

Effect of intramammary infection by *Staphylococcus caprae* on somatic cell counts and milk composition in goats

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(Received 21 March 2001 and accepted for publication 13 September 2001)

KEYWORDS: *Staphylococcus caprae*, somatic cell count, milk composition, goat.

Since its identification by Devirese *et al.* (1983), *Staphylococcus caprae* has been frequently isolated from udder halves of goats with subclinical intramammary infection (IMI) (Contreras *et al.* 1995; Poutrel *et al.* 1996). Several different studies have demonstrated the capacity of *Staph. caprae* to persist throughout lactation (Poutrel *et al.* 1996; Contreras *et al.* 1997; Bedidi-Madani *et al.* 1998a) and during the dry period (Poutrel, 1984). Bedidi-Madani *et al.* (1998a) found that isolates of *Staph. caprae* from udders of uninfected goats express potential virulence factors (different exoproteins and slime); other researchers have occasionally isolated it from clinical cases of goat mastitis (Deinhofer & Pernthaner, 1995), or from human clinical specimens associated with bone and joint infection (Vandenesh *et al.* 1995; Shuttleworth *et al.* 1997). In spite of these forms of pathogenicity, some studies have not shown significant increases in somatic cell count (SCC) of milk from goat udder halves infected by *Staph. caprae* (Deinhofer & Pernthaner, 1995; Poutrel *et al.* 1996). Moreover, there is a lack of information on the effect of infection by this mammary pathogen on milk yield and composition. Knowledge of repercussions for hygiene and production would be useful in mastitis control programmes in view of the high frequency of isolation (higher than 22% of the staphylococci isolated) of *Staph. caprae* in dairy goat herds (Contreras *et al.* 1995; Poutrel *et al.* 1996). The aim of the present investigation was to ascertain the significance of persistent subclinical IMI by *Staph. caprae* on SCC, milk yield and physical-chemical parameters of Murciano-Granadina goat milk at mid-lactation.

MATERIALS AND METHODS

Animals and sample collection

Ten Murciano-Granadina goats were studied in a well-managed dairy-goat herd. The herd, including 80 lactating goats, was free of brucellosis, tuberculosis and

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mycoplasmosis. Goats were machine-milked once a day (8:00) and, after milking, teats were dipped in iodine solution (0.5%).

The bacteriological status of the udders of all lactating goats in the herd was determined on day 90 of lactation. From 100 to 120 d postpartum, 40 test-day observations were made for milk yield, SCC, and contents of fat, total protein, true protein, whey protein, casein, lactose, dry matter, dry matter non fat, ash, pH, and Dornic acidity. Samples were taken every 5 d from ten goats: five goats with natural and bilateral subclinical IMI by *Staph. caprae* and five uninfected goats. On test-days, a 100-ml sample of milk was taken for the analysis of each milking. Additionally, before milking, 10 ml milk was collected aseptically from each udder half for bacteriological analysis. All goats selected were in their second parity, had similar body weights (30 ± 0.8 kg) and were seronegative for caprine arthritis encephalitis virus. In the next lactation (3 d after parity) milk samples were taken for bacteriological analyses ($n = 20$).

Bacteriological procedure, MSCC and physical-chemical determinations on milk

Ten microlitres of each sample were plated on blood agar plates (5% washed sheep erythrocytes). Plates were incubated aerobically at 37 °C and examined after 24, 48, 72, and 168 h. Subclinical IMI was defined as 500 cfu/ml. Bacteria were identified according to the recommendations of the National Mastitis Council (Harmon *et al.* 1990). Identification of staphylococci was made using commercial micromethods (API STAPH; Bio Merieux, Lyon, France).

SCC was determined in a Fluoro-opto-electronic counter (Fossomatic 90. Foss Electric, Hillerød, Denmark). Physicochemical parameters of the milk (fat, total protein, true protein, whey protein, casein, lactose, dry matter, non-fat dry matter, ash, pH, Dornic acidity) were determined by near infrared reflectance spectroscopy (NIRS, InfraAlyzer 500 D, Bran Luebbe, Germany) previously calibrated according to official milk methods.

Statistical analysis

Data were analysed by analysis of variance, using the general linear model of SAS (Statistical Analysis System Institute Incorporation, 1990). Least square means generated in the general linear model procedure of SAS were used in the comparison of treatments. Uninfected and infected groups comparisons were made using Student's *t* test.

RESULTS AND DISCUSSION

Prevalence of subclinical IMI in the herd reached 16.9% of udder halves. Most (96%) of the pathogens isolated were coagulase-negative staphylococci (CNS). Among the halves infected by CNS, *Staph. caprae* was the main pathogen and accounted for 57.7% of the isolates. In selected goats that were bilaterally infected by *Staph. caprae*, all the bacteriological analyses were positive with excretion densities higher than 4000 cfu/ml. Persistence capability of *Staph. caprae* IMI throughout the experiment agrees with other reports (Poutrel *et al.* 1996; Contreras *et al.* 1997; Bedidi-Madani *et al.* 1998a). All *Staph. caprae* isolated in the selected goats had the same biotype (API-Staph scoring 6716103), which was the third most frequently isolated by Bedidi-Madani *et al.* (1998b). Despite the fact that the most common profiles of *Staph. caprae* usually express numerous exoproteins with virulence capability in the udder (Bedidi-Madani *et al.* 1998a), non-significant effects were detected on SCC when healthy and infected goats were compared in the present

