

Udder infection and milk somatic cell count, NAGase activity and milk composition—fat, protein and lactose—in Israeli-Assaf and Awassi sheep

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Abstract

The present study aimed to identify the pathogens that cause subclinical udder infections in Israeli dairy sheep and evaluate their influence on milk yield and composition. Eight hundred and fifteen Israeli-Assaf and Israeli-Awassi dairy sheep were surveyed. More than half of the sheep were in their third or higher lactation (513/815 sheep) and in 14 out of 20 flocks; the sheep were in their peak (second to third month) of lactation. The percentage of bacteriological infected udders in the flocks ranged from 8.6 to 64.3%. The effect of the bacteriological infection on somatic cells count (SCC) was significant ($P > 0.001$). Various species of coagulase-negative staphylococci (CNS) mainly *S. chromogenes* and *S. epidermidis*, formed the main pathogen group in infected udders. Lactation number did not significantly influence either the infection rate of udder halves or the milk SCC, although the percentage of udder halves with no bacteriological findings found was higher at first lactation than at the second and third lactation. Milk yield was significantly higher in uninfected than in infected halves. Milk composition—fat, proteins and lactose—varied among flocks, with mean total protein lower in uninfected halves than in infected ones and lactose higher in uninfected than in infected halves.

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1. Introduction

Udder infection in dairy sheep has a major effect in reducing both yield and quality of milk, leading to greater economic losses than those reported for dairy cattle (Watson and Buswell, 1984). Our earlier

study (Leitner et al., 2001) identified the pathogens causing udder infections in Assaf dairy sheep, the changes occurring throughout lactation and the correlation between the pathogens and the severity of the infection, as measured by somatic cells count (SCC) and *N*-acetyl- β -D-glucosaminidase (NAGase), activity. One of the findings in that study was that there were almost no changes in the bacteriological status of the udder halves in the course of the lactation period; 90.6% of the halves did not change in status

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after the first sampling, 1–3 weeks into lactation. This indicates that a single test of the milk, after the first 2 weeks following lambing, is a highly accurate means of determining the subclinical bacteriological status of individual sheep and its prevalence in the flock. Subclinical mastitis, defined as an inflammation that is not readily detected clinically but that adversely affects milk production, increases the SCC and affects milk quality. Prevalence of subclinical mastitis is not as evident in sheep as in dairy cows, because of the lack of regular testing and regulations governing the payment of milk-quality premiums. In dairy sheep flocks, mastitis prevalence of 22–48% (Fthenakis, 1994) and up to 40% (Leitner et al., 2001) has been reported. In the case of dairy cows, payments related to SCC milk are implemented in most industrial countries. In dairy sheep the SCC method is starting to be included in some testing programs (Gonzalo et al., 1994), but the acceptable SCC levels in healthy udders under different management systems and for different breeds is still controversial (Maisi et al., 1987; Fthenakis et al., 1991; Gonzalez-Rodriguez et al., 1995). Various threshold values for SCC have been suggested as a base level for the estimation of subclinical mastitis in a flock (Fthenakis et al., 1991; Fthenakis, 1994). Although intramammary infection is the most important factor influencing the SCC (Gonzalez-Rodriguez et al., 1995; Mavrogenis et al., 1999), other factors such as stage of lactation, lactation number, time of day, lentivirus infection, and management (review by Menzies and Ramanoon (2001)) could be of relevance. Some studies have yielded evidence that sheep normally have higher SCCs than cows (Maisi et al., 1987; Fthenakis et al., 1991; Baro et al., 1994; Gonzalo et al., 1994; Gonzalez-Rodriguez et al., 1995), although others found similar values to those in cows (De La Cruz et al., 1994). In our previous study with Israeli-Assaf sheep (Leitner et al., 2001), the mean SCC of the healthy glands was low ($<4 \times 10^5$ cells/ml), with no significant changes in the course of the lactation. However, the mean SCC found in the healthy uninfected glands in that study was twice as high as those found in Assaf sheep sampled in mid-lactation in Spain (Gonzalez-Rodriguez et al., 1995). Coagulase-negative staphylococci (CNS) is the most common type of udder infection; however, their association with increased SCC differs among studies (review by Menzies and Ramanoon (2001)). In our

previous study (Leitner et al., 2001) the three main CNS: *S. haemolyticus*, *S. chromogens*, and *S. intermedius* had similar effects on the SCC in the mammary glands, increasing it to above 1.2×10^6 cells/ml. The high SCCs in the CNS-infected halves contrasted with the moderate SCCs found in similarly infected dairy cows, suggesting that the sheep's udder has a lower resistance to and an augmented immunological response towards this group of bacteria than that of the cow. Although SCC is the main factor influencing milk quality, other milk components, such as fat, protein and lactose have been found to have a negative correlation with bacterial udder infection (Burriel, 1997). The present study aimed to identify the pathogens causing subclinical udder infections in Israeli dairy sheep, and determine their influence on both quantity and quality of milk.

