

DETECTION OF ENTEROTOXIGENIC *STAPHYLOCOCCUS* ISOLATED FROM THE GOAT MILK SOLD AT SÃO PAULO STATE¹

(DETECÇÃO DE *STAPHYLOCOCCUS* ENTEROTOXIGÊNICOS ISOLADOS DE LEITE DE CABRA COMERCIALIZADO NO ESTADO DE SÃO PAULO)

(DETECCIÓN DE *STAPHYLOCOCCUS* ENTEROTOXIGÉNICOS AISLADOS EN LECHE DE CABRA COMERCIALIZADO EN EL ESTADO DE SÃO PAULO, BRASIL)

M. L. POIATTI², R. P. SCHOCKEN-ITURRINO³, A. V. F. RAGAZANI⁴,
M. C. HATAYDE⁴, G. R. GARCIA⁴

SUMMARY

The aim of this work was to isolate *Staphylococcus* sp. and detect its capacity in producing enterotoxins. Two hundred fifty five samples of either raw, pasteurized or frozen goat milk from three farms located in the hinterland of São Paulo State, Brazil, were analyzed from April through October 2000. The method described by APHA (1992) was used to isolate the *Staphylococcus*. The production of toxins was analyzed by the Optimum Sensitivity technique in plates according to ROBBINS and colleagues (1974). Out of 143 positive samples for *Staphylococcus* sp, 32% were coagulase-positive and 67.3% were determined to be coagulase-negative. Among the coagulase-positive *Staphylococci*, 27.1% came from raw milk; 1.3% from the pasteurized one, and 4.3% from the frozen milk. Among the isolated raw samples, 10.6% presented *Staphylococcal* enterotoxins (SE) A and B, whereas 6.5% had the A, B and C enterotoxins. These results disclose the potential risk this product could offer to its consuming population, especially because raw milk is mostly used to make several milk products.

KEY-WORDS: Goat's milk. Coagulase-positive *Staphylococci*. Microbiological quality.

RESUMEN

El trabajo tuvo como objetivo aislar *Staphylococcus* sp y detectar su capacidad de producción de enterotoxinas, a partir de 255 muestras de leche caprino "in natura", pasteurizado y congelado, provenientes de 3 propiedades localizadas en el Estado de São Paulo, Brasil, durante los meses de abril a octubre de 2000. Para el aislamiento de *Staphylococcus* fue seguida la metodología de la APHA (1992). La verificación de la producción de toxinas fue realizada por la técnica de sensibilidad óptima en placas, de acuerdo con ROBBINS et al. (1974). Fueron aisladas 143 muestras de *Staphylococcus* sp, de las cuáles 32,7% se identificaron como *Staphylococcus* coagulasa positiva y 67,3% fueron coagulasa negativa. De los coagulasa positiva, 27,1% eran procedentes de la leche "in natura", 1,3% de la leche pasteurizada y 4,3% de la leche congelada. De las muestras aisladas de la leche "in natura", 10,6% produjeron enterotoxinas estafilocócicas (SE) A y B, y 6,5% de A, B y C. Estos resultados toman grande importancia, una vez que evidencian el riesgo potencial que este producto puede representar para la salud pública, sobre todo considerando que la leche "in natura" es utilizada para la

¹Part of the MS thesis of the first author, Zootecnia, FCAV – UNESP, Campus Jaboticabal (SP) – Brazil e-mail: luiza@fcav.unesp.br

² Faculdade de Ciências Agrárias e Veterinárias, Zootecnia, Campus Jaboticabal (SP)

³ Department of Veterinarian Pathology, FCAV – UNESP, Campus Jaboticabal (SP)

⁴ Faculdade de Ciências Agrárias e Veterinárias, Microbiologia, Campus Jaboticabal (SP)

fabricación de varios productos artesanales.

