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Staphylococcal toxin genes in strains isolated from cows with subclinical mastitis[1]

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ABSTRACT.- Freitas M.F.L., Luz I.S., Silveira-Filho V.M., Júnior J.W.P., Stamford T.L.M., Mota R.A., Sena M.J., Almeida A.M.P., Balbino V.Q. & Leal-Balbino T.C. 2008. **Staphylococcal toxin genes in milk samples from cows diagnosed with subclinical mastitis.** *Pesquisa Veterinária Brasileira 28(12):617-621.* Centro de Pesquisas Aggeu Magalhães, Fiocruz, Av. Prof. Moraes Rego s/n, Campus da Cidade Universitária, Recife, PE 50670-420, Brazil. E-mail: cristina@cpqam.fiocruz.br

The present study was carried out in 11 dairy herds in four municipal districts of the rural area of the State of Pernambuco, Brazil. Out of 984 quarter milk (246 cows), 10 (1.0%) were positive for clinical mastitis, 562 (57.1%) for subclinical mastitis and 412 (41.9%) were negative. A total of 81 *Staphylococcus* spp. isolates were obtained from milk samples from the cows diagnosed with subclinical mastitis. From these, 53 (65.0%) were *S. aureus*, 16 (20.0%) coagulase-positive staphylococci (CPS) and 12 (15.0%) coagulase-negative staphylococci (CNS). The isolates were further investigated for the presence of toxin genes by multiplex and uniplex PCR. The main gene observed was *seg* followed by *seh*, *sei* and *sej*. The distribution of these observed genes among the isolates obtained from different areas showed a regional pattern for the SEs. The presence of toxin genes in the strains isolated from bovine milk demonstrates a potential problem for public health.

INDEX TERMS: Staphylococcal toxins, toxin genes, milk, dairy cows.

RESUMO.- [**Detecção de genes de toxinas em linhagens de estafilococos isolados de vacas com mastite subclínica.**] O presente estudo foi realizado em 11 rebanhos leiteiros de quatro municípios da área rural do estado de Pernambuco, Brasil. Dos 984 quartos mamários examinados (246 vacas), 10 (1,0%) foram positivos para a mastite clínica, 562 (57,1%) para a mastite subclínica e 412 (41,9%) foram negativos para mastite. Foram isoladas 81 linhagens de *Staphylococcus* spp. do leite de vacas com mastite subclínica. Destes, 53 (65,0%) foram *S. aureus*, 16 (20,0%) estafilococos coagulase-positivo (SCP) e 12 (15,0%) estafilococos coagulase-negativo (SCN). O principal gene observado nos estafilococos foi o *seg* seguido pelo *seh*, *sei* e *sej*. Foi constatada distribuição regional dos genes dos estafilococos isolados dos animais nos municípios estudados. A presença dos genes das toxinas nas linhagens isoladas do leite de vacas representa risco potencial para a Saúde Pública.

TERMOS DE INDEXAÇÃO: Toxinas estafilocócicas, genes das toxinas, leite, vacas leiteiras.

INTRODUCTION

Staphylococci cause several human and animal diseases. These pathogenic groups of microorganisms play an important role in the etiology of infectious bovine mastitis. Classically, staphylococci are related predominantly in subclinical form of bovine mastitis. Subclinical mastitis is very important because of its high prevalence among

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herds, decreases milk production, difficult therapy and diagnosis (Bradley et al. 2002).

Some staphylococci produce staphylococcal enterotoxins (SEs) involved in staphylococcal food poisoning syndrome in humans, especially in the toxic shock syndrome toxin 1 (TSST-1), in humans patients and the exfoliate toxins (ETA and ETB) that cause staphylococcal scalded skin syndrome in children and newborns. Recently, 19 serologically distinct SEs have been identified. SEA, B, C, D and E are the classical five major types. However, other new enterotoxins have been described (Arbuthnott et al. 1990, Loncarevic et al. 2005, Thomas et al. 2007).

Several studies reported the production of SEs or the presence of toxin genes in *Staphylococcus aureus* from milk and derivates associated with mastitic cows in different countries (Ercolini et al. 2004, Loncarevic et al. 2005, Normanno et al. 2005, Rosec et al. 1997, Zschöck et al. 2004). However, in Brazil little attention has been dispended in order to evaluate the prevalence of staphylococcal toxins in strains isolated from bovine mastitis.

