APPLIED AND ENVIRONMENTAL MICROBIOLOGY, May 1990, p. 1323–1326
0099-2240/90/051323-04$02.00/0

Vol. 56, No. 5

Enterotoxin Production by Staphylococci Isolated from Healthy Goats

JORGE VALLE,[1] ESPERANZA GOMEZ-LUCIA,[2] SEGUNDO PIRIZ,[1] JOAQUIN GOYACHE,[2] JOSE A. ORDEN,[2] AND SANTIAGO VADILLO[1]*

U.D. Microbiología e Inmunología, Facultad de Veterinaria, Universidad de Extremadura, 10.071 Cáceres,[1] and U.D. Microbiología e Inmunología, Facultad de Veterinaria, Universidad Complutense, 28.040 Madrid,[2] Spain

Received 18 October 1989/Accepted 23 January 1990

The ability of 342 staphylococcal isolates from different anatomical sites in healthy goats to produce staphylococcal enterotoxins (SE) was investigated. SE were produced by 74.3% of the 70 coagulase-positive strains and by 22% of the coagulase-negative strains studied. Most enterotoxigenic strains were isolated from the skin of udders and teats and from milk. SEC was the SE type most frequently produced, either alone (67.9%) or in combination with others. Five coagulase-negative species not previously reported as SE producers were identified (*Staphylococcus chromogenes*, *S. warneri*, *S. sciuri*, *S. saprophyticus*, and *S. lentus*). SEA, SEB, and SEC were detected in the milk of 17 of the 133 healthy goats studied. These results suggest that the goat is an important reservoir of enterotoxigenic staphylococci, most of which produce SEC.

Staphylococcal enterotoxins (SE) are exoproteins which, when ingested by humans, give rise to symptoms of acute gastroenteritis. Several types of staphylococcal enterotoxins have been identified on a serological basis and are named SE A through E (SEA through SEE). Data regarding the enterotoxigenicity of staphylococcal strains isolated from healthy animals indicate that only a relatively small number of strains are enterotoxigenic (2, 15, 20, 25, 36, 43). Studies of goats have focused on determining the enterotoxigenicity of *Staphylococcus aureus* isolated from normal or infected milk (8, 23), although staphylococci are usually found in the skin and mucosae of humans and other animals (27, 32). It is important to determine the enterotoxin-producing ability of staphylococci usually found in the skin, mucosae, and milk of animals, because they may give rise to contamination of food destined for human consumption.

On the other hand, staphylococci isolated from animal skin, mucosae, and milk are mainly coagulase-negative strains (CNS) (9, 11, 27, 30, 32, 39); specifically, subclinical infections of the goat's udder are predominantly produced by coagulase-negative staphylococci (23, 30, 39, 44), and very few studies have been done on the enterotoxigenicity of these strains (3, 5, 6, 37, 38; M. S. Bergdoll, K. F. Weiss, and M. J. Muster, Bacteriol. Proc., p. 12, 1967).

This paper reports our studies on the ability of staphylococci isolated in the skin, nasal mucosa, and milk of 133 healthy goats to produce enterotoxins (SE) and determines the direct presence of SE in milk from these animals, using the enzyme-linked immunosorbent assay method.

MATERIALS AND METHODS

Isolation of staphylococci. A total of 133 female goats from 11 different flocks were studied. Animals (2 to 7 years old) were in the lactation stage. Samples were taken on sterile swabs from the nasal mucosa and the skin of the axillary fold, the udder surface, and the teat of each animal. Milk (25 ml from each compartment) was collected aseptically in sterile containers. Samples were immediately transferred to the laboratory in isothermic containers at 4 to 6°C. Mucosa and skin samples were streaked onto sheep blood agar (5%) (Oxoid Ltd., Hampshire, United Kingdom), rubbing the swab onto the upper third of petri dishes and then streaking. Milk (100 μl) was surface spread on sheep blood agar (5%) and on Schleifer-and-Krämer medium (42), and the remaining milk was frozen at −20°C for subsequent direct detection of SE. Plates were incubated in aerobic conditions at 37°C for 24 to 48 h. Colonies with cell morphology resembling that of gram-positive, catalase-positive cocci were subsequently selected, subcultures being made when 10 colonies of the same morphological type were present in the primary culture. One or two colonies were selected to obtain pure cultures on brain heart infusion agar (Difco Laboratories, Detroit, Mich.).

