JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 1989, p. 2444–2447
0095-1137/89/112444-04$02.00/0

Vol. 27, No. 11

Antibodies to Staphylococcal DNases in Sera from Different Animal Species, Including Humans

STEINAR HØIE[1]* AND KÅRE FOSSUM[2]

National Veterinary Institute, P.O. Box 8156 Dep. 0033, Oslo 1,[1] and Department of Microbiology and Immunology, Norwegian College of Veterinary Medicine, Oslo,[2] Norway

Received 27 March 1989/Accepted 7 August 1989

An agar diffusion method using microtiter plates was used to detect antibodies to the DNases produced by *Staphylococcus aureus*, *S. intermedius*, and *S. hyicus*. Antibodies to DNase from *S. aureus* were demonstrated in most of the sera from the species investigated, except dogs, only 11% of whose sera were positive. Positive titers to *S. intermedius* DNase were found in 84% of dog sera, 61% of Icelandic pony sera, 41% of pig sera, 21% of human sera, and 20% of cow sera but in only 2 and 4% of goat and sheep sera, respectively. Although antibodies to DNase from *S. hyicus* were not found in sera from humans, dogs, goats, or sheep, 84% of sera from pigs and cows and 29% of sera from Icelandic ponies were positive in this respect. The good accordance between the findings from bacteriological investigations performed elsewhere and the results of serologic tests performed in this study indicates that the results obtained with the serological method in this study properly reflect the actual antigenic exposure to and distribution of the three *Staphylococcus* spp. in animals and humans.

The presence of antibodies to various components and products of a bacterial species in animal sera may reflect the distribution of the organism in question. Sandvik (15) regarded the humoral immune response to exposure to staphylococcal DNases as strong. According to Gudding (7), the DNases produced by *Staphylococcus aureus*, *S. intermedius*, and *S. hyicus* are serologically different. Ness (14) examined the pattern of antibodies against staphylococcal DNases in 205 dog sera and found high antibody titers to *S. intermedius* DNase in 33.8% of sera from animals assumed to be healthy and in 46.4% of sera from animals with a history of skin disorders. High antibody titers to *S. aureus* and *S. hyicus* DNases were detected in these sera on only one occasion in each case.

The aim of the present study was to investigate the presence of antibodies to the DNases (anti-DNases) produced by *S. aureus*, *S. intermedius*, and *S. hyicus* in sera from humans and various domestic animal species by an agar diffusion method using microtiter plates (S. Høie and R. Gudding, Acta Vet. Scand., in press).

MATERIALS AND METHODS

Sera. The sera and their origins were as follows: 62 human serum samples collected from the blood bank of a hospital in Oslo, Norway; 64 dog serum samples collected from the polyclinic of the Norwegian College of Veterinary Medicine, Oslo, Norway; 63 goat serum samples (12 from kids and 51 from lactating goats); 54 sheep serum samples (36 from lambs and 18 from ewes); 64 lactating cow serum samples; 64 pig serum samples (14 from slaughter pigs and 50 from sows); 64 Icelandic pony (2 years of age or more) serum samples from Norway; 64 Icelandic pony serum samples from Iceland. The animal sera originated from several different herds or flocks.

Determination of anti-DNases. An agar diffusion method using microtiter plates with toluidine blue DNA agar as the basic substrate was used to monitor the levels of anti-DNases in sera (Høie and Gudding, in press). The following *Staphylococcus* spp. were used for DNase production: *S. aureus* NVH (culture collection of the Department of Microbiology and Immunology, Norwegian College of Veterinary Medicine) 3610 (from goat mastitis), *S. intermedius* NVH 3670 (from dog skin), and *S. hyicus* subsp. *hyicus* ATCC (American Type Culture Collection, Rockville, Md.) 11249 (from pig exudative epidermitis). DNase samples were added to melted toluidine blue DNA agar with constant stirring at 56°C. Altogether, 100 μl of toluidine blue DNA agar with added DNase was placed into wells of F-shaped microtiter plates. The plates were stored at 4°C for a maximum of 4 h before 20-μl volumes of serum dilutions were applied. Twofold dilutions of the test sera were made in 0.05 M Tris hydrochloride buffer (pH 9.0). The plates were preincubated for 18 h at 4°C and subsequently incubated at 37°C for 24 h. After incubation, the plates were inspected against a white background with lighting from below. This examination was also performed after the plates were stored for 24 h at 4°C. Each test serum was examined only once.