The purpose of our study was to determine the presence and distribution of the *se, tst, eta* and *etb* toxin genes in *Staphylococcus* spp. from milk obtained from cows diagnosed with subclinical mastitis, in the rural area of the State of Pernambuco, Brazil.

MATERIALS AND METHODS

Source of the staphyloccocal strains

The study was carried out in 11 dairy herds in four municipal districts (Angelim, São Bento do Una, Caetés and Correntes) located in the rural area of the State of Pernambuco, Brazil. Cows were screened for clinical mastitis by the Tamis Test (Radostitis 2007) and for subclinical mastitis by the California Mastitis Test - CMT (Schalm & Noorlander 1957). Milk samples were collected for the analysis from the quarter milk of cows that tested positive for clinical or subclinical mastitis.

Phenotypic diagnosis of Staphyloccocal strains were performed by the classical microbiological test, including hemolysis and pigmentation production in sheep blood agar, Gram staining and the biochemical tests of coagulase and acetoin production, thermonuclease, catalase, glucose (anaerobic) and mannitol (anaerobic and aerobic) fermentation.

Genomic DNA extraction

Staphylococcal DNA was extracted from 1mL of culture grown in brain heart infusion (BHI) broth, centrifuged at 14,000rpm at 4°C. The pellet was homogenized in 500µL of TE buffer along with the addition of 10µL of lysozyme (10mg/mL) and 10µL of proteinase K (5mg/mL). The suspension was incubated at 60°C for 20 min followed by the addition of 100µL of STE buffer (2.5% SDS, 10mM Tris-HCl, pH 8, 0.25 M EDTA) and incubation for 15min at 60°C, 5min at room temperature and 5min in an ice bath. The reaction was neutralized with 130µL of 7.5 M ammonium acetate, kept in an ice bath for 15min and then centrifuged for 5min. Approximately 700µL of the supernatant was transferred to another tube and mixed with the same volume of phenol-chloroform-isoamyl alcohol (25:24:1), followed by centrifugation for 5min. The supernatant was transferred to a new tube, and DNA was precipitated with approximately 420µL of isopropanol at either -70°C for 30min or -20°C for 24 h. After centrifugation, the supernatant was discarded, and the precipitate was resuspended in 10µL of sterile deionized water and kept at -20°C. The DNA product was quantified using the 1D Image Analysis Software program, version 3.5 from Kodak Digital Science, DC 120 zoom Digital Camera, after electrophoresis in 1% agarose gel using ë *Hind*III DNA as standard.

Detection of toxin genes by PCR

Multiplex-PCR. Two multiplex-PCR essays were developed: one to detect the *sea, seb, sec, sed, see* genes and the other for *eta, etb* and *tst*. Reactions were prepared for a 25µL final volume, with 20 pmol of each primer, 10mM Tris-HCl, pH 9.0, 50mM KCl, 160µM of each dNTP, 3mM $MgCl_2$, 20çg of genomic DNA and 1.2 U of Taq DNA polymerase (Invitrogen, Brazil). Amplifications were carried out in a thermocycler (Biometra) programmed for 30 cycles, each consisting of 95ºC for 1 min (denaturation), 55ºC for 1 min (annealing), and 72ºC for 2 min (extension). Primer sequences and the size of the expected products are shown in Table 1. The following FRI (Food Research Institute, Madison, Wisconsin, USA) *S. aureus* strains were used as positive controls: FRI 361 harboring *sec, sed, seg, sei* and *sej* genes; FRI MN8, *tst* gene; FRI 722, *sea* gene; FRI S6, *seb* gene; and FRI 1151, *sed* gene. The amplified products were separated by electrophoresis in 1.5% (w/v) agarose gel, stained with ethidium bromide (10mg/mL) for 15min, visualized under a UV transilluminator and photographed.

