphylococcus epidermidis* accounted for most CNS and they were found in all the flocks tested (Table 3). Other major CNS were *Staphylococcus chromogenes* and *Staphylococcus simulans*. The presence of CNS elicited strong inflammatory responses, increasing SCC to 10^6 cells/ml with no significant difference among the various strains (Table 3). Statistical analysis of each of the five CNS species in the model showed no significant effects for all the dependent variables.

Milk fat, protein and lactose concentrations ranged among flocks from 19·9 to 55·6 g/l for fat, 34·0 to 51·1 g/l for total protein and 47·4 to 51·1 g/l for lactose. Statistical analysis for the six goat herds that were included in the model showed that the mean total protein and fat were lower in uninfected halves than that in infected ones (39·1 *v.* 39·9 g/l for protein and 37·5 *v.* 42·0 g/l for fat), whereas lactose was higher in uninfected than in infected halves (49·6 *v.* 47·2 g/l). Effects of bacteriological status (infected or uninfected) for the six goat herds were not significant for fat but significant ($P < 0·01$ and $< 0·001$) for protein and lactose, respectively (Table 4). Flock effect on all of the parameters was significant ($P < 0·001$) whereas effect of lactation number was significant for protein and lactose but not for fat, and effect of DIM was significant for fat and protein but not for lactose (Table 4).

Discussion

CNS, mainly *Staph. caprae, Staph. epidermidis, Staph. chromogenes* and *Staph. simulans*, were the most abundant bacterial isolates, and were found in almost all flocks tested. *Escherichia coli*, *Staph. aureus*, streptococci and

Table 4. Mean values and SE for milk fat, protein, and lactose according to udder-half bacteriological status in six goat herds, and the effects of udder bacteriological status, flock, lactation number, time of milking and days in milk

Status	Fat, g/l	Protein, g/l	Lactose, g/l
Uninfected (n=327)	37·5±0·09	39·1±0·03	49·6±0·02
Infected (n=273)	42·0±0·09	39·9±0·03	47·2±0·03
Statistical significance of effects of:			
Bacteriological status	NS	<0·001	<0·001
Flock	<0·001	<0·001	<0·001
Lactation	NS	<0·001	<0·001
Days in milk	<0·001	<0·001	NS

Pseudomonas spp. are commonly isolated from cases of clinical mastitis (Menzies & Ramanoon, 2001) and hence the numbers of goats that were found to be infected with these bacteria in the present study did not reflect their frequencies in the flocks. Moreover, in the last 5 years, sheep and goat flocks in Israel have suffered outbreaks of mastitis caused by *Pseudomonas aeruginosa* (Rapoport et al. 1998) and those with acute clinical symptoms were removed from the flocks.

All CNS isolates in this study similarly affected the mammary glands, as indicated by SCC, which increased above 1000×10^3 cells/ml. This trend is discussed by Haenlein (2002), who reports over 10^6 cells/ml in response to CNS IMI in different countries. Other factors such as lactation, DIM and caprine arthritis-encephalitis virus infection are associated with increased SCC in goats (Ryan et al. 1993; Haenlein & Hinckley, 1995; Wilson et al. 1995). However, avoiding sampling goats immediately after parturition and not more than 130 d after kidding reduced the contribution of lactation number and DIM in the present work and strongly suggested that increased SCC is associated with IMI during this period. Therefore, it is strongly suggested that within the limit of the above period, SCC or NAGase activity should be complemented with bacterial testing to assess IMI. Moreover, since in Israel most of the milk is yielded during this period (25–130 d), the increase in SCC regardless of IMI towards the end of lactation has less effect on bulk milk SCC. A similar association between infection with CNS and high level of SCC occurs in sheep (Fthenakis, 1994; Gonzalez-Rodriguez et al. 1995; Las Heras et al. 1999; Leitner et al. 2001), indicating that the immune response in sheep and goats is more acute than in cows, although normal SCC in goat and sheep is higher than for cows (Paape & Capuco, 1997).

IMI, even if only subclinical, decreases milk yield in sheep (McCarthy et al. 1988; Fthenakis & Jones, 1991; Leitner et al. 2003). A basic feature of mammary secretion is that the total osmotic pressure of the secretion is approximately constant and equal to that of blood (Holt, 1993). As lactose is the main osmotic component in milk, the secretion volume closely follows changes in the secretion of lactose (Shamay et al. 2000). Thus, based on the decrease in lactose concentration in the infected glands, it is assumed that subclinical mastitis is associated with reduced milk yield also in goats. The decrease in lactose concentration in the infected halves is consistent with similar findings in sheep (Burriel, 1997; Leitner et al. 2003). However, it remains unclear whether the decrease in lactose concentration is related to microbial activity or to the effect of plasmin-induced casein-derived products on mammary epithelial cells (Shamay et al. 2002, 2003).

The milk from 6 of the 10 farms in the present study is processed by the owners into fermented milk products and cheese, so any changes in the dry matter, mainly casein concentration, have economic impact. In the present study, levels of fat and total protein were significantly higher in the infected halves than in the uninfected ones, consistent with recent findings in sheep (Leitner et al. 2003). In dairy cows, milk with high SCC shows an extended coagulation time and forms a weak coagulum (Auldist et al. 1996), so that the cheese is higher in moisture and dry matter yield is reduced. Milk from a mastitic cow's udder has increased proteolytic activity and is associated with a reduced concentration of caseins and an increased concentration of whey protein (Schaar & Funke, 1986). Therefore, measurements of casein yield, casein proportions and curd yield and parameters reflecting cheese quality are essential to assess the effect of subclinical mastitis on cheese yield and quality in goats.

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