PALABRAS-CLAVE: Leche de cabra. *Staphylococcus* coagulasa positivo. Calidad microbiológica

RESUMO

O trabalho teve por objetivo isolar *Staphylococcus* sp e detectar a sua capacidade de produção de enterotoxinas, a partir de 255 amostras de leite caprino “in natura”, pasteurizado e congelado, advindas de 3 propriedades localizadas no Estado de São Paulo, durante os meses de abril a outubro de 2000. Para o isolamento de *Staphylococcus*, seguiu-se a metodologia da APHA (1992). A verificação da produção de toxinas foi realizada pela técnica de sensibilidade ótima em placas, de acordo com ROBBINS et al. (1974). Foram isoladas 143 amostras de *Staphylococcus* sp, das quais 32,7% se foram coagulase positivos e 67,3% coagulase negativos. Dos coagulase positivos, 27,1% eram procedentes do leite “in natura”, 1,3% do leite pasteurizado e 4,3% do leite congelado. Das amostras isoladas do leite “in natura”, 10,6% produziram enterotoxinas estafilocócicas (SE) A e B, e 6,5% de A, B e C. Esses resultados assumem grande importância, uma vez que evidenciam o risco potencial que o leite pode representar à saúde pública, sobretudo se considerado que é utilizado “in natura” para manufatura de vários produtos artesanais.

PALAVRAS-CHAVE: leite de cabra, *Staphylococcus* coagulase positivo, qualidade microbiológica

INTRODUCTION

The milk is in our daily diet, providing it with fat supply, proteins, carbohydrates, minerals, and vitamins. The caprine milk is usually addressed to people having some kind of problem regarding the bovine milk consumption, and as such, destined to the sucking, the elderly, and convalescents due mainly to the characteristics of digestibility of this food, reason that has augmented substantially its consumption in latest decades.

The milk contamination during its extraction, or even during the milk process of extraction can constitute a handicap factor to the final quality of this product. Some hygienically and sanitary aspects regarding the production of it might, besides contributing to the reduction of its nutritional value, favor the vehiculation of pathogens to its consumers' health, in the form of toxins and microorganisms (COSTA, 1999).

The presence of microorganisms, mainly the *Staphylococcus aureus* and its toxins, represents a serious issue to the public health due to its largely used by both industry and dairy farms (BERGDOLL, 1989; ISHIKAWA et al. 1996). Some strains are capable of producing toxins stable to warming that allow conveyance via food, what might certainly contribute to the appearance of some kind of intoxication (PRATA et al., 1998). It was reported 593,212 cases of food intoxication between 1984 and 1997, in accordance with the data provided by the Brazilian Health Ministry (BRASIL, 1998). However, the data did not mention which specific toxins, microorganisms or source were responsible for these cases (CARDOSO et al., 2000). Several kinds of food have already been epidemiologically penalized and reported as capable of supporting both the natural and artificial development of the *Staphylococcus*

aureus as well as the production of its correspondent enterotoxins. Takeushi et al. (1998) evaluated the *S. aureus* capacity of producing toxins isolating samples coming from expansion containers duly used for both milk cooling and storage, what made sure its presence in 75.4% of them.

Among the food subtracts, milk products such as cheese, crude milk, pasteurized or powdered, butter and ice creams, are pointed out potentially capable of transmitting *Staphylococcus aureus* enterotoxins. (PEREIRA et al., 2001). Thus, this work aimed to isolate *Staphylococcus* sp and detect its capacity of producing enterotoxins in the “in natura” caprine milk, pasteurized and frozen, traded in the State of São Paulo, Brazil.

MATERIAL AND METHODS

During the period of April to October of 2000, microbiological analysis were carried out in 255 samples of caprine milk in several stages of its processing, such as: “in natura” (just extracted, held 4°C), pasteurized (63.5C/ 30 min) and frozen, ready for consumption (-15° C / 6 h, held under the same conditions as far as its distribution) coming from 3 milk farms from the State of São Paulo, Brazil.

The samples were accommodated aseptically in duly sterilized glassy flasks and transported in isothermal boxes to the Laboratory of Microbiology of the Department of Veterinarian Pathology, FCAV – UNESP, Jaboticabal, São Paulo, Brazil.

All of these samples were submitted to 10⁻⁵ fold dilution (SPECK, 1984). For the isolation of *Staphylococcus* sp, 0.1 mL of the dilutions were inoculated on the surface of the plates with Agar Baird Parker

(DIFCO[®]), incubated at 32 – 35° for 24 – 48 hours. The colony traits were identified via coloration through the (Gram's Method), free coagulase test, catalase and acetoin production (VP) and the use on aerobiosis and anaerobiosis of maltosis and trealosis (APHA, 1992). The isolates were held in brain heart infusion broth - BHI (OXOID[®]) with glycerol at 50%, under – 80°C temperature until the materialization of all the tests occurred.

The isolates were sent to the Research Laboratory in *Staphylococci* of FUNED, Belo Horizonte, Minas Gerais, Brazil, where the analyses were realized with the application of the Optimum Sensitiveness Technique on Plate (OSP)(ROBBINS et al., 1974).

