Brazilian Journal of Microbiology 44, 2, 493-498 (2013)
ISSN 1678-4405

Research Paper

Comparative analysis of *agr* groups and virulence genes among subclinical and clinical mastitis *Staphylococcus aureus* isolates from sheep flocks of the Northeast of Brazil

Lara M. de Almeida[1], Mayra Zilta P.R.B. de Almeida[2], Carla L. de Mendonça[2], Elsa M. Mamizuka[1]

[1]Departamento de Análises Clínicas e Toxicológicas, Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, São Paulo, SP, Brazil.
[2]Clínica de Ruminantes, UniversidadeFederal de Pernambuco, Recife, PE, Brazil.

Submitted: July 27, 2011; Approved: September 10, 2012.

Abstract

Staphylococcus aureus is one of the most frequent mastitis causative agents in small ruminants. The expression of most virulence genes of *S. aureus* is controlled by an accessory gene regulator (*agr*) locus. This study aimed to ascertain the prevalence of the different *agr* groups and to evaluate the occurrence of encoding genes for cytotoxin, adhesins and toxins with superantigen activity in *S. aureus* isolates from milk of ewes with clinical and subclinical mastitis in sheep flocks raised for meat production The *agr* groups I and II were identified in both cases of clinical and subclinical mastitis. Neither the *arg* groups III and IV nor negative *agr* were found. The presence of *cflA* gene was identified in 100% of the isolates. The frequency of *hla* and *lukE-D* genes was high - 77.3 and 82.8%, respectively and all isolates from clinical mastitis presented these genes. The *sec* gene, either associated to *tst* gene or not, was identified only in isolates from subclinical mastitis. None of the following genes were identified: *bbp*, *ebpS*, *cna*, *fnbB*, *icaA*, *icaD, bap, hlg*, *lukM-lukF-PV* and *se-a-b-d-e*.

Key words: *Staphylococcus aureus*, *agr* groups, virulence genes, mastitis, sheep flock.

Introduction

Mastitis in sheep has a major economic impact for the farmer when compared to the effects on cow and goat. It can lead to loss of the mammary gland and even death of ewe and/or lamb (Menzies and Ramanon, 2001). The incidence of clinical mastitis in dairy sheep is usually lower than 5% per year. In a low percentage of herds, the incidence is higher and may exceed 30-50% of the animals, causing mortality or culling of up to 70% of the herd (Bergonier *et al.*, 2003). If untreated, it also constitutes a serious problem in ewes raised for meat production. The Santa Inês breed stands out because gains weight fast and it also reproduces quickly all through the year, representing thus an excellent matrix for breeding. However, Santa Inês breed presents characteristics that render a very efficient milk yield. In semi-intensive and intensive handling with a richer diet, there is greater predisposition to infection of the mammary gland because the surplus of milk is not consumed by the lamb (Sousa *et al.*, 2005).

Staphylococci are one of the most frequent mastitis causative agents in small ruminants (Contreras *et al.*, 2007). Even though the most common Staphylococci is coagulase-negative, the occurrence of *Staphylococcus aureus* is concerning because this pathogen can cause gangrenous mastitis due to the production of specific toxins such as α-toxin that produces necrosis in the alveoli (Santos *et al.*, 2007). The expression of hemolysins is the main factor that contributes to bacterial infection and inhibition of the immune response of the host (Silva *et al.*, 2005). Moreover, *S. aureus* has the ability to produce many other virulence factors (Fitzgerald *et al.*, 2000), such as bi-component leukotoxins and toxins with superantigen activity. The toxins of *S. aureus* can cause vascular thrombosis, gangrene and, consequently, the affected gland gets gradually

Send correspondence to L.M. Almeida. Department of Clinical Analysis, Pharmaceutical Science Faculty, University of São Paulo, São Paulo, SP, Brazil. E-mail: larameal@gmail.com.

isolated from the surrounding tissue (Winter 2001). This pathogen may also produce specific adhesins that bind to a variety of host proteins, especially in the extracellular matrix, such as collagen, fibrinogen and fibronectin (Novick 2000). The interaction with the host tissue represents a critical role in the establishment of mastitis by *S. aureus* (Kerro Dego *et al.*, 2002).

