Acta vet. scand. 1995, *36,* 423-431.

Relations Between Udder Infection and Somatic Cells in Camel *(Camelus dromedarius)* Milk

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Abdurahman, O. A. Sh., H. Agab, B. Abbas and G. Åström: Relations between udder infection and somatic cells in camel (camelus dromedarius) milk. Acta vet. scand. 1995, 36, 423-431. – Quarter milk samples (n = 391) from 101 camels were examined to study the occurrence and causes of mastitis in traditionally managed camels in eastern Sudan and to evaluate the value of the California Mastitis Test (CMT), somatic cell count (SCC) and adenosine triphosphate (ATP) in the detection of subclinical mastitis in the camel.

One hundred and seventy (43.5%) of the quarter milk samples yielded pathogenic bacteria. *Streptococcus agalactiae,* other *Streptococcus* spp., *Staphylococcus aureus,* coagulase-negative staphylococci, and *Escherichia coli* were isolated from milk. Thirty-two (8.2%) quarter milk samples yielded mixed cultures, and 189 (48.3%) yielded no growth.

Mean values for CMT, SCC and ATP were higher for quarters infected with major pathogens. However, a significant number of quarter milk samples had elevated values in these tests but were from quarters from which no bacteria were isolated. The ability of the tests to predict a positive bacteriology increased slightly when 2 or 3 tests were combined.

inflammation; diagnostic tests; Mastitis; CMT; ATP; bacteriology; Sudan.

Introduction

There is extensive literature on bovine mastitis and somewhat less reported research on ovine and caprine mastitis. Very little is known about mastitis in the camel, because the disease was thought to be rare in this species (*Bolbol* 1982). However, cases of mastitis in the camel have recently been reported from several countries, including Egypt (*Mostafa et al.* 1987, *Saad & Thabet* 1993), Saudi Arabia (*Barbour et al.* 1985, *Hafez et al.* 1987), Somalia (*Abdurahman et al.* 1991, *Arush et al.* 1984), Sudan (*Obeid* 1983, *Bakhiet et al.* 1992), and the United Arab Emirates (*Quandil & Oudar* 1984).

Clinical mastitis in the camel can be detected by examination of the udder, the milk, or both. Detection of subclinical mastitis, however, is difficult and depends mainly on various test procedures. Bacterial infections are considered to be the primary cause of mastitis in domestic animals. Camels under traditional management systems are usually kept far from urban areas where laboratory facilities are scarce or non-existent. Hence, bacteriological examination of milk presents considerable difficulties.

Inflammation of the mammary gland results in major alterations in the composition of milk, including an increase of somatic cells in milk (*Schalm et al.* 1971). Methods measuring increased numbers of somatic cells and related compositional changes have been used widely

as aids in the diagnosis of subclinical mastitis in cows. Diagnostic accuracy of these methods has been evaluated for cows (*Emanuelson et al.* 1987), ewes (*Maisi et al.* 1987), and goats (*Dulin et al.* 1982), but not for camels. We conducted a survey to study the occurrence and causes of mastitis in traditionally managed camels in the Sudan as well as to assess the value of inflammatory markers like the California Mastitis Test (CMT), somatic cell count (SCC) and the level of adenosine triphosphate (ATP) in the diagnosis of camel mastitis.

Materials and methods

Camels

We examined 101 lactating camels among 778 breeding females from 20 herds in Rufa and Kassala districts in eastern Sudan. All camels that were lactating and accessible during the visit were sampled. Camels were kept by nomadic pastoralists under traditional management. Information on age, parity, lactation stage and previous history of mastitis was gathered.

Sampling

The udder was examined for visible abnormalities, palpated, and the secretion inspected. Teat ends were cleaned vigorously and rubbed with cotton moistened in 70% alcohol. The first streams of milk from each quarter were discarded, and about 5 to 10 ml of foremilk were collected into 10-ml polyethylene tubes and kept on ice.