Staphylococci were differentiated by means of the lysostaphin sensitivity test (28), the lysosyme resistance test (41), and the furazolidine agar growth test (45).

Staphylococcal strains. Coagulase-positive strains (CPS) were identified by following the criteria proposed by Devriese and Hájek (10). CNS were identified by the criteria suggested by Devriese and co-workers (9, 11). Sensitivity to novobiocin (Sigma Chemical Co., St. Louis, Mo.) was determined by dilution in agar, using Mueller-Hinton medium (Difco) at concentrations of 0.5, 1, 1.5, 4, 8, and 16 μg/ml. Plates were incubated at 37°C for 48 h. The MIC for novobiocin-sensitive CNS was ≤1 μg/ml (40).

The following reference strains were used for biochemical and physiological analyses of CNS: *S. cohnii* DSM 20260, *S. haemolyticus* DSM 20263, *S. xylosus* DSM 20266, *S. sciuri* ATCC 29062, *S. lentus* ATCC 29070, *S. caprae* CCM 3573, *S. arlettae* BP 47, *S. kloosii* DSM 20676, *S. warneri* ATCC 27836, and *S. simulans* ATCC 27848.

Enterotoxin production on solid media. A 500-μl sample of the 18-h broth inoculated with the staphylococcal strains was poured on the surface of cellophane membranes (Spectrapor membrane tubing; Spectrum Medical Industries Inc., Los Angeles, Calif.) on brain heart infusion agar, as described by Hallander (21), and harvested with 2.5 ml of 0.01 M Na_2HPO_4.

Enterotoxin detection. SEA, SEB, SEC, SED, and SEE present in extracts and milk were detected by the enzyme-

* Corresponding author.

TABLE 1. Enterotoxigenicity of staphylococci isolated, by site of isolation

Location	Enterotoxigenic strains		Nonenterotoxigenic strains	Coagulase positive			Coagulase negative		
				Enterotoxigenic strains		Nonenterotoxigenic strains	Enterotoxigenic strains		Nonenterotoxigenic strains
	No.	%		No.	%		No.	%	
Nasal	13	36.2	23	6	85.7	1	7	24.1	22
Axilla		0	21		0			0	21
Udder skin	27	30.7	61	9	90	1[a]	18	30	60
Nipple skin	51	33.2	103	19/1[a]	59.4	13/3[a]	32	28.6	90
Milk	21	48.8	22	18/1[a]	85.7	3	3	13.6	19
Total	112	32.7	230	52	74.3	18	60	22	212

[a] *S. hyicus* CPS.

linked immunosorbent assay described by Freed et al. (14), with a detection limit of 0.625 ng of SE per ml. To remove staphylococcal protein A, samples or extracts were diluted 1:1 in normal rabbit serum with inactivated complement (13). The serum-sample mixture was incubated at 4°C for 1 h. Reference toxins and antisera were obtained from M. Bergdoll, Food Research Institute, Madison, Wis., to whom we are greatly indebted.

RESULTS AND DISCUSSION

A total of 342 staphylococcal strains were isolated from the different sites, corresponding to 70 CPS and 272 CNS. Of these, 112 (32.7%) were enterotoxigenic, 52 CPS (74.3%) and 60 CNS (22%) (Table 1). The percentage of enterotoxigenic CPS isolated in this study was considerably higher than that reported for *S. aureus* strains isolated in different animals species (2, 15, 20, 25, 36, 43) and higher than that reported for *S. aureus* strains in infected cow's milk (1, 16, 22, 26, 35), but it was similar to that reported by Hàjek (19) and Gutiérrez et al. (18) for *S. aureus* strains isolated from infected sheep's milk. Similarly, De Buyser et al. (8) and Harvey and Gilmour (23) reported a high percentage of enterotoxigenic *S. aureus* strains in infected (75%) and normal (35%) goat's milk. Most enterotoxigenic strains were isolated from the skin of the mammary gland and from milk, with very high percentages of CPS (udder, 90%; teat, 59.4%; milk, 85.7%), and lower percentages CNS (udder, 30%; teat, 28%; milk, 13.6%). Fewer SE-producing strains were isolated in the nasal mucosa, although enterotoxigenicity percentages were very high (85.7% of CPS and 24.1% of CNS). No SE-producing strains were isolated from the skin of the axillary fold.