RESULTS AND DISCUSSION

The results showed that among the 255 samples analyzed, 85.8% (73) of the “in natura” milk, 37.6% (32) of the pasteurized one, and 21.2% (18) of frozen milk were confirmed to have *Staphylococcus* sp. After the examination of the biochemical tests, 27.1% of the milk samples “in natura” came up with *Staphylococcus* coagulase positive, 13% in the pasteurized form and another 4.3% in the frozen one, 63.7% corresponded to the *Staphylococcus* coagulase negative (Figure 1). These findings showed that pasteurization was effective in the elimination of this group of microorganisms. Our results are similar the ones found by Carvalho (1998) that in turn also showed the thermal efficiency in the reduction of the *Staphylococcus* coagulase positive.

Among the isolated microorganisms in the “in natura” milk, 10.6% referred to the producers of the A and B toxins, and 6.5% to the types A, B and C (Figure 2). The same strain of *S. aureus* revealed being capable of producing more than one type of enterotoxin, as registered in this work. These findings are similar to others registered in the literature (MORGAN et al., 1986; BOGNI et al., 1997; TAKEUSHI et al., 1998; CARDOSO et al., 2000). The detection of toxins of the Toxic Shock Syndrome type (TSST – 1) and the *Staphylococci* enterotoxins (SE) in

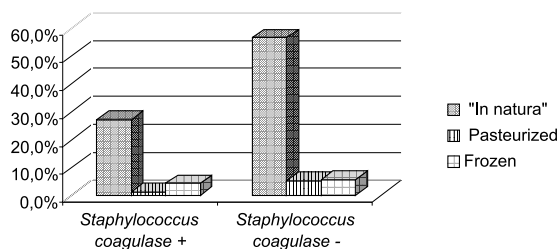


Figure 1 - Isolation percentage of *Staphylococcus aureus* coagulase positive and negative obtained from samples of goat milk “in natura”, pasteurized and frozen, coming from 3 farms located in the State of São Paulo.

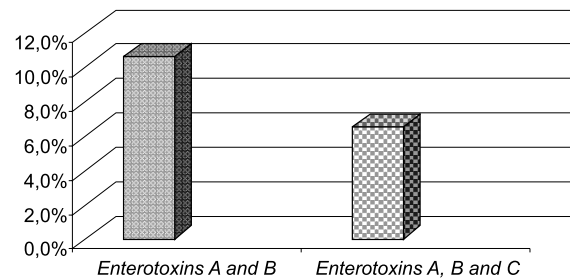


Figure 2 - Distribution of the *Staphylococcal* enterotoxins production in goat milk “in natura” samples, coming from 3 farms located in the State of São Paulo, Brazil.

isolated samples of the bovine mastitis in Brazil are reported by Bogni et al. (1997), Takeushi et al. (1998) and Cardoso et al. (2000). However, these authors do not discuss the importance of these findings. The co-production of these different types of toxins could increase the toxigenic effect of these antigens, suggesting that it is important to the pathogeny of the produced infections by these microorganisms (SOARES et al., 1997; CARDOSO et al., 2000). The reason for an existing association among the specific enzymatic activity, the enterotoxigenity activity, and the resistance of the *Staphylococcus aureus* to several antibiotics, mainly in those samples producing more than one kind of enterotoxin was demonstrated by Refai et al. (1988) and stressed out by Cardoso et al. (2000).

The detection of the *Staphylococcal* enterotoxins (SE) in the present work is crucial since there is substantive shortage of data in the Brazilian literature regarding these findings, mainly those associated to the caprine milk. Bergdoll (1989) and Murdough et al. (1996) reported how important this group of microorganisms is, because its capacity to produce enterotoxins and thermal treatments, such as pasteurization, are not capable of inactivating these toxins, leading to potential risks to the consumers.

The simple presence of the toxigenical samples of *Staphylococcus aureus* in the final product does not imply the occurrence of intoxication; there must be, however, a risk, mainly if we take into consideration that this microorganism is the most important in the intramammary infections of bovines and caprines, and that the milk is essential for the growth of the *S. aureus*, besides a high degree of contamination caused by this agent CARDOSO et al. (2000). Thus, we can conclude that contaminated milk presents a potential risk of intoxication. Our findings are even more important because the caprine milk is usually used in the making of cheese and some other milk products that normally are less inspected.