Table 1. Effect of sub-clinical infection with *Staphylococcus caprae* on the yield and composition of goat milk in mid-lactation(Values are means with SEM for $n = 10$)

Milk variables	Uninfected	Infected	SEM
Milk yield, kg/d	2.10	2.05	0.483
Log SCC	5.38	5.39	0.459
Fat, g/l	51.9	50.2	7.69
Total protein, g/kg	34.2	34.5	5.13
True protein, g/kg	32.7	32.3	4.51
Casein, g/kg	27.6	26.7	2.97
Whey protein, g/kg	6.1	5.1	2.99
Lactose, g/l	53.1	52.3	0.99
Dry matter, g/kg	137.9	139.3	9.22
Non-fat dry matter, g/kg	88.2	88.2	4.43
Ash, g/kg	7.6	7.4	0.23
pH	6.70	6.69	0.034
Dornic acidity (°D)	13.28	13.62	1.034

experiment (Table 1), which makes bacteriological analysis necessary for diagnosis. Serum protein content also remained unchanged as a result of infection and therefore no inflammatory responses were found. In addition, the absence of effects on milk yield, and on milk components synthesized by the udder infected by *Staph. caprae*, (Table 1) are consistent with the absence of lesions on the secretory epithelium. In spite of this, five of the ten halves infected by *Staph. caprae* maintained the infection in three goats in the next lactation, which agrees with the results of Poutrel (1984). Persistence of *Staph. caprae* during the dry period could be a risk factor for the next lactation because of possible expression of virulence factors during the periparturient period when defence mechanisms of the udder are diminished (Sordillo *et al.* 1997).

Even though our results showed neither health nor production repercussions in *Staph. caprae*-infected goat udders, further studies are necessary to define the dynamics of subclinical IMI by *Staph. caprae* during lactation and possible clinical implications.

The study was supported by the Dirección General de Enseñanza Superior e Investigación Científica (I + D Programa FEDER) and resulted from co-ordination of the two sub-projects, 1FD97-1010-C02-01 and 1FD97-1010-C02-02. NIRS calibration was supported by the Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), project CAL00-046-C3-2.

REFERENCES

- Bedidi-Madani, N., Greenland, T. & Richard, Y. 1998a Exoprotein and slime production by coagulase-negative staphylococci isolated from goat's milk. *Veterinary Microbiology* **59** 139–145
- Bedidi-Madani, N., Kodjo, A., Villard, L. & Richard, Y. 1998b Ribotyping of *Staphylococcus caprae* isolated from goat milk. *Veterinary Research* **29** 149–158
- Contreras, A., Corrales, J. C., Sierra, D. & Marco, J. 1995 Prevalence and etiology of nonclinical intramammary infection in murciano-granadina goats. *Small Ruminant Research* **17** 71–78
- Contreras, A., Corrales, J. C., Sánchez, A. & Sierra, D. 1997 Persistence of subclinical intramammary pathogens in goats throughout lactation. *Journal of Dairy Science* **80** 2815–2819
- Deinhofer, M. & Pernthaner, A. 1995 *Staphylococcus* spp. as mastitis-related pathogens in goat milk. *Veterinary Microbiology* **43** 161–166
- Devirese, L. A., Poutrel, B., Kilpper-Bälz, R. & Schleifer, K. H. 1983 *Staphylococcus gallinarum* and *Staphylococcus caprae*, two new species from animals. *International Journal of Systematic Bacteriology* **33** 480–486
- Harmon R. J., Eberhart, R. J., Jasper, D. E., Langlois, B. E. & Wilson, R. A. 1990 Microbiological procedures for the diagnosis of bovine udder infection. Arlington, Virginia, USA: National Mastitis Council

- Poutrel, B. 1984 Udder infection in goats by coagulase-negative staphylococci. *Veterinary Microbiology* **9** 131–137
- Poutrel, B., De Crémoux, R., Pillet, R., Heuchel, V. & Ducheilliez, M. 1996 In *Caprine mammary infections with respect to cell counts in milk*, pp. 61–64 (Ed. R. Rubino). Wageningen, The Netherlands: EAAP Publication No. 77
- SAS/SAT® 1990 *User's Guide*, Version 6.12, 4th edn, Volume 1. Cary, NC: Statistical Analysis System Institute Incorporation
- Shuttleworth, R., Behme, R. J., McNabb, A. & Colby, W. D. 1997 Human isolates of *Staphylococcus caprae*: association with bone and joint infections. *Journal of Clinical Microbiology* **35** 2537–2541
- Sordillo, L. M., Shafer-Weaver, K. & Derosa, D. 1997 Immunobiology of the mammary gland. *Journal of Dairy Science* **80** 1851–1865
- Vandenesch, F., Eykyn, S. J., Bes, M., Meugnier, H., Fleurette, J. & Etienne, J. 1995 Identification and ribotypes of *Staphylococcus caprae* isolates isolated as human pathogens and from goat milk. *Journal of Clinical Microbiology* **33** 888–892