Uniplex-PCR. Detection of *seg, seh, sei* and *sej* was carried out separately in uniplex-PCR reactions. Reaction mix consisted of a mixture of 20pmol of each primer, 160µM of each dNTP, 1.5mM of $MgCl_2$, 10mM Tris-HCl pH 9.0, 50mM KCl, 20çg of genomic DNA and 1U of Taq DNA polymerase (Invitrogen, Brazil) for a final volume of 25µL. Amplifications were performed in a thermocycler (Biometra) programmed for 30 cycles, each one consisting of 94ºC for 3min, 94ºC for 30 sec, 60ºC for 30 sec and 72ºC for 30 sec to amplify *seg, seh* and *sej*. For *sei* amplification, the cycle was modified to 94ºC for 30 sec, 60ºC for 30 sec and 72ºC for 60 sec. Primer's sequences and the size of the expected products are shown in Table 1. *S. aureus* strain FRI 361 was used as positive control. Amplification products were analyzed as previously described.

Restriction analysis and sequencing

To confirm their identity, the PCR-generated fragments for *seh*, *seg, sei* and *sej* were sequenced, and their restriction profiles were analyzed. The selection of the enzymes, based on the restriction sites, was determined by the Generunner DNA Sequence Analyses software, version 3.05, available for free on the internet. The amplified fragment of *seh* was digested with *Dra*I, and *seg, sei* and *sej* fragments were digested with *Rsa*I. The size of the fragments obtained after digestion was determined by electrophoresis in 1.8% agarose gels.

The amplified fragments were purified using the PureLink PCR Purification commercial kit (Invitrogen, Brazil) and analyzed in an automatic sequencer ABI 3100 (Applied Biosystem, USA). The sequences obtained were examined using the program *S. aureus* sequencing assembly-forward and reverse (Huang & Madan 1999) and compared to the GenBank sequences and by the Blast search program version 2.2.12 (Altschul et al. 1990).

Table 1. Sequence of the primers, genes, size of the expected segments and references

Primer	Sequence (5'→3')	Gene	Product (bp)	References
SEA-3b	cct ttg gaa acg gtt aaa acg	*sea*	127	Becker et al. 1998
SEA-4b	tct gaa cct tcc cat caa aaa c			
SEB-1c	tcg cat caa act gac aaa cg	*seb*	477	Becker et al. 1998
SEB-4b	gca ggt act cta taa gtg cct gc			
SEC-3b	ctc aag aac tag aca taa aag cta gg	*sec*	271	Becker et al. 1998
SEC-4b	tca aaa tcg gat taa cat tat cc			
SED-3b	cta gtt tgg taa tat ctc ctt taa acg	*sed*	319	Becker et al. 1998
SED-4b	tta atg cta tat ctt ata ggg taa aca tc			
SEE-3b	cag tac cta tag ata aag tta aaa caa gc	*see*	178	Becker et al. 1998
SEE-2c	taa ctt acc gtg gac cct tc			
TST-3	aagccctttgttgcttgcg	*tst*	445	Becker et al. 1998
TST-6	atcgaactttggcccatacttt			
ETA-3b	ctagtgcatttgttattcaagacg	*eta*	119	Becker et al. 1998
ETA-4b	tgcattgacaccatagtacttattc			
ETB-3b	acg gct ata tac att caa ttc aat g	*etb*	262	Becker et al. 1998
ETB-4b	aaa gtt att cat tta atg cac tgt ctc			
SEG-1	acgtctccacctgttgaagg	*seg*	400	Rosec & Gigaud 2002
SEG-2	tgagccagtgtcttgctttg			
SHE-1	tcacatcatatgcgaaagcag	*seh*	357	Rosec & Gigaud 2002
SHE-2	tagcaccaatcacccttcc			
SEI-1	ggtgatattggtgtaggtaac	*sei*	454	Omoe et al. 2002
SEI-2	atccatattctttgcctttaccag			
SEJ-1	cagcgatagcaaaaatgaaaca	*sej*	426	Rosec & Gigaud 2002
SEJ-2	tctagcggaacaacagttctga			

Table 2. Distribution of toxin genes in *Staphylococcus* spp. from milk samples from cows diagnosed with subclinical mastitis

Genotype	Origin									
	Angelim	São Bento do Una			Caetés			Correntes		
	Sa[a]	Sa	CPS	CNS	Sa	CPS	CNS	Sa	CPS	CNS
seg	-	10	4	2	3	4	-	-	-	-
seh	-	-	-	-	-	-	-	4	2	4
sei	6	-	-	-	-	-	-	-	-	-
seg+sej	-	6	-	-	2	-	-	-	-	-
seg+she	-	-	-	-	2	-	3	-	-	-
seg+sei	-	-	-	-	4	2	-	-	-	-
seh+sei	-	-	-	-	-	-	-	1	1	-
seg+sei+sej	-	-	-	-	1	-	-	-	-	-
seg+seh+sei	-	-	-	-	1	-	3	-	-	-
No gene amplified	3	9	2	-	-	1	-	1	-	-
Total of isolates	9	25	6	2	13	7	6	6	3	4