The amount (titer) of anti-DNase in the sera tested was defined as the log of the reciprocal value of the highest dilution of serum that prevented any change of the toluidine blue DNA agar from blue to pink. A titer of 0.3 or more was denoted a positive titer, and mean titers were calculated from the values of positive samples.

RESULTS

The animal species examined, the numbers of sera of each species tested, the percentages of positive sera, and the mean titers of antibodies to *S. aureus*, *S. intermedius*, and *S. hyicus* are presented in Table 1. Figure 1 shows the distribution of the titers of the sera from humans, dogs, goats, cows, pigs, and ponies from Norway to the three staphylococcal DNases.

The titer distribution patterns of the sera from sheep and goats were similar, as were the distribution patterns of the sera from the ponies from Norway and Iceland. Antibodies to the DNase from *S. aureus* were found in most of the sera from the species investigated, except for dogs, only 11% of whose sera were positive. Altogether, 60 of the 62 human

* Corresponding author.

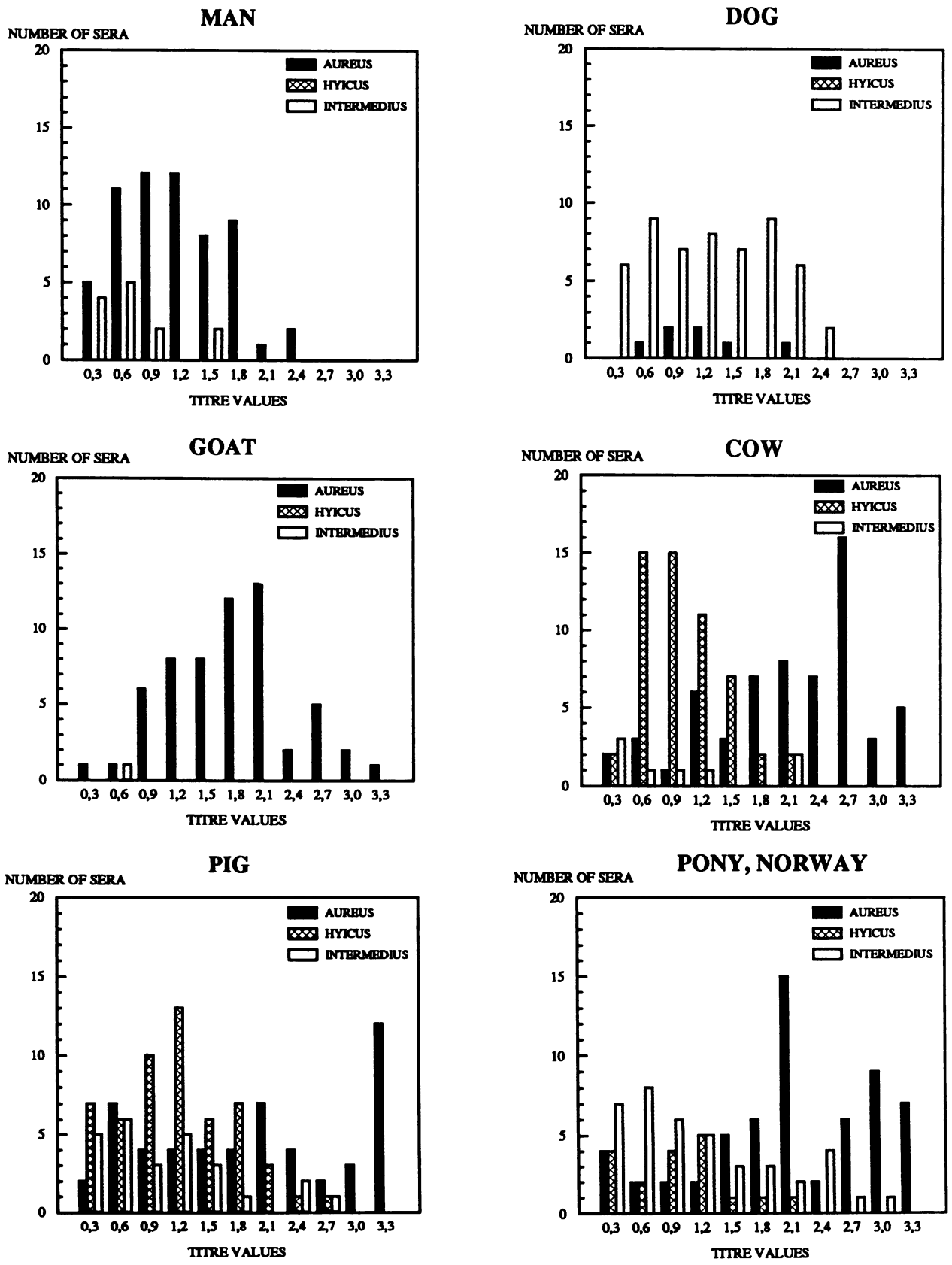

FIG. 1. Distributions of the titers of the various sera to DNases from *S. aureus*, *S. intermedius*, and *S. hyicus*.