Two to 6 h after milk collection, a portion (about 20 µl for ATP and 100 µl for bacteriology) of each quarter milk sample was transferred to 2 filter paper discs in a cassette designed to collect and transport milk samples (MASTISTRIP; SVA, Uppsala, Sweden) for later analysis of ATP and bacteriology (*Nilsson et al.* 1994). The cassette has 4 projectable pins, one for each teat. A box containing silica gel in the cassette ensures rapid drying of the discs to stabilize samples. A portion of each milk sample was used for the CMT test and preparation of smears for SCC. The remaining milk was frozen at - 20°C and, together with the MASTISTRIP cassettes and smears, was transported to Sweden for further analysis.

California Mastitis Test (CMT)

CMT was carried out using the method described by *Schalm & Noorlander* (1957). Reactions were graded 1, 2, 3, 4, or 5 according to the Scandinavian recommendations (*Klastrup & Schmidt Madsen* 1974).

Somatic cell count (SCC)

The milk samples were allowed to reach room temperature and were shaken carefully before smearing. Milk samples (0.01 ml) were spread over 1 cm^2 of an ordinary alcohol precleaned glass slide by means of a microsyringe (Applied Research Institute, New York, USA). Four such squares were prepared from each sample. The film was dried, stained with methylene blue (*Prescott & Breed* 1910) and examined under oil immersion using a light microscope (1000X) .

Adenosine Triphosphate (ATP)

The MASTISTRIP discs for the ATP (n = 320) were cut down and shaken in a Tris-EDTA buffer and measured with a bioluminescence technique using an ATP monitoring reagent (Wallac/LKB, Stockholm, Sweden) as described earlier (*Olsson et al.* 1986).

Bacteriological Examination

The MASTISTRIP paper discs for bacteriology were chopped into test tubes and 0.5 ml of RPMI 1640 culture medium was added. The test tubes were shaken and 50 µl of the mixture were cultured on bovine blood agar plate. Isolation and identification of bacteria were carried

Table 1. Distribution of age (years), parity (number) and lactation stage (months) in 100 camels.

	Age year/priority number/lactation months											
Factor	1	2	3	4	5	6	7	8	9	10	11	≥12
Age					8	5	14	16	20	10	12	15
Parity	11	28	28	19	11	3						
Lactation	30	21	10	8	3	7	7	5	1	3	0	5

out according to the Scandinavian recommendations on examination of quarter milk samples (*Klastrup* 1975).

Quarters were classified as not infected (NI) if no organisms were isolated, infected with major pathogens (MAP) if *Streptococcus agalactiae,* other *Streptococcus* spp., *Staphylococcus aureus,* and *Escherichia coli* were isolated, and infected with minor pathogens (MIP) if coagulase-negative staphylococci were isolated. Quarters were also classified as "normal" or "abnormal". Quarters were normal if no organism was isolated, the udder had no injuries or indurations, the appearance of milk was normal, and no previous history of mastitis was recorded, and "abnormal" otherwise. Mixed cultures were not included in the analysis.

Statistical Analysis

Because of the skewed distribution, SCC and ATP values were log transformed (base 10) prior to statistical calculations. The effect of various factors on mastitis indicators was evaluated with an ordinary least squares means analysis of variance as applied in PROC GLM of SAS (SAS Inst. Inc. 1986). The statistical model included effects of bacteriological group (MAP, MIP, NI), age (5-11 or ≥12) and parity (1-3 or ≥4) of the camel, lactation month (1-10 or ≥12), previous history of mastitis (yes or no), udder and quarter appearance (clinically normal, or abnormal). The effects of age, parity and lactation stage were included in the model in order to adjust for the effect of bacteriological grouping on the mastitis indicators and will not be discussed further.

The ability of mastitis indicators to distinguish between "normal" and "abnormal" quarters was assessed by means of logistic regression analyses using PROC LOGIST of SAS (SAS Inst. Inc. 1985). Mastitis indicators were evaluated separately and in combinations. The model included, in addition to mastitis indicators, effects of age, parity, and lactation stage. Status of the quarter was predicted using parameters estimated in the logistic regression analyses, and sensitivity, specificity and percentages of classifications that were correct, false positive and false negative rates were calculated accordingly (SAS Inst. Inc. 1985).