[a] Sa = *Staphylococcus aureus;* CPS = coagulase positive *Staphylococcus;* CNS = coagulase negative *Staphylococcus.*

RESULTS

Out of 984 quarter milk samples from 246 cows investigated, 10 (1.0%) were positive for clinical mastitis, 562 (57.1%) for subclinical mastitis and 412 (41.9%) were negative for mastitis. A total of 81 *Staphylococcus* spp. isolates were obtained from milk samples from the cows diagnosed with subclinical mastitis. Among them, 53 (65.0%) were *S. aureus*, 16 (20.0%) were coagulase-positive staphylococci (CPS) and 12 (15.0%) were coagulase-negative staphylococci (CNS). Table 2 shows the differences on distribution of the isolates based on the municipal district.

None of the isolates analyzed amplified of the classical *sea-see, tst, eta* and *etb* toxin genes. Sixty-five (80.2%) isolates amplified the *seg, seh, sei* and *sej* genes, whereas 16 isolates (19.8%) amplified no toxin gene (Table 2). The *seg, seh* and *sei* genes were found alone or in combinations of two or three genes (Table 2). Accordingly the expected segments were amplified by the reference strains used as positive controls.

*Dra*I restriction fragments of *seh* and *Rsa*I restriction fragments of *seg, sei* and *sej* generated the expected segments confirming the identity of the PCR amplified fragments (Fig.1).

The nucleotide sequences obtained for *seg, seh, sei* and *sej* were deposited in the GenBank with the accession numbers: DQ916163, DQ917579, DQ917580 and DQ917581, respectively. Comparison of these sequences

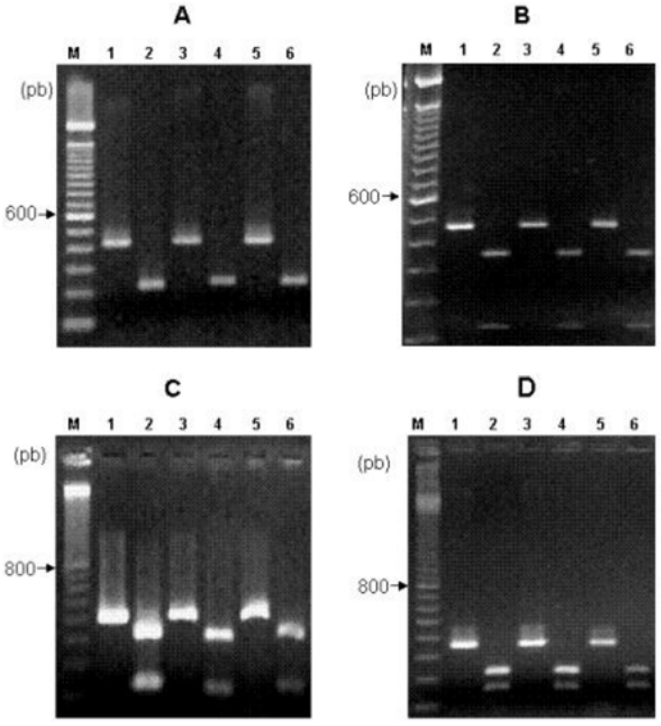

Fig.1. PCR amplification of the genes *sej, sei, seg* and *seh* (lanes 1, 3 and 5: A, B, C and D); and their restriction pattern (lanes 2, 4, 6) with *Rsa*I: A, B and C, and *Dra*I: D.

using the Blast software revealed homology of 98.0%, 99.0%, 99.0% and 95.0% respectively with the sequences available in GenBank.