2. Materials and methods

2.1. Animals

Nineteen Israeli-Assaf and one Israeli-Awassi dairy sheep flocks were surveyed. The flocks were in different locations and of various sizes (250–1250 sheep). Sheep were in various stages of lactation (1, 2 and >3 lactations), from a minimum of 25 days up to 120 days after lambing. Lambs were removed immediately after birth and the sheep were milked mechanically twice a day. The average milk yield in the flocks was 350–500 l per sheep per lactation with a daily yield of 0.5–4 l. In each flock, 35–60 sheep, depending on flock size were randomly chosen (a total of 823 sheep) for inclusion in this study. Udder halves were tested for udder bacterial condition, SCC, NAGase activity and milk composition. Milk yield was recorded by the owner up to 2 weeks before or after sampling time. Food was offered in mangers located in free stall barns.

2.2. Milk sampling

Sheep were sampled during the morning or evening milking. Udder halves were cleaned and disinfected prior to sampling with a non-woven towelette moistened with chlorhexidine, cetrimide and ethanol (Medi-Wipes, Al Baad Massuot Itzhak, Israel). The first three squirts of milk were discarded and approximately

5 ml of milk were taken in a sterile tube for bacteriological tests, SCC measurement with a Coulter[®] Counter model Z1 (Coulter Electronics, Beds., UK), and NAGase activity testing. Foremilk (50 ml) was taken from each half to determine SCC and milk composition (fat, proteins and lactose) with a Fossomatic 360 at the Milk Control Laboratory, Cattle Breeders Association, Caesarea. Samples were taken aseptically according to the International Dairy Federation (1985), and kept at 4–8 °C until submitted to the laboratory (1–5 h). SCC and milk composition were determined according to the revised protocol of the A2B sub-group of mastitis experts of the International Dairy Federation (1991).

2.3. Bacteriological examinations

Bacteriological analysis was performed according to accepted standards (Hogan et al., 1999). From each milk sample, 0.01 ml 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 to be 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., Marcy-l'Etoile, France). When the percentage of micrococci-like bacteria that matched the test strain exceeded 90%, the strain was regarded as specified. If the percentage fell below 90%, the strain was registered as unidentified CNS. Gram negative colonies were identified with the API 20 E or API NE kit (bioMerieux S.A., Marcy-l'Etoile, France).

2.4. NAGase test

The concentration of NAGase, a lysosomal enzyme in milk, was fluorometrically determined according to the ADL MILK NAGase test (ADC Applied Diagnostics, Helsinki, Finland) with a computerized microplate setting. A value of 100 corresponds to the release of product at about 5 $\mu\text{mol l}^{-1} \text{min}^{-1}$ at 25 °C.

2.5. Statistical analysis

Data were analyzed with the (SAS, 2002) User's Guide: Statistics, Software Release 8.2 (SAS Inst.,

Cary, NC). Dependent variables were SCC and log SCC (Coulter[®] Counter), SCC and log SCC (Fossomatic 360), NAGase, milk, fat, protein and lactose. The independent variables—bacteriological status, flock, lactation number, milking time and days in milk—were examined according to the model:

$$Y_{ijklm} = u + B_i + H_j + L_k + TM_l + \text{DIM}_{ijklm} + (\text{DIM} \times \text{DIM})_{ijklm} + e_{ijklm}$$

where Y is the dependent variable, U the overall mean, B the bacteriological status ($i = 1, 2$), H the flock ($j = 1, \dots, 20$), L the lactation number ($k = 1, 2, 3+$), TM the time of milking ($l = 1, 2$), DIM the days in milk is a continuous variable and e the error. The results for the Israeli-Awassi (one flock) did not differ from those for the Israeli-Assaf, therefore all analyses disregarded the breed as a variable. Also, no significant difference was found between analyses based on SCC and those based on log SCC, therefore, the results presented are those based on the arithmetic SCC.

