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Description of 14 New Capsular Types of *Streptococcus suis*

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Fourteen new capsular types of *Streptococcus suis* (types 9 to 22) are described. All reference strains are morphologically and biochemically similar to types previously described. Reference strain types 9 to 13, 15, 16, and 22 were isolated from diseased pigs, whereas types 17 to 19 and 21 came from clinically healthy pigs; type 14 was isolated from a human case of meningitis, and type 20 was isolated from a diseased calf. The group T streptococcus of de Moor has been included in the typing system as type 15. Two-way cross-reactions between types 6 and 16 and a one-way cross-reaction between types 2 and 22 have been demonstrated. In addition, several cross-reactions probably not due to capsular material were detected among different types by using the coagglutination test. This test should not be used alone; weak or multiple positive reactions must be confirmed by the capsular reaction test or the capillary precipitation test.

Streptococcus suis is an important swine pathogen isolated in almost all countries where the pig industry is developed. It has been associated mainly with bronchopneumonia and meningitis and less frequently with endocarditis, arthritis, and other infections (2, 10, 18, 19, 25, 26, 28). It is also known to cause meningitis in humans (1, 6, 30).

Originally defined as Lancefield groups R, S, RS, and T by de Moor (7), strains of *S. suis* were later shown to share antigens with group D streptococci (8). The capsule on *S. suis* cells suggested a type-specific antigenicity, and groups S, R, and RS were regrouped as *S. suis* types 1, 2, and 1/2, respectively (8, 29). Recently, however, *S. suis* was shown to be a genetically homogeneous species which is different from other members of Lancefield group D (16). Until now, the group T of de Moor, which is capsulated and defined biochemically and genetically as *S. suis*, had been omitted. In 1983, Perch et al. (23) presented six new types of *S. suis* (types 3 to 8) isolated from diseased pigs, and they postulated the existence of other types. Since then, untypeable isolates (not associated with any of the nine published types) have frequently been isolated from healthy and diseased pigs (5, 13, 24, 26, 28; L. M. Wilkins, Proc. Am. Assoc. Swine Pract. 1989, p. 365–367). The variety of new types mentioned by several authors has introduced some confusion in the literature (4, 24, 25; E. Sanford, Proc. Am. Assoc. Swine Pract. 1989, p. 193–195). This study aims at clarifying the present knowledge regarding *S. suis* typing and includes the characterization of 14 new capsular types of *S. suis*.

MATERIALS AND METHODS

Bacterial strains and field isolates. Bacterial strains used in this study included reference strains of *S. suis* types 1 to 8 and 1/2 (23) and 14 other strains representing proposed types 9 to 22. One of these fourteen strains was the de Moor group T (7), which is proposed as type 15. The 13 other strains were chosen because (i) they were biochemically *S. suis* (22); (ii) they possessed the Lancefield group D antigen, as demonstrated with a coagglutination reagent (Phadebact; Pharmacia Diagnostic, Uppsala, Sweden); and (iii) they were capsulated, as indicated by homogeneous growth in broth (22), but untypeable with all antisera available. Descriptions of the 14 proposed reference strains, including their sources of isolation and countries of origin, are given in Table 1. Because of some particularities inherent in type 22, five field isolates of this type were used along with the other strains.

Production of antisera. Antisera against all the reference strains and field isolates were raised in rabbits. Antigens for immunization were prepared as previously described (20). When required, absorption of antisera was carried out with a 10% (wt/vol) suspension of cells for 2 h at 37°C.

Typing. Typing was based on three different techniques: capsular reaction (Neufeld), capillary precipitation, and coagglutination tests. The capsular reaction test was carried out as described earlier (20) and observed with a phase-contrast microscope (magnification, ×1,000). The capillary precipitation test was performed with antigens extracted with 0.066 N hydrochloric acid (23). Preparation of reagents and the coagglutination technique were carried out as described by Mittal et al. (21). The results of the latter test were described semiquantitatively as 0, 1+, 2+, 3+, and 4+. Only reactions of 2+ or higher were considered positive. One strain of *Enterococcus faecalis* (ATCC 19433), representing Lancefield group D, and a suspension of staphylococcal cells coated with normal rabbit serum were used as controls.

Electron microscopy. Preparation for transmission microscopy was carried out as described previously (15). Capsular material was stabilized with homologous and cross-reacting heterologous antisera; normal rabbit serum was used as a negative control. Thin sections were examined with an electron microscope (Philips 201) at an accelerating voltage of 60 kV.

RESULTS

Fourteen new capsular types (9 to 22) are described. All strains had a colonial morphology, including alpha-hemolysis on bovine blood agar, and a biochemical pattern similar to that of *S. suis*.

In both the capsular reaction and the capillary precipitation tests, clear and specific reactions were obtained for types 9 to 15 and 17 to 21. With both of these techniques, two-way cross-reactions between types 6 and 16 were detected, and a one-way cross-reaction was detected with serum produced against type 2 and the antigen from the reference strain and field isolates of type 22. All these cross-reactions were eliminated after absorption of antisera

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TABLE 1. Description of the 14 strains proposed as reference strains for types 9 to 22 of *S. suis*

Strain no.	Type	Source of isolation	Origin
22083	9	Diseased pig	Denmark[a]
4417	10	Diseased pig	Denmark[a]
12814	11	Diseased pig	Denmark[a]
8830	12	Diseased pig	Denmark[a]
10581	13	Diseased pig	Denmark[a]
13730	14	Diseased human	The Netherlands[b]
NCTC 10446	15	Diseased pig	The Netherlands[c]
2726	16	Diseased pig	Denmark[a]
93A	17	Clinically healthy pig	Canada[d]
NT77	18	Clinically healthy pig	Canada[d]
42A	19	Clinically healthy pig	Canada[d]
86-5192	20	Diseased calf	United States[e]
14A	21	Clinically healthy pig	Canada[d]
88-1861	22	Diseased pig	Canada[d]