DISCUSSION

Staphylococcal toxin genes have a broad distribution worldwide. Differences in the geographical distribution of these genes and toxin gene combinations have been reported (Omoe et al. 2002, Cabral et al. 2004, Lim et al. 2004, Salasia et al. 2004, Katsuda et al. 2005, Silva et al. 2005). In our study, the prevalence of genes encoding the most recently described enterotoxins SEG, SEH, SEI and SEJ was very high (80.2%) in the isolates of *Staphylococcus* spp. obtained from milk samples from cows diagnosed with subclinical mastitis. Interestingly, none of the genes encoding the classical toxins, TSST-1, ETA and ETB, were found.

The gene *seg* alone was predominant. It was found in 35.0% (23/65) of the isolates, but it was also found in combination with genes *sej*, *sei* and *seh*. The prevalence of SEG and combination of the *seg* and *sei* has reported elsewhere (Abe et al. 2000, Jarraud et al. 2001, Omoe et al. 2002, Cabral et al. 2004, Katsuda et al. 2005, Zschöck et al. 2005). The association *seg* and *sei* is attributed to their localization in tandem orientation in the enterotoxigenic gene cluster (*egc)*. Nonetheless, a low incidence of the *seg* and *sei* combination has also been described (Jorgensen et al. 2005), indicating probably that they are not always associated with the same strain.

The *seh* gene was detected in 32.0% (21/65) of the isolates, either alone or associated. This gene is rarely reported. However, similar study found *seh* genes in isolates from municipal districts of Caetés and Correntes, Brazil (Jorgensen et al. 2005). The occurrence of *seh* in these two areas can be explained by their geographical proximity and similar management of the dairy farms studied.

The gene *sej* has been found only in *Staphylococcus aureus* associated with *seg* and *sei*. The occurrence of multiple toxin genes in *S. aureus* is considered rare (Jorgensen et al. 2005). However, the prevalence of *seg* in *S. aureus* has been noted (Abe et al. 2000), indicating the potential importance of these gene in pathogenicity of *Staphylococcus* spp. strains in occurrence of subclinical bovine mastitis.

Usually CNS is not taken into account in most of the investigations and its toxigenic ability is seldom analyzed (Su & Wong 1996). Interestingly, in the present study all CNS samples analyzed harbored toxin genes. Each strain harbored either *seg*, *seh* or combinations of genes. These findings suggest a toxigenic involvement of CNS in subclinical bovine mastitis.

In conclusion, our results show evidence of a regional distribution of enterotoxin genes in staphylococci strains isolated from milk from cows with subclinical mastitis in the rural area of the State of Pernambuco, Brazil, reinforcing the geographical distribution of toxigenic isolates. The studies of the gene pattern of the staphylococcal strains isolated from bovine mastitis highlighted in present study can contribute in the knowledge of pathogenicity of microorganism, and consequent measures indicated for control of contagious mastitis in dairy herds. Furthermore, the presence of toxigenic staphylococci in milk samples isolated from cows, especially in subclinical mastitis, represent a public health threat.

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REFERENCES

Abe J., Ito Y., Onimaru M., Kohsaka T. & Takeda T. 2000. Characterization and distribution of a new enterotoxin-related superantigen produced by *Staphylococcus aureus*. Microbiol. Immunol. 44:79-88.

Altschul S.F., Gish W., Miller W., Myres E.W. & Lipman D.J. 1990. Basic local alignment search tool. J. Mol. Biol. 215:403-410.

Arbuthnott J.P., Coleman D.C. & De Azevedo J.S. 1990. Staphylococcal toxins in human disease. In: The Staphylococci: an introduction. J. Appl. Bacteriol. 69:101-107.

Bradley A.J. 2002. Bovine mastitis: An evolving disease. Vet. J. 164:116-128.

Becker K., Roth R. & Peters G. 1998. Rapid and specific detection of toxigenic *Staphylococcus aureus*: Use of two multiplex PCR enzyme

immunoassays for amplification and hybridization of staphylococcal enterotoxin genes, exfoliative toxin genes, and toxic shock syndrome toxin 1 gene. J. Clin. Microbiol. 36:2548-2553.

Cabral K.G., Lämmler C., Zschöck M., Langoni H., Sá M.E.P., Victória C. & Da Silva A.V. 2004. Pheno and genotyping *Staphylococcus aureus*, isolated from bovine milk samples from São Paulo State, Brazil. Can. J. Microbiol. 50:901-909.

Ercolini D., Blaiotta G., Fusco V., & Coppola S. 2004. PCR-based detection of enterotoxin *Staphylococcus aureus* in the early stages of raw milk cheese making. J. Appl. Microbiol. 96:1090-1096.