The virulence factors of *S. aureus* are not expressed constantly, some are more important than others, according to different stages of the infection (Kalorey *et al.*, 2007). The expression of most virulence genes of *S. aureus* is controlled by an accessory gene regulator (*agr*) locus, which encodes a two-component signal transduction system that leads to down-regulation of surface proteins and up-regulation of secreted proteins during growth (Zhang *et al.*, 1998). Four allelic groups of the *agr* system have been identified in human isolates (Ji *et al.*, 1997). In *S. aureus* isolates from bovine mastitis, variations in the nucleotide sequences of *agr*B and *agr*D genes were identified (Takeuchi *et al.*, 2001), other than those described by Ji *et al.* (1997).

This study aimed to ascertain the prevalence of the different *agr* groups and to evaluate the occurrence of encoding genes for cytotoxin, adhesins and toxins with superantigen activity in *S. aureus* isolates from milk of ewes with clinical and subclinical mastitis in flocks of sheep raised for meat production in the Northeast of Brazil.

Materials and Methods

Clinical examination and sample collection

From August 2004 to October 2005, 31 herds located in 15 districts of State of Pernambuco (Northeast Brazil) were surveyed and 135 primiparous and multiparous ewes Santa Inês in different stages of lactation were sampled. 270 mammary glands were examined clinically following the recommendations of Diffay *et al.* (2002).The collection of milk samples and bacteriological culture was performed according to standard laboratory procedures of the National Mastitis Council (1999).

Identification of *S. aureus* isolates

S. aureus was isolated from sheep milk in 29.03% out of herds, located in 46.67% of the districts surveyed, accounting for 18 isolates - 6 from clinical mastitis samples and 12 from subclinical mastitis cases (Table 1). These isolates represent a population of *S. aureus* from 31 sheep flocks distributed in 15 different districts located in an area of 22,500 km^2.

Conventional methods that included Gram staining, colony morphology, catalase and coagulase tests were used (Quinn *et al.*, 1994). The colonies identified as *S. aureus* were confirmed by polymerase chain reaction (PCR) performed on the *nuc* gene using the primers F:5'TATGGTCCTGAAGCAAGTG3' and R:5'GCCACGTCCATATTTATCAG3' that were designed based on sequences of genomic DNA of MRSA strain 252 (GenBank database accession number NC_002952).

Table 1 - *S. aureus* isolates from sheep milk in Pernambuco, Brazil (2004-2005).

Isolate	Origin of isolate	Sequence type (ST)*
1	Clinical mastitis	ST750
2	Clinical mastitis	ST750
3	Subclinical mastitis	ST750
4	Subclinical mastitis	ST750
5	Subclinical mastitis	ST750
6	Subclinical mastitis	ST750
7	Subclinical mastitis	ST750
8	Clinical mastitis	ST1729
9	Subclinical mastitis	ST1729
10	Clinical mastitis	ST1728
11	Clinical mastitis	ST1728
12	Clinical mastitis	ST1730
13	Subclinical mastitis	ST1728
14	Subclinical mastitis	ST1728
15	Subclinical mastitis	ST1728
16	Subclinical mastitis	ST1728
17	Subclinical mastitis	ST1728
18	Subclinical mastitis	ST1728

*STs determined in a previous study (1).

DNA extraction and amplification of *agr* and virulence genes

The extraction of chromosomal DNA of isolates was performed using the technique of phenol-chloroform extraction adapted from Sambrook *et al.* (1989). The primers and PCR conditions used for amplification of the *hla, hlb*, *hlg*, *lukE-D*, *pvl*, *eta*, *etb*, *tst* and *sea-e* genes were described by Jarraud *et al.* (2002); for *bbp*, *cna*, *eno*, *ebp*, *fnbA*, *fnbB*, *fib*, *clfA*, *clfB icaA*, *icaD* and *bap* genes, by Tristan *et al.* (2003) and Vancraeynest *et al.* (2004) and for *agr* groups by Gilot *et al.* (2002). The reactions were carried out in Veriti Thermal Cycler 6.5 (Applied Biosystems). PCR products were analyzed by electrophoresis through 1% agarose gels.