Table 1

Distribution of the 815 Israeli-Assaf and Israeli-Awassi dairy sheep, according to flock, lactation number and average (\pm S.D.) number of days in milk

Flock	Breed	Lactation				DIM
		1	2	>3	Total	
1	Assaf	–	–	35	35	115 \pm 2
2	Assaf	–	–	35	35	56 \pm 9
3	Assaf	8	5	22	35	63 \pm 3
4	Assaf	7	27	21	55	56 \pm 56
5	Assaf	26	–	8	34	UK ^a
6	Assaf	2	8	25	35	UK
7	Assaf	12	16	32	60	37 \pm 3
8	Assaf	2	18	40	60	77 \pm 21
9	Assaf	12	11	37	60	37 \pm 13
10	Assaf	–	–	35	35	UK
11	Assaf	6	9	20	35	65 \pm 9
12	Assaf	15	10	10	35	50 \pm 5
13	Awassi	9	6	35	50	31 \pm 24
14	Assaf	10	1	29	40	49 \pm 13
15	Assaf	–	–	34	34	70 \pm 8
16	Assaf	11	2	27	40	70 \pm 5
17	Assaf	19	3	13	35	86 \pm 37
18	Assaf	12	3	20	35	50 \pm 6
19	Assaf	5	10	18	33	47 \pm 11
20	Assaf	16	1	17	34	124 \pm 48
Total		172	130	513	815	

^a Day of lambing not recorded (unknown—UK).

3. Results

Eight hundred and fifteen Israeli-Assaf and Israeli-Awassi dairy sheep were distributed according to flock, lactation number and average (\pm S.D.) number of days in milk as summarized in Table 1. More than half of the sheep were in their third or higher lactation (513/815 sheep). In 14 out of the 20 flocks, the sheep were in their peak (second to third month) of lactation, in three flocks they were at the end of their lactation, and in three flocks the time of lambing had not been recorded. The percentage of bacteriologically infected udders in the flocks ranged from 8.6 to 64.3% (Table 2). Means and standard errors (S.E.) of NAGase activity, log SCC (with the Coulter[®] Counter (all 20 flocks) or the Fossomatic 360 (12 flocks)) according to flock is presented in Table 2. The correlation coefficients between the SCC as measured with the Coulter[®] Counter and with the Fossomatic was $r = 0.83$. In general, SCC according to the Fossomatic was 10–20% higher than that counted with the Coulter[®] Counter. No interaction was found between bacteriological status and flock, in their effects on somatic cells counted with either

the Coulter[®] Counter or the Fossomatic 360, or on NAGase activity. The effect of the bacteriological status (infected or uninfected) on all of these parameters was significant ($P < 0.001$), whereas the flock effect was significant ($P < 0.001$) for the SCC with Coulter[®] Counter and for NAGase activity, but not for the SCC with Fossomatic 360 (Table 3). Lactation number, time of milking and days in milk did not affect significantly SCC and NAGase activity. Bacteriological status and SCC over flock is summarized in Table 4. No bacterial growth was detected in the milk of 68% (1109 out of the 1630) of the halves, and those were accordingly classified as uninfected. *S. aureus* was detected in 10 flocks, *Pseudomonas* in four flocks, and both in only one to four sheep per flock, and they caused strong responses in somatic cells, which increased to $(2-5) \times 10^6$ cells ml⁻¹. Various species of CNS formed the main pathogen group in infected udders, and for the majority of the CNS species, infection of one-half did not affect the other. *S. chromogenes* and *S. epidermidis* formed the majority of the CNSs, and they were found in 16–18 of the flocks tested. CNSs elicited strong responses, with the SCC increasing to 10^6 cells ml⁻¹, and no

Table 2

Percentage of udder halves infected (status), NAGase activity, log SCC by Coulter[®] Counter or Fossomatic 360, according to flock