Most of the enterotoxigenic strains produced SEC either alone (67.9%) or in combination with other toxins (SEA+C, 2.7%; SEB+C, 2.7%; SEC+E, 2.7%; SEA+B+C, 0.9%) (Table 2). These results agree with those obtained in sheep by Hàjek (19) and and Gutiérrez et al. (18) and in goats by De Buyser et al. (8) and Harvey and Gilmour (23), which seems to suggest that staphylococcal strains isolated from sheep and goats produce mainly SEC. The other enterotoxins produced occurred in low percentages, especially SED, which was only synthesized by one strain of *S. caprae* in combination with SEE (Table 2).

Two species of enterotoxigenic CPS (*S. aureus* and *S. hyicus*) and nine species of enterotoxigenic CNS (*S. chromogenes*, *S. haemolyticus*, *S. warneri*, *S. epidermidis*, *S.*

TABLE 2. Types of enterotoxin (SE) produced by identified staphylococcal species

Species	No. of strains	No. producing given SE										Total	%
		SEA	SEB	SEC	SEE	SEAB	SEAC	SEBC	SECE	SEDE	SEABC		
S. aureus	64	5		39			3	1	1		1	50	78.1
S. hyicus	13/6[a]			2[a]								2	15.4
S. chromogenes	23			3								3	13.0
S. haemolyticus	64		1	13	3	1		1	1			20	31.3
S. warneri	45	1	2	5								8	17.8
S. epidermidis	32	2		5								7	21.9
S. caprae	18		1	1		1				1		4	22.2
S. xylosus	23			3	1				1			5	21.8
S. sciuri	20			1	3							4	20.0
S. saprophyticus	13			2	2			1				5	38.5
S. cohnii	6											0	0.0
S. lentus	3				2							2	66.6
S. equorum	3											0	0.0
S. kloosii	3											0	0.0
Unidentified[b]	12			2								2	16.6
Total	342	8	4	76	11	2	3	3	3	1	1	112	
%[c]		7.1	3.6	67.9	9.9	1.8	2.7	2.7	2.7	0.9	0.9	100	

[a] *S. hyicus* CPS.
[b] CNS.
[c] Average over all enterotoxin-producing strains.

TABLE 3. Enterotoxigenic staphylococci isolated from goat milk

Species	No. of strains[a]	No. producing:			
		SEA	SEC	SEAC	SECE
S. aureus	20	1	13	2	1
S. hyicus	1[b]		1		
S. warneri	5				
S. chromogenes	5		2		
S. caprae	5				
S. epidermidis	4		1		
S. haemolyticus	2				
S. xylosus	1				

[a] Counts, 600 CFU/ml in Schleifer and Krämer (43) medium.
[b] *S. hyicus* CPS.

caprae, *S. xylosus*, *S. sciuri*, *S. saprophyticus*, and *S. lentus*) were identified, while two SEC-producing CNS remained unidentified. Difficulty in determining the enterotoxigenicity of staphylococci has led researchers to seek some property in staphylococci which may be related to enterotoxin production. The product most commonly associated with enterotoxigenicity is coagulase (12), although other products, such as thermonuclease, have also been reported (7, 29, 33). It is widely accepted that SE production is characteristic of CPS, and most studies have dealt with *S. aureus*, with the result that less work has been done on coagulase-negative biovarieties. However, SE production by CNS has been reported by some authors (5, 7, 29, 31, 38; Bergdoll et al., Bacteriol. Proc.), and with particular reference to *S. epidermidis* (4–6, 24, 37), *S. haemolyticus* (37), *S. capitis* (37), *S. hyicus* (24), *S. cohnii* (3), and *S. xylosus* (3). In the present study, some of the enterotoxigenic strains isolated belonged to species not previously reported as SE producers, such as *S. chromogenes*, *S. warneri*, *S. sciuri*, *S. saprophyticus*, and *S. lentus* (Table 2).