TABLE 1. Percentages of sera from animals and humans with positive titers to DNase from *S. aureus*, *S. intermedius*, and *S. hyicus*

Species (no. of sera)	% of sera (mean titer) with titers of ≥0.3 to DNase from:		
	S. aureus	*S. intermedius*	*S. hyicus*
Human (62)	97 (1.14)	21 (0.69)	0 (0.00)
Dog (64)	11 (1.20)	84 (1.24)	0 (0.00)
Goat (63)	94 (1.77)	2 (0.60)	0 (0.00)
Sheep (54)	56 (1.57)	4 (1.65)	0 (0.00)
Cow (64)	95 (2.13)	20 (0.90)	84 (1.01)
Pig (64)	83 (1.98)	41 (1.06)	84 (1.17)
Pony[a] (64)	94 (2.13)	63 (1.19)	28 (0.97)
Pony[b] (64)	97 (1.87)	59 (1.14)	30 (0.65)

[a] Icelandic ponies from Norway.
[b] Icelandic ponies from Iceland.

serum specimens showed positive titers to *S. aureus* DNase. Positive titers to *S. intermedius* DNase were detected in 13 serum specimens, and 1 of these had a lower titer to *S. aureus* DNase. A positive titer to *S. intermedius* DNase was found in 84% of the dog sera. Only one of the serum samples from a goat and two of the serum samples from sheep showed positive titers to *S. intermedius* DNase, and then only at a low level compared with the high titers to *S. aureus* DNase in these three sera. Antibodies to DNase from *S. hyicus* were not found in the sera from humans, dogs, goats, or sheep, although 84% of the sera from pigs and cows and 29% of the sera from Icelandic ponies were positive.

DISCUSSION

The presence of antibodies to the DNases of *S. aureus*, *S. hyicus*, and *S. intermedius* in animal sera may reflect the distribution of these bacteria among the animal species concerned. The findings of the present study confirm the consistent finding previously reported by other workers (1, 2, 8) that *S. intermedius* is the major *Staphylococcus* sp. isolated from dogs. In a study performed by Ness (14) using a semiquantitative agar diffusion test, antibodies to *S. aureus* DNase were found in all of the sera examined. The inhibitory activity of these antibodies was, however, reported to be weak against *S. aureus* DNase and strong against *S. intermedius* DNase in most of the sera. This may reflect a higher specificity of the present method, especially since the sera originated from the same geographical region.

According to Devriese and Hajek (5), *S. intermedius* and *S. hyicus* do not occur in humans. However, the present study, apart from indicating a wide distribution of *S. aureus* in humans, also indicates that humans are subject to a certain exposure to *S. intermedius* antigens. The human sera in this study were collected from the blood bank of a hospital in Oslo. The results may, therefore, reflect the close contact between animals and people in an urban society, since *S. intermedius* is a common and often predominant staphylococcal species inhabiting the nasal membranes and skin of dogs (11). The present results do not, however, give any indications regarding the possible pathogenicity of *S. intermedius* for humans.

The results of investigation of goat and sheep sera indicate that of the three *Staphylococcus* spp., only *S. aureus* is widely distributed within these populations of animals. In various studies (10, 12, 13, 16), it has been found that of the three *Staphylococcus* spp., *S. aureus* is the one most frequently isolated from bovine milk samples and *S. hyicus* is rarely found. According to Devriese and Derycke (4), *S. hyicus* appears to contribute to the normal skin flora of healthy cattle. The above-mentioned findings may reflect a wide distribution of *S. aureus* and *S. hyicus* among cattle and are in accordance with those of the present investigation. The present results also indicate a certain exposure of cattle to *S. intermedius*; this is in accordance with the findings of Hodges et al. (9) and Watts et al. (16).

The present study suggests that *S. aureus* and *S. hyicus* are widely distributed among pigs. The results concerning *S. hyicus* are consistent with the findings of an investigation performed by Devriese (3) in which *S. hyicus* was frequently isolated from healthy pigs and lesions in pigs. The present study also indicates a certain exposure of pigs to *S. intermedius*.