The ATCC 25923 strain was used as positive control for *hla*, *hlg*, *tst*, *sec*, *lukE-D*, *bbp*, *cna*, *eno*, *ebp* and *agr*III genes; N315 strain for the *icaA*, *icaD* and *agr*II genes; 10/8520 for *agr*I gene; Mu50 for *sea* gene; CC63 for *seb* gene; RN4220 for *sed* gene; T47 for *see* gene; ZM for *eta* gene; N5 for *etb* gene; MR108 for *pvl* gene; RN4420 for *hlb* gene and, for *clfA*, *clfB*, *fnbA*, *fnbB* and *fib* genes, some isolates of our collection that had their PCR products sequenced and compared with published sequences in GenBank (access numbers: Z18852, AJ224764, X95848,

X62992 and X72014, respectively) using the Blast software.

Results and Discussion

Some regulatory systems, such as *agr*, *sar, sigB*, *sae, arl* and six *SarA* homologous are involved in the expression of genes encoding for the virulence factors of *S. aureus*. This fact makes it difficult to define the role of *agr* locus in staphylococcal infections and to underscore the multifactorial aspect of virulence of this pathogen. The pathogenesis of *S. aureus* is complex and it probably involves the synthesis of surface-associated proteins along with the secretion of exotoxins, resulting in damaging effects on the host cells (Takeuchi *et al.*, 2001).

In our previous study, MLST analysis of these isolates from sheep milk showed the occurrence of four STs (ST-750, ST-1728, ST-1729 and ST-1730) associated both with clinical and subclinical mastitis cases, being the last three recently reported in http://saureus.mlst.net. ST-750 and ST-1728 isolates were the most prevalent - 38.9% and 50%, respectively - occurring in all the districts surveyed (Almeida *et al.*, 2011). In the present study, we observed that the *agr* group I was identified in *S. aureus* isolates belonged to ST-750 and ST-1729, whereas the *agr* group II was identified in ST-1728 and ST-1730 (Table 2).

The occurrence of *agr* group I have already been observed in *S. aureus* isolates of other animal species. Gilot *et al.* (2002) identified 12 distinct *agr* alleles in an epidemiologically unrelated collection of bovine mastitis isolates. The majority of these isolates was represented by one particular *agr* allele from *agr* group I, suggesting the occurrence of either host-adapted or tissue-adapted *S. aureus* isolates in which the *agr* restriction type (allele) may play a significant role. Our results indicate that *S. aureus* isolates with *agr* group I are also putatively able to infect and to adapt to the sheep host causing either clinical or subclinical mastitis.

The *agr* group II was the most prevalent in this study (ST-1728 and ST-1730), accounting for 50% of isolates. Its distribution ranged from isolates that presented greater combination of virulence factor genes - including the association of *sec* and *tst* genes - to isolates carrying only one gene for adhesin and none for exotoxins. Similarly, the *agr* group I, representing the other 50% (ST-750 and ST-1729), was detected both in isolates carrying multiple virulence genes and in isolates carrying few of these genes. This puts in doubt the specificity of the relations of these *agr* groups exclusively with combinations of multiple genes encoding virulence factors, but suggests that the alleles I and II have an important role in the ability of *S. aureus* isolates to invade and survive in different cell types of sheep host. Buzzola *et al.* (2007) reported that bovine *S. aureus* isolates with *agr* group I showed increased abilities to invade MAC-T cells. Conversely, isolates of *agr* groups II, III and IV were internalized less efficiently, suggesting that these isolates may be more susceptible to attack by the host immune response because they tend to remain in larger amounts in the extracellular environment.