Huang X. & Madan A. 1999. A DNA sequence assembly program. Genome Res. 9:868-877.

Jarraud S., Peyrat M.A., Lim A., Tristan A., Bes M., Mougel C., Etienne J., Vandenesch F., Bonneville M. & Lina G. 2001. *egc*, a highly prevalent operon of enterotoxin gene, forms a putative nursery of superantigens in *Staphylococcus aureus*. J. Imunol. 166:669-677.

Jorgensen H., Mathisen T., Lovseth A., Omoe K., Qvale K.S. & Loncarevic S. 2005. An outbreak of staphylococcal food poisoning causef by enterotoxin H in mashed potato made with raw milk. FEMS Microbiol. Lett. 252:267-272.

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. 105:301-305.

Lim S.K., Joo Y., Moon J., Lee A., Nam H., Wee S. & Koh H. 2004. Molecular typing of enterotoxigenic *Staphylococcus aureus* isolated from bovine mastitis in Korea. J. Vet. Med. Sci. 66:581-584.

Loncarevic S., Jorgensen H.J., Lovseth A., Mathisen T. & Rorvik L.M. 2005. Diversity of *Staphylococcus aureus* enterotoxin types within single samples of raw milk and raw milk products. J. Appl. Microbiol. 98:344-350.

Normanno G., Firinu A., Virgilio S., Mulab G., Dambrosioa A., Poggiu A., Decastelli L., Mionid R., Scuotae S., Bolzonif G., Di Giannataleg E., Salinetti AP., La Salandrai G., Bartolij M., Zucconb F., Pirinob T., Siasb S., Parisii A., Quagliaa N.C. & Celano G.V. 2005. Coagulase-positive Staphylococci and *Staphylococcus aureus* in food products marketed in Italy. Int. J. Food Microbiol. 98:73-79.

Omoe K., Ishikawa M., Shimoda Y., Hu D.L., Ueda S. & Shinagawa K. 2002. Detection of *seg*, *seh* and *sei* genes in *Staphylococcus aureus* isolates and determination of the enterotoxin productivities of *S. aureus* isolates harboring *seg, seh* or *sei* genes. J. Clin. Microbiol. 40:857-862.

Radostitis O.M. 2007. Clínica Veterinária. 10ª ed. Guanabara Koogan, Rio de Janeiro, p.424-463.

Rosec J.P., Guiraud J.P., Dalet C. & Richard N. 1997. Enterotoxin production by staphylococci isolated from foods in France. Int. J. Food Microbiol. 35:61-70.

Salasia S.I.O., Khusnan Z., Lämmler C. & Zschöck M. 2004. Comparative studies on pheno- and genotypic properties of *Staphylococcus aureus* isolated from bovine subclinical mastitis in central Java in Indonesia and Hessen in Germany. J. Vet. Sci. 5:103-109.

Schalm O.W. & Noorlander B.S. 1957. Experiments and observations leading to development of the California Mastitis Test. J. Am. Vet. Med. 130:199-207.

Silva E.R., Carmo L.S. & Silva N. 2005. Detection of the enterotoxin A, B, and C genes in *Staphylococcus aureus* from goat and bovine mastitis in Brazilian dairy herds. Vet. Microbiol. 106:103-107.

Su Y.C. & Wong A.C.L. 1996. Detection of staphylococcal enterotoxin H by an Enzyme- Linked Immunosorbent Assay. J. Food Protection, Des Moines. 59:327-330.

Thomas D., Chou S., Dauwalder O. & Lina G. 2007. Diversity in *Staphylococcus aureus* enterotoxins. Chem. Immunol. Allergy 93:24-41.

Zschöck M., Ribe K. & Sommerhäuser J. 2004. Occurrence and clonal relatedness of *sec/tst*-gene positive *Staphylococcus aureus* isolated of quartermilk samples of cows suffering from mastitis. Lett. Appl. Microbiol. 38:493-498.

Zschöck M., Kloppert B., Wolter W., Hamann H.P. & Lämmler C. 2005. Pattern of enterotoxin genes *seg, seh, sei* and *sej* positive *Staphylococcus aureus* isolated from bovine mastitis. Vet. Microbiol. 108:243-249.