Detection of SE in goats' milk. Counts of >600 CFU of staphylococci per ml were isolated from the milk of 43 (32.3%) of the 133 animals tested. Of the 43 strains isolated and identified, 48.8% were enterotoxigenic, and most produced SEC (Table 3). These results show that a large number of animals showing no clinical symptoms were subclinically infected by enterotoxigenic staphylococcal strains. Many authors have indicated the staphylococcal origin of subclinical infections in goat udders (8, 23, 30, 39, 45). SE (SEA, SEB, and SEC) were directly detected in the milk of 17 of the 133 animals tested. SEB, an enterotoxin not produced by any of the strains isolated in the milk, was found in the milk of 10 of the 17 animals (Table 4). This suggests that SE production may take place directly in the affected udder. Niskanen et al. (34) detected SEA and SEB production in the milk of cows experimentally inoculated in the udder with *S. aureus* strains producing these enterotoxins. That no SEB-producing strains were isolated in milk, despite the presence of SEB in the milk itself, suggests that, (i) due to inadequate selection of staphylococci on culture media, SEB-producing strains were not isolated; or, (ii) in enterotoxin production on solid media on the surface of cellophane membranes (21), the strains failed to synthesize SEB, whereas they did synthesize it in natural conditions in the udder. Gómez-Lucía et al. (17) reported that enterotoxin production depends on the natural substrate, so that different results may be achieved when the strain is grown on cellophane over agar to determine its toxigenicity.

We may conclude that a high percentage of goats should be considered as carriers of enterotoxin-producing staphylococci, secreting mainly SEC. This fact may be of relevance to human health, as goats may be the origin of contamination at some point in the food chain, especially milk and dairy products from this species.

TABLE 4. Detection of staphylococcal enterotoxins in the milk of clinically healthy goats

Goat no.	ng of SE per ml		
	SEA	SEB	SEC
6		4.7	
7		2.3	
15	5.8		
16			2.1
17	3.8		
28			4.3
43		3.0	
45		3.0	
47			2.0
49		2.6	
50		2.0	
51		5.4	2.3
53		2.0	
101		10.1	
105		5.1	
112	2.3		
118	2.3		

ACKNOWLEDGMENT

We are grateful for the technical assistance provided by E. Fernández Corrales.

LITERATURE CITED

1. **Abbar, F. M., M. Tahir Mohammed, and S. H. Arslain.** 1986. Selected biological properties of enterotoxigenic staphylococci isolated from milk. J. Food Prot. **49**:871–873.
2. **Adekeye, D.** 1980. Enterotoxin production by strains of *Staphylococcus aureus* isolated from animals and man in Nigeria. Vet. Microbiol. **5**:143–150.
3. **Bautista, L., P. Gaya, M. Medina, and M. Nuñez.** 1988. A quantitative study of enterotoxin production by sheep milk staphylococci. Appl. Environ. Microbiol. **54**:566–569.
4. **Bergdoll, M. S., B. A. Crass, R. F. Reiser, R. N. Robbins, and J. P. Davis.** 1981. A new staphylococcal enterotoxin, enterotoxin F, associated with toxic shock syndrome *Staphylococcus aureus*. Lancet **i**:1017–1021.
5. **Breckinridge, J. C., and M. S. Bergdoll.** 1971. Outbreak of food borne gastroenteritis due to a coagulase negative enterotoxin producing *Staphylococcus*. N. Engl. J. Med. **284**:541–543.
6. **Crass, B. A., and M. S. Bergdoll.** 1986. Involvement of coagulase-negative staphylococci in toxic shock syndrome. J. Clin. Microbiol. **23**:43–45.
7. **Danielsson, M. L., and B. Hellberg.** 1977. The biochemical activity of enterotoxin and non-enterotoxin producing staphylococci. Acta Vet. Scand. **18**:266–273.
8. **De Buyser, M. L., F. Dilasser, R. Hummel, and M. S. Bergdoll.** 1987. Enterotoxin and toxic shock syndrome toxin-1 production by staphylococci isolated from goat's milk. Int. J. Food Microbiol. **5**:301–309.
9. **Devriese, L. A.** 1986. Coagulase negative staphylococci in animals, p. 51–57. *In* P. A. Mardh and K. H. Schleifer (ed.), Coagulase negative staphylococci. Almqvist and Wiksell International, Stockholm.
10. **Devriese, L. A., and V. Hájek.** 1980. Identification of pathogenic staphylococci isolated from animals and foods derived from animals. J. Appl. Bacteriol. **49**:1–11.
11. **Devriese, L. A., K. H. Schleifer, and G. O. Adegoke.** 1985. Identification of coagulase negative staphylococci from farm animals. J. Appl. Bacteriol. **55**:45–55.