Neither the *agr* groups III and IV, nor negative *agr* were found among the *S. aureus* isolates from ewes in this study, results that differ from a recent survey (Vautor *et al.*, 2008), which identified the *agr* group III in a predominant clone found only in sheep and goats.

Staphylococcus aureus isolates from clinical and subclinical mastitis belonging to the same ST showed no differences in genetic background related to adhesins and proteins associated with biofilm formation. The presence of *cflA* gene (receptors for fibronectin) in 100% of the isolates of this study suggests its involvement in the colonization process of the mammary gland, regardless of the clinical picture of mastitis subsequently developed. The presence and expression of this gene may promote the adherence of *S. aureus* to the tissues of the mammary gland (Que *et al.*, 2001), but it was unable to correlate the presence of *cflA* gene with the clinical manifestation of the disease, since it was identified in isolates of the same ST both in clinical and subclinical mastitis.

None of the following genes were identified in the isolates: *bbp* (receptor for bone sialoprotein), *ebpS* (elastin-binding protein), *cna* (collagen-binding protein), *fnbB* (fibronectin-binding protein), *fib* (fibrinogen-binding pro-

Table 2 - Distribution of *agr* groups and virulence genes among *S. aureus* isolates from ewes with clinical and subclinical mastitis in Pernambuco, Brazil.

Origin of isolate	Sequence type (ST) - MLST	*agr* group	Adhesins and proteins related to biofilm formation	Citotoxins and toxins with superantigen activity	Number/total of isolates (%)
Clinical mastitis	ST750	I	*clfA*	*hla, lukE-D* and *tst*	2/ 11 1
Subclinical mastitis	ST750	I	*clfA*	*hla*, *hlb, lukE-D* and *sec*	5/ 27 5
Clinical mastitis	ST1729*	I	*clfA*	*hla* and *lukE-D*	1/ 5 5
Subclinical mastitis	ST1729*	I	*clfA*	*lukE-D*	1/ 5 5
Clinical mastitis	ST1728*; ST1730*	II	*clfA* e *clfB*	*hla* and *lukE-D*	3/ 16 6
Subclinical mastitis	ST1728*	II	*clfA* e *clfB*	*hla, lukE-D*, *tst* and *sec*	3/ 16 6
Subclinical mastitis	ST1728*	II	*clfA*	-	3/ 16 6

* Novel STs recently reported in http://saureus.mlst.net.

tein), *eno* (laminin-binding protein), *icaA*, *icaD* and *bap* (proteins related to biofilm formation).

The absence of *cna* gene, which encodes the collagen binding protein, is consistent with the reports of Smeltzer *et al.* (1997) that *S. aureus* isolates from animals do not usually have this gene. No isolates presented the *fnbB* gene, but this does not exclude the possibility of presence of variants of this gene (Sung *et al.*, 2008) involved with cases of acute gangrenous mastitis. Vautor *et al.* (2009) reported that *fnbB* gene, which can bind to host proteins such as fibrinogen, fibronectin and elastin was missing in the *S. aureus* strain responsible for a case of acute gangrenous mastitis and was also less common in high virulence isolates, being associated with a smaller spread of infections (Vautor *et al.*, 2008).

Our results showed greater diversity in the combination of exotoxin genes. Besides presenting adhesin genes, 55.2% also presented genes for cytotoxins and toxins with superantigen activity. Combinations of the staphylococcal exotoxins, as well as the amount of their secretion may define the pathogenic potential of the bacteria. The pore-forming exotoxins induce pre-inflammatory changes in mammalian cells, inactivating the immune system and degrading tissues, thus providing the bacteria with nutrients facilitating their dispersal in other sites (Projan and Novick, 1997). Some authors have suggested the involvement of α-toxin with gangrenous mastitis in cattle (Anderson 1983), but relations between this toxin and severe manifestations of the disease are still under scrutiny. In sheep, data correlating the presence of *hla* gene in *S. aureus* and the occurrence of clinical mastitis are scarce. The frequency of *hla* and *lukE-D* genes among the isolates of this study was high - 77.3 and 82.8%, respectively, and all *S. aureus* isolates from clinical mastitis presented the combination of the genes encoding for α-toxin and LUKE-D leukocidin.