Results

Data on age, parity and lactation stage are shown in Table 1. Examination of the udder revealed the presence, in some animals, of a device used for regulating milk consumed by the

Table 2. Bacteriological findings in quarter milk samples of camels.

Microorganisms isolated	No.	%
No growth	189	48.3
Coagulase-negative staphylococcus	68	17.4
Staphylococcus aureus	21	5.4
Streptococcus agalactiae	69	17.6
Streptococcus spp.	10	2.6
Escherichia. coli	2	0.5
Mixed cultures	32	8.2
Total	391	100

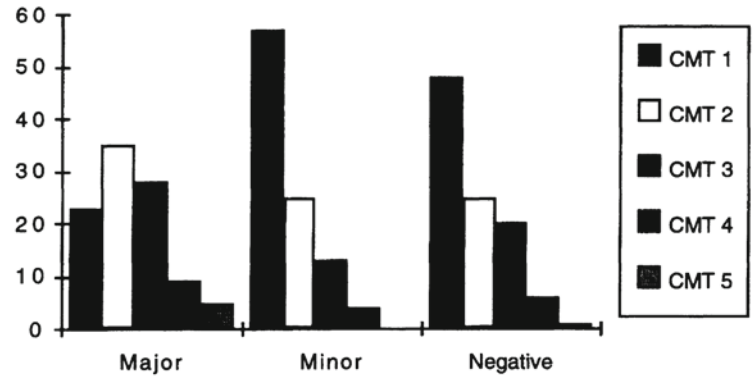

Figure 1. Percentage distribution of CMT scores in relation to udder infection status.
ATP = Adenosine triphosphate. CMT = California Mastitis Test. MAP = major pathogen, MIP= minor pathogen, NI= non-infected. SCC = somatic cell count.

calf. As the calf is not separated from its dam, the objective of the device is to prevent the calf from suckling all the milk. A string is used to tie up 2 or more teats together with a sharp stick.

Table 3. Number of observations, means, and standard deviations of log ATP, log SCC and CMT in milk from quarters infected with major pathogens (MAP), minor pathogens (MIP) and from bacteriologically negative quarters (NI).

	No	Mean	SD.
Major pathogen:			
logATP	83	0.74	0.64
logSCC	98	6.18	0.67
CMT	100	2.38	1.08
Minor pathogen:			
logATP	63	0.47	0.61
logSCC	67	5.85	0.62
CMT	68	1.64	0.78
Non-infected:			
logATP	143	0.54	0.79
logSCC	180	5.96	0.76
CMT	180	1.85	0.98

ATP = Adenosine triphosphate
CMT = California Mastitis Test
SCC = somatic cell count

Furthermore, most of the camel udders were infested with ticks and showed lesions on the teats and udder skin. The teat lesions often had a circular form, were located at the upper half of the teat and, were mostly old, non-penetrating superficial wounds. Ten (9.9%) camels had stenosis in one or more teats, and the passage of milk was obstructed. Of the 101 dromedary udders examined for mastitis, 6 (5.9%) had clinical mastitis manifested by swollen udder and supramammary lymph nodes, severe thelites, and visible alteration of the color and consistency of milk. In 2 of these, the udder was hardened, and the secretion was grossly altered with oozing floccules of pus. Two camels also had teat fistulae.

Of 391 quarter milk samples examined, 170 (43.5%) yielded pathogenic bacteria. Results of the bacteriological examination are shown in Table 2. Microorganisms recovered were *Streptococcus agalactiae,* other *Streptococcus* spp., *Staphylococcus aureus,* coagulase-negative staphylococci, and *Escherichia coli.* Thirty-two (8.2%) quarter milk samples yielded unspecified mixed cultures, and 189 (48.3%) yielded no bacterial growth.