12. **Evans, J. B., L. G. Beuttner, and C. F. Niven.** 1950. Evaluation of the coagulase tests in the study of staphylococci associated with food poisoning. J. Bacteriol. **60**:481–484.
13. **Fey, H., H. Pfister, and O. Rüegg.** 1984. Comparative evaluation of different enzyme-linked immunosorbent assay systems for the detection of staphylococcal enterotoxins A, B, C, and D. J. Clin. Microbiol. **19**:34–38.
14. **Freed, R. C., M. L. Evenson, R. F. Reiser, and M. S. Bergdoll.** 1982. Enzyme-linked immunosorbent assay for detection of staphylococcal enterotoxin in foods. Appl. Environ. Microbiol. **44**:1349–1355.
15. **Fukuda, S., H. Tokuno, H. Ogawa, M. Sasaki, T. Kishimoto, J. Kawano, A. Shimizu, and S. Kimura.** 1984. Enterotoxigenicity of *Staphylococcus intermedius* strains isolated from dogs. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A **258**:360–367.
16. **García, M. L., B. Moreno, and M. S. Bergdoll.** 1980. Characterization of staphylococci isolated from mastitic cows in Spain. Appl. Environ. Microbiol. **39**:548–553.
17. **Gómez-Lucía, E., J. Goyache, J. A. Orden, J. L. Blanco, J. A. Ruiz-Santa Quiteria, L. Domínguez, and G. Suárez.** 1989. Production of enterotoxin A by supposedly nonenterotoxigenic *Staphylococcus aureus* strains. Appl. Environ. Microbiol. **55**: 1447–1451.
18. **Gutiérrez, L., I. Menes, M. L. García, B. Moreno, and M. S. Bergdoll.** 1982. Characterization and enterotoxigenicity of staphylococci isolated from mastitic ovine milk in Spain. J. Food Prot. **45**:1282–1286.
19. **Hàjek, V.** 1978. Identification of enterotoxigenic staphylococci from sheep and sheep cheese. Appl. Environ. Microbiol. **35**: 264–268.
20. **Hàjek, V., and E. Marsàlek.** 1973. The occurrence of enterotoxigenic *Staphylococcus aureus* strains in host of different animal species. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A **223**:63–68.
21. **Hallander, H. O.** 1965. Production of large quantities of enterotoxin B and other staphylococcal toxins on solid media. Acta Pathol. Microbiol. Scand. **63**:299–305.
22. **Harvey, J., and A. Gilmour.** 1985. Application of current methods for isolation and identification of staphylococci in raw bovine milk. J. Appl. Bacteriol. **59**:207–221.
23. **Harvey, J., and A. Gilmour.** 1988. Isolation and characterization of staphylococci from goats milk produced in Northern Ireland. Lett. Appl. Microbiol. **7**:79–82.
24. **Hoover, D. G., S. R. Tatini, and J. B. Maltais.** 1983. Characterization of staphylococci. Appl. Environ. Microbiol. **46**:649–660.
25. **Kato, E., Y. Kaji, and K. Kaneko.** 1978. Enterotoxigenic staphylococci of canine origin. Am. J. Vet. Res. **39**:1771–1773.
26. **Kato, E., and T. Kume.** 1980. Enterotoxigenicity of bovine staphylococci isolated from California mastitis test-positive milk in Japan. Jpn. J. Vet. Res. **28**:75–85.
27. **Kloos, W. E.** 1980. Natural populations of the genus *Staphylococcus*. Annu. Rev. Microbiol. **34**:559–592.
28. **Lachica, V. R. F., P. D. Hoeprich, and C. Genigeorgis.** 1971. Nuclease production and lysostaphin susceptibility of *Staphylococcus aureus* and other catalase-positive cocci. Appl. Microbiol. **21**:823–826.