The *hlb* gene, which encodes β-hemolysin, was identified only in isolates from subclinical mastitis, accounting for 27.5% of total isolates. The presence and possible expression of this gene may explain the relation between *S. aureus* isolates carrying *hlb* gene and the occurrence of chronic cases, because this gene can promote the escape of bacteria from the host immune system and assist in its process of obtaining nutrients (Huseby *et al.*, 2007), helping the survival of the pathogen.

The presence of *sec* gene (enterotoxin C) was identified in 44.1% of isolates, all from subclinical mastitis (ST-750 and ST-1728). These results are consistent with the prevalence of this gene in *S. aureus* isolates from the same animal species observed by Orden *et al.* (1992). The *tst* gene (toxic shock syndrome toxin) was found in 27.7% of isolates (16.6% associated with *sec* gene). This association was unique to isolates from subclinical mastitis (ST-1728), but the presence of *tst* gene was also identified in isolates from cases of clinical mastitis (ST-750). The frequency of the combination of *sec* and *tst* genes was lower (16.6%) than the 74% found by Orden *et al.* (1992) in sheep. Almost all genes for toxins with superantigen activity are related to pathogenicity islands or other mobile genetic elements, some coexisting in the same isolate. In cattle, a putative pathogenicity island encoding multiple superantigens, the SaPIbov, was identified in the genome of a bovine isolate (Fitzgerald *et al.*, 2001).

The production of toxic shock syndrome toxin (TSST-1) in staphylococcal isolates from different anatomical sites of healthy sheep and the detection of antibodies to this toxin in milk and whey were studied by Valle *et al.* (1991), suggesting a frequent contact these animals with isolates producing of the TSST-1. In *S. aureus* isolates from cattle, the genes for toxins with superantigen activity have been linked to persistent intramammary infections (Haveri *et al.*, 2008). These toxins may contribute to spread of bacteria within a host, and even between hosts, since they are secreted during periods of high bacterial density (Katsuda *et al.*, 2005). Our tests showed that *S. aureus* isolates from clinical and subclinical mastitis - 11.1% and 16.6% respectively - carried the *tst* gene. Takeuchi *et al.* (2001) suggested that the *agr* locus variations of *S. aureus* bovine isolates may be related to the low production of α-toxin and TSST-1 among these isolates. However, little is known about this in *S. aureus* isolates from sheep.

The *hlg* (gamma-hemolysin), *lukM-lukF-PV* (Panton-Valentine bi-component leukotoxin - PVL), *eta-b* (Exfoliative toxins A-B) and *sea-b-d-e* (A, B, D, E enterotoxins) genes were not identified in any of the isolates.

Conclusions

We identified the distribution of the *agr* groups I and II in *S. aureus* isolates from ewes both with clinical and subclinical mastitis raised for meat production suggesting that these alleles are involved in overcoming host defenses and to establish an intramammary infection in sheep. Our data support a significant role of the *cflA* gene in the establishment of mastitis by *S. aureus*, such as the associations of the *hla*/*lukE-D* genes and *tst*/*sec* genes on spread of infection in clinical and subclinical mastitis, respectively.

Acknowledgments

This study was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

References

Almeida LM, Almeida MZPRB, Mendonça CL, Mamizuka EM (2011) Novel sequence types (STs) of *Staphylococcus aureus* isolates causing clinical and subclinical mastitis in flocks of sheep in the northeast of Brazil. J Dairy Res 78:1-6.

Anderson JC (1983) Veterinary aspects of staphylococci. *In*: Easmon CSF and Adlam C (ed) Staphylococci and Staphylococcal Infections. vol 1. Academic Press, London, pp 193-241.

Bergonier D, de Cremoux R, Rupp R, Lagriffoul G, Berthelot X (2003) Mastitis of dairy small ruminants. Vet Res 34:689-716.

