Arq. Bras. Med. Vet. Zootec., **v.64, n.2, p.495-498, 2012**

Communication

[Comunicação]

Lactation stage and udder health status of Santa Ines ewes

[*Estágio da lactação e saúde da glândula mamária em ovelhas da raça Santa Inês*]

M.G. *Blagitz*, N.R. *Benites*, P.A. *Melville*, C.F. *Batista*, P.S. *Betiol*, M.R. *Azedo*, V. *Gomes*, F.N. *Souza*, A.M.M.P. *Della Libera*[*]

Faculdade de Medicina Veterinária e Zootecnia - USP - São Paulo, SP

Mastitis is an important disease of sheep with serious economic losses, even in meat-producing sheep (Clements *et al*., 2003; Moroni *et al*., 2007). It is well known that the composition of ewe milk undergoes marked changes throughout the lactation (Cuccuru *et al*., 1997; Bergonier *et al*., 2003; Sevi *et al*., 2004). These changes during the lactation may trigger periods of increased or decreased susceptibility to mastitis, or may even turn out qualitative changes in milk (Cuccuru *et al*., 1997; Bergonier *et al*., 2003). Differences among breeds were also reported. Indeed, the majority of the information available for sheep is concerned predominantly with dairy sheep in Mediterranean countries. So, there are few data non-dairy sheep breeds (Conington *et al*., 2008).

Thus, the purpose of the present study was to make an inference about the risk of infection and inflammation regarding the phase of lactation of Santa Ines ewes.

The lactation period of 33 recently lambed Santa Ines ewes from extensive and semi-intensive management systems was followed. Sheep were submitted to physical examination of their udders (Baumgartner, 2005), strip cup test, California Mastitis test (CMT) and collection of milk samples for bacteriological and somatic cell count (SCC). Throughout lactation period of the assessed ewes, samples were grouped, according to days after parturition in: stage 1 – ewes from the 3rd to the 30th day postpartum; stage 2 – from the 31st to the 60th day postpartum; stage 3 – from the 61st to the 90th day postpartum; and stage 4 – from the 91st to the 120th day postpartum.

The first strip was discarded, and then the milk samples for bacteriology investigation were aseptically collected. Milk samples were streaked onto a 5% sheep-blood agar. The plates were aerobically incubated at 37^{o}C and examined at 24 and 48h. Positive samples were submitted to biochemical analyses for the identification of the bacteria (Oliver *et al*., 2004).

The direct microscopic SCC (DMSCC) and differential leukocytes count were performed in duplicate using hematoxylin-eosin. The purpose of the search was to reach a total count from 100 microscopic fields, which the leukocytes were differentiated in mononuclear and polymorphonuclear. The working factor was 3,751 in all cases. Samples analyzed by automatic SCC (Bentley Instruments, Chaska, MN, 55318, USA) were collected in flasks containing bronopol and sent to the Milk Quality Laboratory at the Escola Superior de Agricultura Luiz de Queiroz, ESALQ-USP.

Samples collected for the strip cup test and bacteriological examination were ranked by scores, so that negative samples scored 0 and positive samples scored 1. CMT results were graded in: 1) no precipitate; 2) trace precipitate; 3) distinct precipitate/weak gel formation; 4) distinct gel formation; 5) strong gel formation (Clements *et al*., 2003). During the study, some animals were sold by the owners and others died.

Recebido em 4 de março de 2011
Aceito em 12 de janeiro de 2012
*Autor para correspondência (*Corresponding author*)
E-mail: dellalibera@usp.br

To determine differences between old and new infections, the Cochran Test and the Qui-Square Test were used. The Cochran Test was useful to evaluate the missing teats. During this study, it was considered a new infection, every time a new bacterium was identified. To compare tests used during this study, ANOVA, Friedman test and pearson correlation coefficient test, were applied.

Most of the milk samples collected were negative (80.2% of all samples). Among positive samples, most of the isolates were *Staphylococcus* spp. (94.3%) (Table 1), which are the main etiological agents of intramammary infections (IMI) in small ruminants (Bergonier *et al*., 2003; Moroni *et al*., 2007).