29. **Lachica, V. R. F., K. F. Weiss, and R. H. Deibel.** 1969. Relationships among coagulase, enterotoxin, and heat-stable deoxyribonuclease production by *Staphylococcus aureus*. Appl. Microbiol. **18**:126–127.
30. **Lerondelle, C., and B. Poutrel.** 1984. Characteristics of nonclinical mammary infections of goats. Ann. Rech. Vet. **15**:105–112.
31. **Lotter, L. P., and C. A. Genigeorgis.** 1975. Deoxyribonucleic acid base composition and biochemical properties of certain coagulase-negative enterotoxigenic cocci. Appl. Microbiol. **29**: 152–158.
32. **Mardh, P. A., B. Hovelius, K. Hovelius, and P. O. Nilsson.** 1978. Coagulase negative, novobiocin resistant staphylococci on the skin of animals and man, on meat and in milk. Acta Vet. Scand. **19**:243–253.
33. **Niskanen, A., and L. Koiranen.** 1977. Correlation of enterotoxin and thermonuclease production with some physiological and biochemical properties of staphylococcal strains isolated from different sources. J. Food Prot. **40**:543–548.
34. **Niskanen, A., L. Koiranen, and K. Roine.** 1978. Staphylococcal enterotoxin and thermonuclease production during induced bovine mastitis and the clinical reaction of enterotoxin in udders. Infect. Immun. **19**:493–498.
35. **Olson, J. C., E. P. Casmam, E. F. Baer, and J. E. Stone.** 1970. Enterotoxigenicity of *Staphylococcus aureus* cultures isolated from acute cases of bovine mastitis. Appl. Microbiol. **20**: 605–607.
36. **Olsvik, O., B. P. Berdal, K. Fossum, and T. Omland.** 1981. Enterotoxin production by *Staphylococcus aureus* related to the origin of the strains. Acta Pathol. Microbiol. Scand. Sect. B **89**:423–426.
37. **Olsvik, O., K. Fossum, and B. P. Berdal.** 1982. Staphylococcal enterotoxin A, B, and C produced by coagulase-negative strains within the family *Micrococcaceae*. Acta Pathol. Microbiol. Immunol. Scand. Sect. B **90**:441–444.
38. **Omori, G., and Y. Kato.** 1959. A staphylococcal food poisoning caused by coagulase negative strain. Biken J. **2**:92–96.
39. **Poutrel, B.** 1984. Udder infection of goats by coagulase negative staphylococci. Vet. Microbiol. **9**:131–137.
40. **Schleifer, K. H.** 1986. Taxonomy of coagulase-negative staphylococci, p. 11–26. *In* P. A. Mardh and K. H. Schleifer (ed.), Coagulase-negative staphylococci. Almqvist and Wiksell International, Stockholm.
41. **Schleifer, K. H., and W. E. Kloos.** 1975. A simple test for the separation of staphylococci from micrococci. J. Clin. Microbiol. **1**:337–338.
42. **Schleifer, K. H., and E. Krämer.** 1980. Selective medium for isolating staphylococci. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe C **1**:270–280.
43. **Shiozawa, K., E. Kato, and A. Shimizu.** 1980. Enterotoxigenicity of *Staphylococcus aureus* strains isolated from chickens. J. Food Prot. **43**:683–685.
44. **Smith, M. C., and M. Roguinsky.** 1977. Mastitis and other diseases of the goat's udder. J. Am. Vet. Med. Assoc. **171**: 1241–1248.
45. **Von Rheinbaben, K. E., and R. M. Hadlok.** 1981. Rapid distinction between micrococci and staphylococci with furazolidone agar. Antonie van Leeuwenhoek J. Microbiol. Serol. **47**:41–51.