Buzzola FR, Alvarez LP, Tuchscherr LP, Barbagelata MS, Lattar SM, Calvinho L, Sordelli DO (2007) Differential abilities of capsulated and noncapsulated *Staphylococcus aureus* isolates from diverse *agr* groups to invade mammary epithelial cells. Infect Immun 75:886-891.

Contreras A, Sierra D, Sánchez A, Corrales JC, Marco JC, Paape MJ, Gonzalo C (2007) Mastitis in small ruminants. Small Ruminant Research 68:145-153.

Diffay BC, McKenzie D, Wolf C, Pugh DG, (2002) Handling and examination of sheep and goats. In: Pugh DG, (ed) Sheep and Goat Medicine. vol. 1. WB Saunders, Philadelphia, pp 1-17.

Fitzgerald Ik, Hartigan PJ, Meaney WJ, Smyth CJ (2000) Molecular population and virulence factor analysis of *Staphylococcus aureus* from bovine intramammary infection. J Appl Microbiol 88:1028-1037.

Fitzgerald JR, Monday S, Foster T, Bohach G, Hartigan P, Meaney W, Smyth C (2001) Characterization of a putative pathogenicity island from bovine *Staphylococcus aureus* encoding multiple superantigens. J Bacteriol 183:63-70.

Gilot P, Una G, Cocfaard T, POUTREL B (2002) Analysis of the genetic variability of genes encoding the RNA III-activating components Agr and TRAP in a population of *Staphylococcus aureus* isolates isolated from cows with mastitis. J Clin Microbiol 40:4060-4067.

Haveri M, Hovinen M, Roslöf A, Pyörälä S (2008) Molecular types and genetic profiles of *Staphylococcus aureus* isolates isolated from bovine intramammary infections and extramammary sites. J Clin Microbiol 46:3728-3735.

Huseby M, Shi K, Brown CK, Digre J, Mengistu F, Seo KS, Bohach GA, Schlievert PM, Ohlendorf DH, Earhart CA (2007) Structure and biological activities of beta toxin from *Staphylococcus aureus*. J bacterial 189:8719-8726.

Jarraud S, Mougel C, Thioulouse J, Una G, Meugnier H, Forey F, Nesme X, Etienne J, Vandenesch F (2002) Relationships between *Staphylococcus aureus* genetic background, virulence factors, *agr* groups (alleles), and human disease. Infect Immun 70:631-641.

Ji G, Beavis R, Novick RP (1997) Bacterial interference caused by autoinducing peptide variants. Science 276:2027-2030.

Kalorey DR, Shanmugam Y, Kurkure NV, Chousalkar KK, Barbuddhe SB (2007) PCR-based detection of genes encoding virulence determinants in *Staphylococcus aureus* from bovine subclinical mastitis cases. J Vet Sci 8:151-4.

Katsuda K, Hata E, Kobayashi H, Kohmoto M, Kawashima K, Tsunemitsu H, Eguchi M (2005) Molecular typing of *Staphylococcus aureus* isolated from bovine mastitic milk on the basis of toxin genes and coagulase gene polymorphisms. Vet Microbiol 25:301-305.

Kerro Dego O, Van Dijk JE, Nederbragt H (2002) Factors involved in the early pathogenesis of bovine *Staphylococcus aureus* mastitis with emphasis on bacterial adhesion and invasion. A review Vet Q 24:181-198.

Menzies PI, Ramanoon SZ (2001) Mastitis of sheep and goats. Vet Clinics of North America: Food Animal Practice 17:333-355.

National Mastitis Council (1999). Laboratory Handbook on Bovine Mastitis. Madison, WI: National Mastitis Council.

Novick RP (2000) Pathogenicity factors and their regulation. *In*: Fischetti, VA; Novick, RP; Ferretti, JJ; Portnoy, DA; Rood, JI (eds). Gram-positive pathogens, ASM Press, Washington, DC, pp 392-407.

Orden JA, Goyache J, Hernandez J, Domenech A, Suarez G, Gomez Lucia E (1992) Detection of enterotoxins and TSST-1 secreted by *Staphylococcus aureus* isolated from ruminant mastitis. Comparison of ELISA and immunoblot. J Appl Bacteriol 72:486-489.