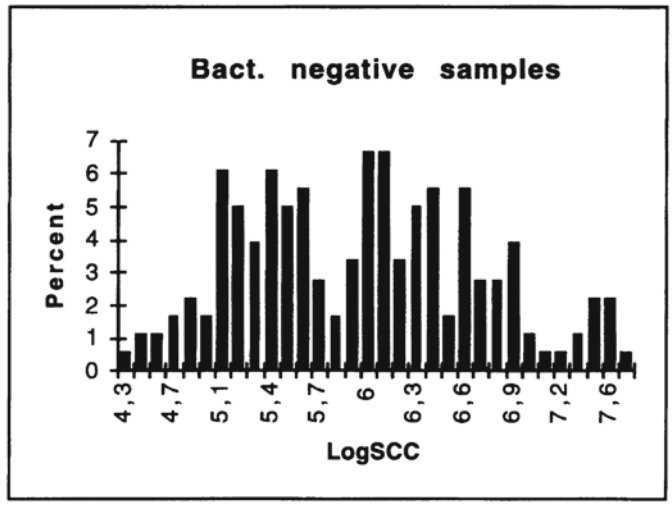

Figure 2. Distribution of somatic cells in non-infected quarter milk samples.

Percentage distribution of CMT scores in relation to the bacteriological groupings is shown in Fig. 1. Forty-two percent of quarters infected with MAP scored 3 or more compared with 14 and 27% of quarters infected with MIP and NI quarters, respectively. A high proportion of NI quarters had higher CMT scores than quarters infected with MIP.

Numbers of observations, means, and standard deviations of CMT, SCC and ATP in quarters classified as MAP, MIP, and NI are shown in Table 3. Quarters infected with MAP had higher mean values for all parameters examined than NI quarters and in quarters infected with MIP. However, values of NI quarters varied considerably (Fig. 2) and means were not significantly different for CMT, SCC and ATP of quarters infected with MIP and of NI quarters.

Inflammatory markers within each bacteriological grouping (Table 4) were significantly correlated ($p < 0.0001$). SCC was more strongly correlated with ATP than with CMT in milk samples from all bacteriological groupings. Both CMT and ATP were significantly influenced by the presence or absence of MAP. However, NI had higher, although not significant, values than MIP (Table 5). CMT, SCC and ATP also had higher values in quarters that had

Table 4. Rank correlations among results obtained by log SCC, CMT and log ATP in milk from quarters infected with major pathogens (MAP), minor pathogens (MIP) and quarters that are not infected (NI) ($p < 0.0001$).

Components	logSCC	logATP
Major pathogen:		
CMT	0.59	0.51
logSCC		0.64
Minor pathogen:		
CMT	0.59	0.59
logSCC		0.65
Non-infected:		
CMT	0.65	0.63
logSCC		0.70

ATP = Adenosine triphosphate
CMT = California Mastitis Test
SCC = somatic cell count

Table 5. Least squares means (± standard error) for the effect of bacteriological group.

Bactgroup	CMT	log_{10} SCC	log_{10} ATP.
MAP	2.47 ± 0.13	6.31 ± 0.09	0.99 ± 0.10
MIP	1.72 ± 0.13	5.95 ± 0.10	0.59 ± 0.10
NI	2.12 ± 0.11	6.15 ± 0.07	0.78 ± 0.08

ATP = Adenosine triphosphate
CMT = California Mastitis Test
MAP = major pathogen,
MIP= minor pathogen,
NI= non-infected.
SCC = somatic cell count

a previous history of mastitis compared with quarters that had no previous mastitis history (Table 6). All 3 parameters were significantly higher for quarters that had been judged to be clinically abnormal. This response occurred independent of bacterial isolations.

Results from the logistic regression analyses are presented in Table 7. Inclusion of the mastitis indicators in addition to the demographic variables of age, parity, and lactation month did not improve the ability to predict whether a quarter was normal, when judged on sensitivity or percentage correctly classified. However,

Table 6. Least square means (± standard error) for the effect of previous history of mastitis.