ACKNOWLEDGEMENT

We are thankful to the Research Laboratory in *Staphylococci* of the FUNED, Belo Horizonte, MG, Brazil, for identifying the enterotoxins found in this research.

ARTIGO RECEBIDO: dezembro/2003
APROVADO: junho/2005

REFERENCES

- AMERICAN PUBLIC HEALTH ASSOCIATION - APHA **Compendium of methods for the microbiological examination**. Washington, 1992. 701p.
- BRASIL. **Ministério da Saúde**. Secretaria Executiva. SIH/SUS. DATASUS, Brasília, 1998.
- BERGDOLL, M. S. *Staphylococcus aureus*. In: DOYLE, M.P. **Foodborne bacterial pathogens**. New York: Marcel Dekker, 1989. p.464-523.
- BOGNI, C., RASPANTI, C., ODIERNO, L. Sintesis de enterotoxinas y resistencia a antibióticos de estafilococos aislados de mastitis bovina. **Revista de Medicina Veterinária**, v. 78, p.236-239, 1997.
- CARDOSO, H. F. T., CARMO, L. S., SILVA, N. Detecção da toxina-1 da síndrome do choque tóxico em amostras de *Staphylococcus aureus* isoladas de mastite bovina. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v. 52, n.1, 2000.
- CARVALHO, M. G. X.. **Características físico-químicas, biológicas e microbiológicas do leite de cabra processado em micro-usinas da região da Grande São Paulo - SP**. São Paulo, SP. 1998. 102p. Tese (Doutorado) Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo.
- COSTA, E. O. Controle e Prevenção de Mastite em Bubalinos. In: BARNABE, V.H.; TONHATI, H.; BARUSELLI, P.S. **Bubalinos: sanidade, reprodução e produção**. Jaboticabal: FUNEP, 1999. p. 68-83.
- ISHIKAWA, M. , ISHIKAWA, T., MIZOMOTO, T. Productivity of enterotoxins and toxic shock syndrome toxin-1, and coagulase type of *Staphylococcus aureus* strains isolated from bovines and humans in the same district. **Animal Science Technology**, v.67, p.780-786, 1996.
- MORGAN, K. L., GRUFFYDD-JONES, E., WIENEKE, A. A. Staphylococcal toxic shock syndrome. **Veterinary Records**, v. 119, p.559, 1986.
- MURDOUGH, P. A., DEITZ, K. E., PANDEY, J. W. Effects of freezing on the viability of nine pathogens from quarters with subclinical mastitis. **Journal of Dairy Science**, v. 79, n. 2, p.334-336, 1996.
- PEREIRA, M. L., CARMO, L. S., PEREIRA, J. L. Comportamento de estafilococos coagulase negativos pauciprodutores de enterotoxinas, em alimentos experimentalmente inoculados. **Ciência e Tecnologia de Alimentos**, v. 21, n.2, May/Aug, 2001.
- PRATA, L. F., RIBEIRO, A. C., REZENDE K. T., CARVALHO, M. R. B., RIBEIRO, S. D. A., COSTA, R. G. Composição, perfil nitrogenado e características do leite caprino (Saanen). Região Sudeste, Brasil. **Ciência e Tecnologia de Alimentos**, v.18, n.4, 1998.
- REFAI, M., NIAZI, Z. M., YOUSSEF, S. A. H. Correlation between antibiotic-resistance, enterotoxigenicity and enzymatic activities of *Staphylococcus aureus* recovered from foods. **Veterinary Medicinal Journal**, v.36, p.107-119, 1988.
- ROBBINS, R., GOULD, S., BERGDOLL, M. Detecting the enterotoxigenicity of *Staphylococcus aureus* strains. **Applied Microbiology**, v. 28, n.6p.946-950, 1974.
- SOARES, M. J. S., TOKUMARU-MIYAZAKI, N. H., NOLETO, A. L. S. Enterotoxin production by *Staphylococcus aureus* clones and detection of Brazilian epidemic MRSA clone (III:B:A) among isolates from food handlers. **Journal Medical of Microbiology**, v.46, p.214-221, 1997.
- SPECK, M. L. **Compendium of methods for the microbiological examination of foods**. Washington, D.C.: American Public Health Association, 1984. p.701
- TAKEUSHI, S., ISHIGURO, K., IKEGAMI, M. Production of toxic shock syndrome toxin by *Staphylococcus aureus* isolated from mastitic cows milk and farm bulk milk. **Veterinary Microbiology**, v. 59, p. 251-258, 1998.