Projan SJ, Novick RP (1997) The molecular basis of pathogenesis. *In*: Crossley K. and Archer GL (Ed). *The Staphylococci in Human Disease*, New York, NY: Churchill Livingstone, p.55-81.

Que YA, Francois P, Haehiger JA, Entenza JM, Vaudaux P & Moreillon P (2001) Reassessing the role of *Staphylococcus aureus* clumping factor and fibronectin-binding protein by expression in *Lactococcus lactis.* Infect Immun. 69:6296-6302.

Quinn PJ, Carter ME, Markey B, Carter GR (1994) Clinical Veterinary Microbiology. Mosby, Philadelphia.

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual. (2nd ed). v.3, Appendix B, Cold Spring Harbor Laboratory Press, USA.

Santos RA, Mendonça CL, Afonso JAB, Simão LCV (2007) Aspectos clínicos e características do leite em ovelhas com mastite induzida experimentalmente com *Staphylococcus aureus*. Pesquisa Veterinária Brasileira 27:6-12.

Silva ER, Boechat JUD, Martins JCD, Ferreira WPB, Siqueira AP, Silva N (2005) Hemolysin production by *Staphylococcus aureus* species isolated from mastitic goat milk in Brazilian dairy herds. Small Ruminant Research, Amsterdam. 56:271-275.

Smeltzer MS, Gillaspy AF, Pratt FLJ, Thames MD, Iandolo JJ (1997) Prevalence and chromosomal map location of *Staphylococcus aureus* adhesin genes. Gene 196:249-259.

Sousa WH, Lobo RNB, Morais RM (2005) Santa Inês: estado de arte e perspectivas. O Berro Uberlândia. 82:78-82.

Sung JM, Lloyd DH, Lindsay JA (2008) *Staphylococcus aureus* host specificity: comparative genomics of human *vs.* animal isolates by multi-strain microarray. Microbiology. 154:1949-1959.

Takeuchi S, Maeda T, Hashimoto N, Imaizumi K, Kaidoh T, Hayakawa Y (2001) Variation of the *agr* locus in *Staphylococcus aureus* isolates from cows with mastitis. Vet Microbiol 79:267-274.

Tristan A, Ying L, Bes M, Etienne J, Vandenesch F, Lina G (2003) Use of multiplex PCR to identify *Staphylococcus aureus* adhesins involved in human hematogenous infections. J Clin Microbiol 41:4465-4467.

Valle J, Vadillo SE, Piriz S, Gomes-Lucia E (1991) Toxic shock syndrome toxin one (TSST-1) production by staphylococci isolated from goats and presence of specific antibodies to TSST-1 in serum and milk. Appl Environ Microbiol 57:889-891.

Vancraeynest D, Hermans K, Haesebrouck F (2004) Genotypic and phenotypic screening of high and low virulence *Staphylococcus aureus* isolates from rabbits for biofilm formation and MSCRAMMs. Vet Microbiol 103:241-247.

Vautor E, Magnone V, Rios G, Le Brigand K, Bergonier D, Lina G, Meugnier H, Barbry P, Thiéry R, Pépin M (2008) Genetic differences among *Staphylococcus aureus* isolates from

dairy ruminant species: A single-dye DNA microarray approach. Vet Microbiol 133:105-114.

Vautor E, Cockfield J, Le Marechal C, Le Loir Y, Chevalier M, Robinson AD, Thiery R, Lindsay J (2009) Difference in virulence between *Staphylococcus aureus* isolates causing gangrenous mastitis *vs.* subclinical mastitis in a dairy sheep flock. Vet Res 40:56.

Winter A (2001) Mastitis in ewes. Pract. 23:160-163.

Zhang S, Iandolo JJ, Stewart GC (1998) The enterotoxin D plasmid of *Staphylococcus aureus* encodes a second enterotoxin (*sej*). FEMS Microbiology Letters, Amsterdam. 168:227-233.