Previous mastitis	CMT	log_{10} SCC	log_{10} ATP.
Yes	2.15 ± 0.12	6.25 ± 0.09	0.87 ± 0.09
No	1.88 ± 0.10	5.87 ± 0.07	0.55 + 0.07

ATP = Adenosine triphosphate
CMT = California Mastitis Test
SCC = somatic cell count

SCC and ATP in combination increased the specificity from 33.7 to 41.3%. Sensitivity and also percentage of quarters correctly classified was very much influenced by the low proportion of quarters considered to be normal (75 out of 305).

Discussion

The microorganisms that were isolated in the present study were regarded as important mastitis pathogens in the camel in the Sudan (*Obeid* 1982, *Bakhiet et al.* 1992) and elsewhere (*Arush et al.* 1984, *Barbour et al.* 1985, *Hafez et al.* 1987, *Quandil & Oudar* 1984) as well as in other animals (*Schalm et al.* 1971). In one study (*Barbour et al.* 1985), *Micrococcus* spp.

Table 7. Sensitivity (SE), specificity (Sp), % correct classified (CO), false positive rate (FP) and false negative rate (FN) in predicting abnormal quarter status[1].

Variables (s)[2]	SE(%)	SP(%)	CO(%)	FP(%)	FN(%)
CMT	95.4	30.4	79.5	19.2	31.7
Log_{10} SCC	95.4	33.7	80.4	18.3	29.7
Log_{10} ATP.	94.6	36.4	80.4	17.8	31.7
CMT+log_{10} SCC	96.4	34.8	79.7	18.6	31.9
CMT+log_{10} ATP	93.2	34.7	79.1	18.2	38.1
Log10 SCC+log_{10} ATP	94.9	41.3	81.9	16.5	27.9
CMT+log_{10} SCC+log_{10} ATP	93.9	41.3	81.0	16.9	31.1

[1]Quarters were defined as normal if no bacteria was isolated, there was no previous history of mastitis, a normal udder, and normal milk, and as abnormal otherwise.
[2]In addition to the effects of age, parity and lactation month, that were included in the model.

ATP = Adenosine triphosphate
CMT = California Mastitis Test
SCC = somatic cell count

was indicated as the main cause of camel mastitis, but in another study (*Obeid* 1983), these species were considered to be non-pathogenic. In the present study, quarters infected with coagulase-negative staphylococci had mean values of inflammatory markers that were equal to or less than the NI quarters. Coagulase-negative staphylococci were thus regarded as MIP.

Milk samples from quarters infected with MAP had higher values for CMT, SCC, and ATP than samples from quarters that were infected with MIP and from NI quarters (Table 5). There were, however, many samples that showed high values for CMT, SCC, and ATP from which bacteria were not isolated. The distribution of logSCC and logATP in milk from quarters infected with MAP, MIP and NI consequently had a wide overlap and was difficult to separate clearly. There was also only small improvement in the ability to predict the status of the quarter by combining several indicators.

The high variability concerning SCC and ATP of the bacteriologically negative samples suggests a heterogeneous population. Some of these samples might be bacteriologically false negatives. From Fig. 2, it can be hypothesized that 2 (or more) distinct populations of samples were represented. The reason is unclear and requires further investigation. Using ELISA, investigators detected bacterial antigens in 67.7% of quarter milk samples from clinical mastitis cases in cows that did not yield bacterial pathogens on culture (*Zorah et al.* 1993). In the present study, we used MASTISTRIP for sampling milk to detect udder infection. This method has 96% agreement with that of bacteriological reference method and offers the advantage of preserving samples during transportation (*Nilsson* 1994). Other possible explanations for the variability of NI quarters could be the occurrence of non-specific subclinical mastitis. This possibility is supported by the fact that most of the examined camels had lesions on the teats and udder skin. Teat lesions in the present camels resulted mainly from the string used for tying teats. Investigators (*Agger & Willeberg* 1986) have previously found a significant association between teat lesions and subclinical mastitis even though the lesions were small and superficial. In addition, close to half of the camels had a previous history of untreated mastitis.