Table 1. Frequency of bacterial genera and species isolated from milk samples collected from 33 Santa Ines ewes throughout lactation period

	Stage 1	Stage 2	Stage 3	Stage 4	Amount of 4 stages
Negative samples	45 (80.4%)	49 (79.0%)	32 (76.2%)	16 (80.0%)	142 (80.2%)
Positive samples	11 (19.6%)	11 (21.0%)	9 (23.8%)	4 (20.0%)	35 (19.8%)
Amount of samples	56	60	41	20	177
P value		P = 0.98			
Isolated bacteria					
Staphylococcus spp.	6 (10.7%)	3 (4.8%)	4 (9.5%)	0 (0.0%)	13 (7.3%)
Staph. kloosii	4 (7.1%)	2 (3.2%)	2 (4.8%)	0 (0.0%)	8 (4.5%)
Staph. hyicus	0 (0.0%)	2 (3.2%)	2 (4.8%)	2 (10.0%)	6 (3.4%)
Staph. haemolyticus	0 (0.0%)	1 (1.6%)	1 (2.4%)	1 (5.0%)	3 (1.7%)
Staph. camosus	1 (1.8%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.6%)
Staph. chromogenes	0 (0.0%)	1 (1.6%)	0 (0.0%)	0 (0.0%)	1 (0.6%)
Staph. warneri	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (5.0%)	1 (0.6%)
Streptococcus dysgalactiae	0 (0.0%)	1 (1.6%)	0 (0.0%)	0 (0.0%)	1 (0.6%)
Streptococcus uberis	0 (0.0%)	1 (1.6%)	0 (0.0%)	0 (0.0%)	1 (0.6%)

No differences were found in the results of bacteriological analyses in relation to the stage of lactation (P=0.98) and regarding new infection (P=0.49; results not showed). This is in agreement with others who described that the prevalence of subclinical IMI throughout lactation period remained relatively constant (Al-Majali and Jawabreh, 2003; Bergonier *et al*., 2003). Indeed, the persistence of subclinical IMI during lactation is often high due to the poor detection and elimination of such infections during lactation, and to their frequent staphylococcal origin (Bergonier *et al*., 2003; Blagitz *et al*., 2008). Conversely, some authors observed that dairy sheep were more susceptible to infections in the beginning of lactation (Bergonier *et al*., 2003), or both in the end and in the beginning of lactation (Menzies and Ramanoon, 2001).

Physical examination of the udder of most ewes did not show any clinical changes (results not showed). No significant difference was found in CMT (P=0.90) and DMSCC by polymorphonuclear cells count (P=0.52), mononuclear cells count (P=0.14) and total cells count (P=0.17) throughout lactation (Table 2). Nevertheless, a tendency toward an increase in automatic SCC during the last stage was observed (P=0.051) (Table 2). Further studies also showed increased cell counts in the end of lactation that may be related to a greater resistance to infection in this period (Cuccuru *et al*., 1997; Bergonier *et al*., 2003; Blagitz *et al*., 2008).

The presence of bacteria did not change the proportion of polymorphonuclear and mononuclear cells throughout lactation, as seen in DMSCC. Thus, the variation of SCC observed in these stages may have been due to physiological reasons.

Table 2. Average, standard variation (SV), median, and range values (cells/mL) of automatic somatic cell count (SCC) and direct microscopic SCC (DMSCC), and California Mastitis Test CMT) results obtained from milk samples collected from 33 Santa Ines ewes throughout lactation period

Automatic SCC	Stage 1	Stage 2	Stage 3	Stage 4
Average	864,491.1	727,525.4	1,458,964.3	3,125,016.7
SV	1,618,484	1,440,557.9	4,303,187.8	4,522,255.4
Median	217,000	208,000	282,000	754,000
Range	8,000– ,062,000	12,000–7,359,000	5,000–27,600,000	155,000–12,978,000
Amount of samples	56	59	42	18
P value			P = 0.051	
DMSCC				
Polymorphonuclear cells				
Average	349,129	205,771.6	84,343.6	123,021
SV	1,158,004.3	822,255.9	165,243.8	207,109.8
Median	10,713	10,713	17,855	57,136
Range	0–7,820,490	0–6,274,247	0–1,007,022	3,571–928,460
Amount of samples	56	61	42	20
P value			P = 0.52	
Mononuclear cells				
Average	141,182	43,496	52,884.8	89,632.1
SV	351,161.7	92,321.6	125,830.1	113,049.1
Median	10,713	7,142	10,713	35,710
Range	0–1,892,630	0–467,801	0–789,191	0–367,813
Amount of samples	56	61	42	20
P value			P = 0.14	
Total cells (DMSCC)				
Average	490,311.1	249,267.5	137,228.4	212,653.1
SV	1,384,944.4	89,409.6	287,871	283,994
Median	24,997	17,855	26,782.5	114,272
Range	3,571–8,641,820	3,571–6,742,048	3,571–1,796,213	3,571–1,171,288
Amount of samples	56	61	42	20
P value			P = 0.17	
CMT				
Negative (grade 1)	36 (64.3%)	40 (65.6%)	22 (52.4%)	9 (45.0%)
Positive samples	20 (35.7%)	21 (34.4%)	20 (47.6%)	11 (55.0%)
Samples graded 2	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Samples graded 3	12 (21.4%)	15 (24.6%)	13 (31.0%)	4 (20.0%)
Samples graded 4	8 (14.3%)	5 (8.2%)	5 (11.9%)	5 (25.0%)
Samples graded 5	0 (0.0%)	1 (1.6%)	2 (4.8%)	2 (10.0%)
Amount of samples	56	61	42	20
P value			P = 0.90	