Flock	Status ^a (% infection)	NAGase	log SCC \pm S.E. (Coulter [®] Counter)	log SCC \pm S.E. (Fossomatic)
1	47.1	52.2 \pm 5.6	6.00 \pm 0.2	6.15 \pm 0.2
2	22.9	27.2 \pm 2.9	5.60 \pm 0.1	5.78 \pm 0.1
3	34.3	53.7 \pm 6.8	5.30 \pm 0.1	5.90 \pm 0.2
4	38.2	56.5 \pm 3.5	5.95 \pm 0.1	6.00 \pm 0.2
5	38.2	39.5 \pm 4.4	5.60 \pm 0.1	5.60 \pm 0.1
6	64.3	54.1 \pm 3.9	5.30 \pm 0.0	NT ^b
7	20.8	21.6 \pm 4.0	5.48 \pm 0.1	5.60 \pm 0.1
8	19.2	40.8 \pm 4.2	5.30 \pm 0.1	5.69 \pm 0.1
9	23.3	36.0 \pm 6.7	5.48 \pm 0.1	5.30 \pm 0.1
10	31.4	52.2 \pm 3.8	5.30 \pm 0.1	5.78 \pm 0.1
11	28.5	41.2 \pm 4.2	5.60 \pm 0.1	5.78 \pm 0.1
12	57.1	74.4 \pm 5.4	6.00 \pm 0.2	NT
13	19.0	31.4 \pm 1.7	5.30 \pm 0.0	5.40 \pm 0.1
14	17.5	27.1 \pm 2.1	5.48 \pm 0.1	NT
15	35.0	26.7 \pm 2.4	5.30 \pm 0.1	5.90 \pm 0.2
16	25.8	21.1 \pm 1.1	5.30 \pm 0.0	NT
17	55.7	68.1 \pm 5.3	6.04 \pm 0.2	NT
18	8.6	36.7 \pm 2.6	5.00 \pm 0.0	NT
19	32.8	51.4 \pm 4.6	5.85 \pm 0.2	NT
20	47.0	43.2 \pm 3.1	5.78 \pm 0.1	NT

^a Total bacterial infection.

^b Not tested.

Table 3

Mean and S.E. of NAGase activity, log SCC by Coulter® Counter or Fossomatic 360, according to udder bacteriology status, flock lactation number, time of milking and days in milk

Status	NAGase	log SCC ± S.E. (Coulter® Counter)	log SCC ± S.E. (Fossomatic)
Uninfected	22.9 ± 1.5	5.08 ± 0.02	5.15 ± 0.06
Infected	73.1 ± 2.7	6.11 ± 0.5	6.32 ± 0.5
Total (bulk)	47.9	5.66	5.81
Effects			
Bacteriology status	<0.0001	<0.0001	<0.0001
Flock	<0.0001	0.0001	NS ^a
Lactation	NS	NS	NS
Time of milking	NS	NS	NS
Day in milk	NS	NS	NS

^a Not significant.

significant difference was found among the various strains. *Corynebacteria* of various species also formed one of the main udder-infecting populations, but caused only a minor response, with the SCC increasing to 0.5×10^6 cells ml⁻¹. *Streptococci* were isolated from 22 halves and elicited a strong response, with the SCC increasing to 10^6 cells ml⁻¹. Lactation number (1, 2, and >3) did not significantly influence either the infection rate of udder halves or the SCC, although the percentage of udder halves with no

bacteria found was higher at first lactation than at the second, which, in turn, was higher than that at the third lactation (70.7, 64.6 and 52.2%, respectively). Milk yield was recorded by the owners at the sheep level, therefore, in cases where only one half was infected, each half was recorded as having the same milk yield. In spite of that sampling limitation, the milk yield was significantly higher in uninfected than in infected halves (2.77 vs. 2.55 kg/day, respectively) (Table 6). Moreover, the average milk yield of sheep with two uninfected halves was somewhat higher than that of sheep with only one half infected, and both were significantly higher ($P < 0.001$) than that of sheep with two infected udder halves (2.88 ± 0.07 , 2.80 ± 0.09 and 1.81 ± 0.12 kg/day, respectively). The effects of flock, lactation and days in milk were also significant ($P > 0.003$), with no significant interactions. Milk composition—fat, proteins and lactose—varied among flocks, with percentages in the ranges of 3.77–7.11 for fat, 4.77–5.90 for total protein, and 4.69–5.51 for lactose (Table 5). The effects of bacteriological status (infected or uninfected) were significant ($P < 0.001$) (Table 6). Mean total protein was lower in uninfected halves than in infected ones (5.13 and 5.50%, respectively) while lactose was higher in uninfected than in infected halves (5.30 and 4.72%, respectively). This effect was $P = 0.06$, with lower fat percentage in uninfected than in infected halves (4.68 ± 0.08 and 5.29 ± 0.14 , respectively), and with a wide variation among sheep. The flock effect on all the parameters was significant ($P < 0.001$), whereas that of lactation number was significant for protein