Devriese et al. (6) identified *S. aureus* most frequently among the *Staphylococcus* spp. isolated from lesions in horses, followed in descending order of frequency by *S. intermedius* and *S. hyicus*. This is in accordance with the results of the present study, in which similar results were obtained concerning antigen exposure of ponies from Norway and Iceland to the three *Staphylococcus* spp. The present study indicates little or no occurrence of *S. hyicus* among dogs, goats, and sheep.

Gudding (7) reported the absence of antigenic cross-reactivity, except for the DNase of an *S. intermedius* strain from pigeons which induced production of anti-DNases which inhibited the DNase activities of the *S. aureus* strains. Lachica et al. (12) reported that one of the three antisera they used, which were originally produced against various other antigens of *S. aureus*, inhibited the DNases of *S. intermedius* and *S. epidermidis*, although only at low serum dilutions. The present findings concerning sera from humans, dogs, goats, and sheep indicate that *S. aureus* and *S. intermedius* DNases do not cross-react with antisera to *S. hyicus* DNase. A certain cross-reactivity between *S. aureus* and *S. intermedius* DNases may be indicated by the findings on goat and sheep sera.

The good accordance between the findings from bacteriological investigations performed elsewhere and the results of serologic tests performed in the present study indicates that the results obtained with the serological method in the present study properly reflect the actual antigenic exposure to and distribution of the three *Staphylococcus* spp. in animals and humans.

LITERATURE CITED

1. **Berg, J. N., D. E. Wendell, C. Vogelweid, and W. H. Fales.** 1984. Identification of the major coagulase-positive Staphylococcus sp. of dogs as Staphylococcus intermedius. Am. J. Vet. Res. **45**:1307–1309.
2. **Biberstein, E. L., S. S. Jang, and D. C. Hirsch.** 1984. Species distribution of coagulase-positive staphylococci in animals. J. Clin. Microbiol. **19**:610–615.
3. **Devriese, L. A.** 1977. Isolation and identification of Staphylococcus hyicus. Am. J. Vet. Res. **38**:787–792.
4. **Devriese, L. A., and J. Derycke.** 1979. Staphylococcus hyicus in cattle. Res. Vet. Sci. **26**:356–358.
5. **Devriese, L. A., and V. Hajek.** 1980. Identification of pathogenic staphylococci isolated from animals and foods derived from animals. J. Appl. Bacteriol. **49**:1–11.
6. **Devriese, L. A., D. Nzuambe, and C. Godard.** 1985. Identification and characteristics of staphylococci isolated from lesions and normal skin of horses. Vet. Microbiol. **10**:269–277.
7. **Gudding, R.** 1983. Differentiation of staphylococci on the basis of nuclease properties. J. Clin. Microbiol. **18**:1098–1101.
8. **Gudding, R., and E. Ness.** 1985. Identification of nuclease positive staphylococci isolated from animals. J. Med. Micro-

biol. **20**:399–402.

9. **Hodges, R. T., Y. S. Jones, and J. T. S. Holland.** 1984. Characterisation of staphylococci associated with clinical and subclinical bovine mastitis. N. Z. Vet. J. **32**:141–145.
10. **Jasper, E., F. Infante, and J. D. Dellinger.** 1985. Accuracy of the API Staph-Indent system for identification of Staphylococcus species from milk. Am. J. Vet. Res. **46**:1263–1267.
11. **Kloos, W. E., and K. H. Schleifer.** 1986. Genus IV. *Staphylococcus*, p. 1013–1035. *In* P. H. A. Sneath, N. S. Mair, M. E. Sharpe, and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 2. The Williams & Wilkins Co., Baltimore.
12. **Lachica, R. V. F., S. S. Jang, and P. D. Hoeprich.** 1979. Thermonuclease seroinhibition test for distinguishing *Staphylococcus aureus* from other coagulase-positive staphylococci. J. Clin. Microbiol. **9**:141–143.
13. **Langlois, B. E., R. J. Harmon, and K. Akers.** 1983. Identification of *Staphylococcus* species of bovine origin with the API Staph-Indent system. J. Clin. Microbiol. **18**:1212–1219.
14. **Ness, E.** 1984. Patterns of antibodies to staphylococcal DNases in dog sera. J. Clin. Microbiol. **20**:806–807.
15. **Sandvik, O.** 1974. The occurrence of antibodies against staphylococcal deoxyribonucleases in blood sera from different species. Acta Vet. Scand. **15**:631–635.
16. **Watts, J. L., W. Pankey, and S. C. Nickerson.** 1984. Evaluation of the Staph-Ident and STAPHase systems for identification of staphylococci from bovine intramammary infections. J. Clin. Microbiol. **20**:448–452.