The ATP content in fresh milk is reported to decrease up to 20% of its value per h if not stabilized (*Olsson et al.*1986). In the present study, 2 to 6 h elapsed before the milk sample was transferred to MASTISTRIP cassettes. Therefore, the values of ATP presented here might be an underestimate of true values. Despite this, ATP values for both infected and NI quarters showed stronger correlation with SCC than with CMT. This result may be due to the presence in mastitis-free camel milk of cell fragments (apart from somatic cells). Cell fragments are annucleate particles similar in size and shape to leucocytes and contain cytoplasmic organelles such as endoplasmic reticulum and mitochondria (*Abdurahman et al.* 1992). The latter is a possible source of ATP other than cellular ATP. The cell fragments may also be counted as cells when using direct microscopic cell count and an electronic counter (*Dulin et al.* 1982). The methylene blue stain commonly used for direct microscopic counting is inappropriate for differential counting of leucocytes, epithelial cells and cellular fragments (*Smith & Sherman* 1994). The electronic counter, on the other hand, enumerates particles according to their size and the cellular fragments, being similar in size to proper cells, are also counted (*Smith & Sherman* 1994). The origin of cell fragments in camel milk has not been studied. However, similar cell fragments found in goat milk are thought to have resulted from the apocrine secretory process in the goat mammary gland (*Wooding et al.* 1970). Studies on the morphol-

ogy of the lactating mammary gland of the camel may elucidate the origin of cell fragments in camel milk and the mechanism of milk secretion in the camel.

There is very little information regarding the cellular content of camel milk and the udder response to mastitis pathogens. An earlier study (*Kospakov* 1978) reported a mean cell count of 1.3 x 10^6 cells/ml of milk during normal lactation, 3.3 x 10^6 cells/ml during pregnancy and 7.9 x 106 cells/ml at the start of the dry period in bacteria-free camel milk samples *(Camelus bactrianus).* In infected camels, a cell count of 7.4 to 12 x 10^6 cells/ml was observed. Another study (*Mostafa et al.* 1987) reported an increase in the number of somatic cells in camel milk in infected quarters. The apparently high rate of intramammary infection in traditionally managed camels warrants further investigation.

Acknowledgements

We are grateful to the camel owners and herders for their cooperation, and to Agr. Dr. Ulf Emanuelson for help with statistical analysis. This study was supported by Swedish Agency for Research and Cooperation.

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Sammanfattning

Samband mellan juverinfektion och somatiska celler i mjölk från kamel (Camelus dromedarius).

Mjölkprov från individuella juverfjädedelar (n = 391) från 101 kameler undersöktes i syfte att studera förekomst av och orsaker till mastit hos traditionallt skötta djur i östra Sudan. Syftet var också att utvärdera olika metoder, California Mastitis Test (CMT), cell räkning (SCC) och bestämning av adenosintrifosfat (ATP) för diagnostik av subklinik mastit.

I mjölken från 170 (43.5%) juverfjärdedelar växte patogena bakterier. Streptococcus agalactiae, andra streptokocker, Staphylococcus aureus, och Escherichia coli isolerades från mjölken. I mjölken från 32 (8,2%) fjärdedelar isolerades blandkulturer. Ingen bakterieväxt noterades i 189 (48.3%) prover.

Medelvärdet för CMT, SCC, och ATP var förhöjda i mjölk från fjärdedelar som var infekterade med utpräglat patogena bakterier (major pathogens). Ett signifikant antal prover från icke infekterade fjärdedelar hade emellertid också förhöjda värden. Möjligheten att med hjälp av dessa test förutsäga huruvida infektion föreligger ökade svagt när resultaten av 2 eller 3 test kombinerades.

(Received March 15, 1995; accepted June 16, 1995).

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