Table 4

Distribution of 815 dairy sheep udders (1630 halves) from 20 different flocks, according to udder bacteriology infection and log SCC

Bacteria	Number of udder half	Number of flock	log SCC ± S.E.
<i>S. aureus</i>	18	10	6.34 ± 0.5
CNS			
<i>S. chromogenes</i>	115	18	
<i>S. epidermidis</i>	87	16	
<i>S. haemolyticus</i>	17	11	
<i>S. simulans</i>	6	6	
<i>S. gallinarum</i>	3	2	
Non-identified CNS	120	20	
Total CNS	348		6.11 ± 0.2
<i>Streptococci</i>	22	10	6.20 ± 0.3
<i>Pseudomonas</i> spp	14	4	>6.70
<i>Corynebacterium</i> spp.	60	15	5.69 ± 0.1
Others and unidentified	77	20	
Total infection	521 (32%)		6.11 ± 0.5
Total uninfected ^a	1110 (68%)		5.00 ± 0.3

^a Uninfected halves, no bacterial growth on blood-agar and MacConkey plates.

Table 5

Means \pm S.E. of milk yield (kg per day), and percentages of fat, protein, and lactose of sheep from 12 different flocks

Flock	Milk (kg per day)	Fat (%)	Protein (%)	Lactose (%)
1	NT ^a	6.54 \pm 0.13	5.83 \pm 0.09	4.69 \pm 0.09
2	NT	5.84 \pm 0.13	5.19 \pm 0.05	5.13 \pm 0.05
3	NT	4.03 \pm 0.14	5.38 \pm 0.08	4.96 \pm 0.08
4	2.37 \pm 0.09	7.11 \pm 0.15	5.19 \pm 0.08	5.05 \pm 0.07
5	NT	5.88 \pm 0.11	5.52 \pm 0.08	5.24 \pm 0.04
7	2.69 \pm 0.08	3.77 \pm 0.11	4.77 \pm 0.04	5.51 \pm 0.05
8	3.80 \pm 0.09	4.30 \pm 0.13	5.20 \pm 0.04	5.40 \pm 0.04
9	2.90 \pm 0.10	3.90 \pm 0.10	5.30 \pm 0.04	5.20 \pm 0.02
10	NT	5.00 \pm 0.13	5.50 \pm 0.06	5.00 \pm 0.07
11	2.50 \pm 0.08	5.90 \pm 0.15	5.40 \pm 0.06	5.00 \pm 0.02
13	2.90 \pm 0.09	5.20 \pm 0.15	5.30 \pm 0.05	5.10 \pm 0.02
15	NT	4.50 \pm 0.18	5.90 \pm 0.07	5.00 \pm 0.05

^a Not tested.

Table 6

Means \pm S.E. of milk (kg per day), and percentages of fat, proteins, and lactose, according to udder half bacteriology status, flock lactation number, time of milking and days in milk effects

Status	Milk (kg per day)	Fat (%)	Protein (%)	Lactose (%)
Uninfected	2.77 \pm 0.04	4.68 \pm 0.08	5.13 \pm 0.03	5.30 \pm 0.02
Infected	2.55 \pm 0.08	5.29 \pm 0.14	5.50 \pm 0.06	4.72 \pm 0.07
Effects				
Bacteriology status	0.0009	0.0595	<0.0001	<0.0001
Flock	0.0002	<0.0001	<0.0001	<0.0001
Lactation	<0.0001	<0.0001	<0.0002	NS ^a
Time of milking	NS	NS	NS	NS
Day in milk	0.0033	NS	0.002	NS

^a Not significant.

and fat, and that of days in milk for protein only while time of milking (morning or evening) did not affect significantly any of those parameters (Table 6).