In conclusion, no differences were observed in the bacteriological examination in any stage of lactation which point out a higher persistence of IMI in these animals. Furthermore, no significance differences in CMT, automatic and microscopic SCC were found during the lactation period, however, a tendency toward a higher SCC was encountered in the last stage.

Keywords: ewe, somatic cell count, lactation stage, mastitis

RESUMO

Avaliou-se o risco de infecção em diferentes fases da lactação em 33 ovelhas da raça Santa Inês. Inicialmente a glândula foi submetida ao exame físico e à prova de fundo escuro. Posteriormente, amostras de leite foram coletadas assepticamente para a realização de exame bacteriológico – California Mastitis *teste* –*, e contagens microscópica e automática de células somáticas. Nenhuma diferença foi observada entre as distintas fases de lactação. Observou-se alta persistência de infecções intramamárias, e tendência a maior contagem de células somáticas no último período de lactação, que pode ser oriunda da maior resistência a infecções neste período.*

Palavras-chave: ovelha, contagem de células somáticas, fase da lactação, mastite

ACKNOWLEDGEMENTS

The authors would like to thank to FAPESP (proc. n. 2003/08582-7) for financial support.

REFERENCES

AL-MAJALI, A.M.; JAWABREH, S. Period prevalence and etiology of subclinical mastitis in Awassi sheep in southern Jordan. *Small Rum. Res.*, v.47, p.243-248, 2003.

BAUMGARTNER, W. *Clinical propedeutic of the internal diseases and skin diseases of the domestic animals*. 6.ed. Berlin-Wien: Parey, 2005. 382 p.

BERGONIER, D.; CRÉMOUX, R.; RUPP, R. *et al*. Mastitis of dairy small ruminants, *Vet. Res.*, v.34, p.689-716, 2003.

BLAGITZ, M.G.; BATISTA, C.F.; SOUZA, F.N. *et al*. Perfil celular e microbiológico do leite de ovelhas Santa Inês no período lactante e pós-desmame. *Pesq. Vet. Bras*., v.28, p.417-422, 2008.

CLEMENTS, A.C.A.; TAYLOR, D.J.; FITZPATRICK, J.L. Evaluation of diagnostic procedures for subclinical mastitis in meat-producing sheep. *J. Dairy Res.*, v.70, p.139-148, 2003.

CONINGTON, J.; CAO, G.; STOTT, A. *et al*. Breeding for resistance to mastitis in United Kingdom sheep, a review and economic appraisal. *Vet. Rec.*, v.162, p.369-376, 2008.

CUCCURU, C.; MORONI, P.; ZECCONI, A. *et al*. Milk differential cell counts in relation to total counts in Sardinian ewes. *Small Ruminant Res.*, v.25, p.169-173, 1997.

MENZIES, P.I.; RAMANOON, S.Z. Mastitis of sheep and goats. *Vet. Clin. N. Am.: Food Anim. Pract.*, v.17, p.333-355, 2001.

MORONI, P.; PISONI, G.; VARISCO, G. *et al*. Effect of intramammary infection in Bergamasca meat sheep on milk parameters and lamb growth. *J. Dairy Res.*, v.74, p.340-344, 2007.

OLIVER, S.P.; LEWIS, M.J.; GILLESPIE, B.E. *et al*. *Microbiological Procedures for the Diagnosis of Bovine Udder Infection and Determination of Milk Quality*. 4.ed. Verona: National Mastitis Council, 2004. 47p.

SEVI, A.; ALBENZIO, M.; MARINO, R. *et al*. Effects of lambing season on stage of lactation on ewe milk quality. *Small Rum. Res.*, v.51, p.251-259, 2004.