4. Discussion

Intramammary infection is the most important of the factors influencing SCC (Gonzalez-Rodriguez et al., 1995; Mavrogenis et al., 1999), which implies that a high SCC indicates a low milk quality. Payment schemes to encourage milk quality maintenance with regard to dairy cows have been implemented in most industrial countries, and such schemes are beginning to be implemented in some countries, with regard to dairy goats and sheep. Although clinical mastitis causes high economic losses, it is easier to eliminate its effects from the bulk milk than those of subclinical

mastitis. The present study aimed to identify the pathogens that cause subclinical udder infections in Israeli dairy sheep and evaluate their influence on milk yield and quality. The CNS, mainly *S. chromogenes* and *S. epidermidis*, were the most abundant bacterial isolates, and were found in almost all flocks tested, while other species of CNS were more endemic to some flocks. However, the effects of all CNS isolates in this study on the mammary glands, as indicated by the SCCs, were similar: they increased the SCC above 1.2×10^6 cells ml⁻¹. These results are in agreement with those of our previous study (Leitner et al., 2001) as well as with the findings of others (Fthenakis, 1994; Gonzalez-Rodriguez et al., 1995; Las Heras et al., 1999). These results indicate that the immune response to bacterial udder contamination, such as CNS, increased the SCC to a much greater extent in sheep than in the dairy cow. The implication of this result

is that for a given percentage of animals with udders contaminated with CNS, the increase in the SCC will be much higher in the bulk milk of dairy sheep than in that of cows. These data suggest that preventive methods, such as hygienic milking, improved management, and dry-off therapy should be required to prevent CNS intramammary infections throughout lactation, in order to maintain a low SCC in sheep milk. Studies of dry-off therapy were performed in the past (Chaffer et al., 2003), although more extensive work studying others preventive methods and their correlation with CNS infections are warranted.

Intramammary infection has been reported to affect milk production by reducing the yield (McCarthy et al., 1998; Fthenakis and Jones, 1990). In the present study milk production was recorded at the individual sheep level, therefore, the influence of intramammary infection was analyzed twice: first on the half level when, in the case of sheep with only one infected half, both halves were credited with the same amount of milk, and second by separating the sheep into three groups according to their udder condition—neither half infected, one half infected, and both halves infected. Both analyses indicated that subclinical udder infection of both halves reduced milk yield significantly. However, the results also suggested that when only one half was infected, the other half would compensate by producing more milk, so that the loss of milk was moderate.

In the case of dairy sheep, almost all the milk is processed to make cheese, so any changes in the dry matter, mainly casein concentration, have stronger influence on the industry. Interestingly in the present study, the percentages of fat and total protein were significantly higher in the infected half than in the uninfected one. These results are most likely reflecting the reduction in the volume of milk, and, with regard to the protein concentration, include albumin and whey, which have no relevant to the industry. In the case of dairy cows, Auldism et al. (1996) found that milk with high SCC exhibited an extended coagulation time and formed a weak coagulum, so that the cheese had increased moisture content and the dry matter yield was reduced. Schaar and Funke (1986) found that milk from a mastitic udder exhibited increased proteolytic activity. This, in turn, reduced the concentration of caseins (Auldism et al., 1996), which were replaced through increases in the albumin and

whey protein concentrations. Therefore, it is essential to elucidate the influence of subclinical mastitis on casein concentration and not on total protein. The decrease in lactose concentration in the infected halves has been reported elsewhere (Burriel, 1997). Although have no direct effect on cheese, it does indicate the low quality of the milk.

Escherichia coli, *Staphylococcus aureus*, *Streptococci* and *Pseudomonas* spp. are commonly isolated from cases of clinical mastitis (reviewed by Menzies and Ramanoon (2001)). Therefore, the number of sheep that were found to be infected with these bacteria, in the present study did not reflect their frequencies in the flocks. In the last 5 years, sheep and goat flocks in Israel have suffered from outbreaks of acute and subclinical mastitis caused by *Pseudomonas aeruginosa* (Rapoport et al., 1998). These outbreaks were devastating to the flocks involved, and resulted in the culling of every infected animal. Although in the present study, *P. aeruginosa* was isolated in only 4 out of the 20 flocks that were sampled, and in every case from subclinically infected udders, this finding was indicative, first, of the loss of milk quality according to the criterion of the SCC in the milk (above $5 \times 10^6 \text{ ml}^{-1}$), and, secondly, of the importance of regular bacteriological testing of the udders. *Corynebacterium* spp. were isolated in most flocks, but their influence on the SCC was only moderate, and they may infect only the teat canal and/or remain in the udder for a short time.

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