[a] K. B. Pedersen, State Veterinary Serum Laboratory, Copenhagen, Denmark.
[b] J. P. Arends, University of Amsterdam, Amsterdam, The Netherlands.
[c] C. E. de Moor (7) (type previously described as group T).
[d] R. Higgins, Faculty of Veterinary Medicine, University of Montreal, Saint-Hyacinthe, Quebec, Canada.
[e] D. D. Johnson, Department of Veterinary Medicine, South Dakota State University, Brookings.

and were related to capsular material. This suggested the presence of common epitopes between these types, which was confirmed by stabilization of the capsule with cross-reacting heterologous antisera observed with electron microscopy (Table 2). Antiserum against type 16 stabilized type 6 capsular material, and type 6 antiserum stabilized type 16 capsular material. Also, the capsule of type 22 was stabilized with the antiserum raised against type 2 but, in this case, to a lesser extent than with the homologous antiserum (Fig. 1). The capsule of type 2 was not stabilized by type 22 antiserum.

The coagglutination test with homologous antiserum gave strong positive reactions for all types. This test revealed not only the cross-reactions mentioned above but also several other cross-reactions among a large number of types that were not detected with the capsular reaction or the capillary precipitation tests (Table 3). This suggests that capsular antigens are not involved in these reactions. In addition, a strong positive reaction obtained only with the coagglutination test using antiserum against type 22 and antigens of the 22 other types of *S. suis*, as well as antigens of *E. faecalis*, suggests cross-reactions due to group D antigen (22). This particular cross-reaction was eliminated after absorption of the antiserum by *E. faecalis*, and the resulting antiserum was specific for capsular material of type 22. Antisera raised against five field isolates of the same type were successfully absorbed in the same manner to eliminate multiple cross-reactions. In all cases, the capsular material observed under electron microcopy completely concealed the cells, as in the case of pneumococci (27).

TABLE 2. Capsule thickness of *S. suis* reference strains for types 6, 16, 2, and 22 stabilized with homologous and heterologous antisera

Strain no.	Type	Antiserum	Capsule thickness (nm)[a]
2524	6	6	190–210
2524	6	16	190–210[b]
2726	16	16	50–60
2726	16	6	40–50[b]
735	2	2	130–150
735	2	22	30–40[c]
88-1861	22	22	200–220
88-1861	22	2	90–110[b]

[a] Each value is based on 20 to 25 measurements per preparation.
[b] No capsular material was observed when normal rabbit serum was used.
[c] A similar capsular thickness was observed when normal rabbit serum was used.

DISCUSSION

Among types 1, 2, 1/2, and 3 to 8, type 2 was most frequently associated with infectious processes in pigs (5, 13, 28). Scandinavian reports showed a higher prevalence of type 7 over other types, including type 2 (3, 23, 26). Streptococci biochemically similar to *S. suis* but untypeable with antisera against the known types have been found in tissues from diseased pigs (5, 13, 26, 28) as well as from clinically healthy pigs (24; L. Brisebois, R. Charlebois, R. Higgins, and M. Nadeau, Can. J. Vet. Res., in press). Recent data from our laboratory indicate that more than 50% of the isolates of *S. suis* retrieved from diseased pigs were not capsular types 1 to 8 or 1/2 (R. Higgins, M. Gottschalk, K. R. Mittal, and M. Beaudoin, Can. J. Vet. Res., in press).

Since the identification of all types of *S. suis* is a prerequisite for further studies on epidemiology, pathogenesis, and control of the infection, the 14 new types identified and characterized by us represent a significant addition to the class of *S. suis* types. Type 15, previously known as de Moor's group T, has been included in the typing system. The type 14 reference strain was isolated from a case of human meningitis (1). More recently, we have isolated this capsular type from diseased pigs in Denmark and Canada. This confirms that *S. suis* type 2 and other capsular types are potential agents of zoonosis (1, 30). The reference strain of type 20 was isolated from a calf, and we have examined two type 9 strains isolated from *Bison bison* with meningitis and a lamb with endocarditis; this indicates that *S. suis* may be pathogenic to a variety of animal species (14). Types 17 to 19 and 21 were isolated from the nasal cavities of clinically healthy pigs, whereas the reference strains of the eight other new types were isolated from diseased pigs, suggesting differences in pathogenicity between types. Until now, types 17 to 19 and 21 have been isolated almost exclusively from healthy pigs (data not shown). However, studies with more strains are needed to validate such a hypothesis.

Cross-reactions due to capsular material have been described for other bacterial species such as *Streptococcus pneumoniae* (20). Cross-reacting capsular material in the *S. suis* species has previously been reported only in type 1/2, which shares antigens with both types 1 and 2 (22). The capillary precipitation and the capsular reaction tests used in this study allowed the detection of two-way capsular cross-reactions between types 6 and 16, which may be removed by absorption of the respective antisera. The finding of a one-way capsular cross-reaction between types 2 and 22 is interesting. As the capsular material of *S. suis* type 2 seems to be implicated in its pathogenesis (9), the presence of common epitopes on these two types may be an indication of similar pathogenic properties. It is noteworthy that most isolates of type 22 were retrieved from diseased pigs in pure culture from multiple tissues (M. Gottschalk, R. Higgins, K. R. Mittal, and M. Jacques, Abstr. Vth Int. Symp. World Assoc. Vet. Lab. Diagn., Guelph, Ontario, Canada, 25 to 30 June 1989, 113). Isolates of type 22 may be incorrectly identified as type 2 when only weak reactions are obtained.

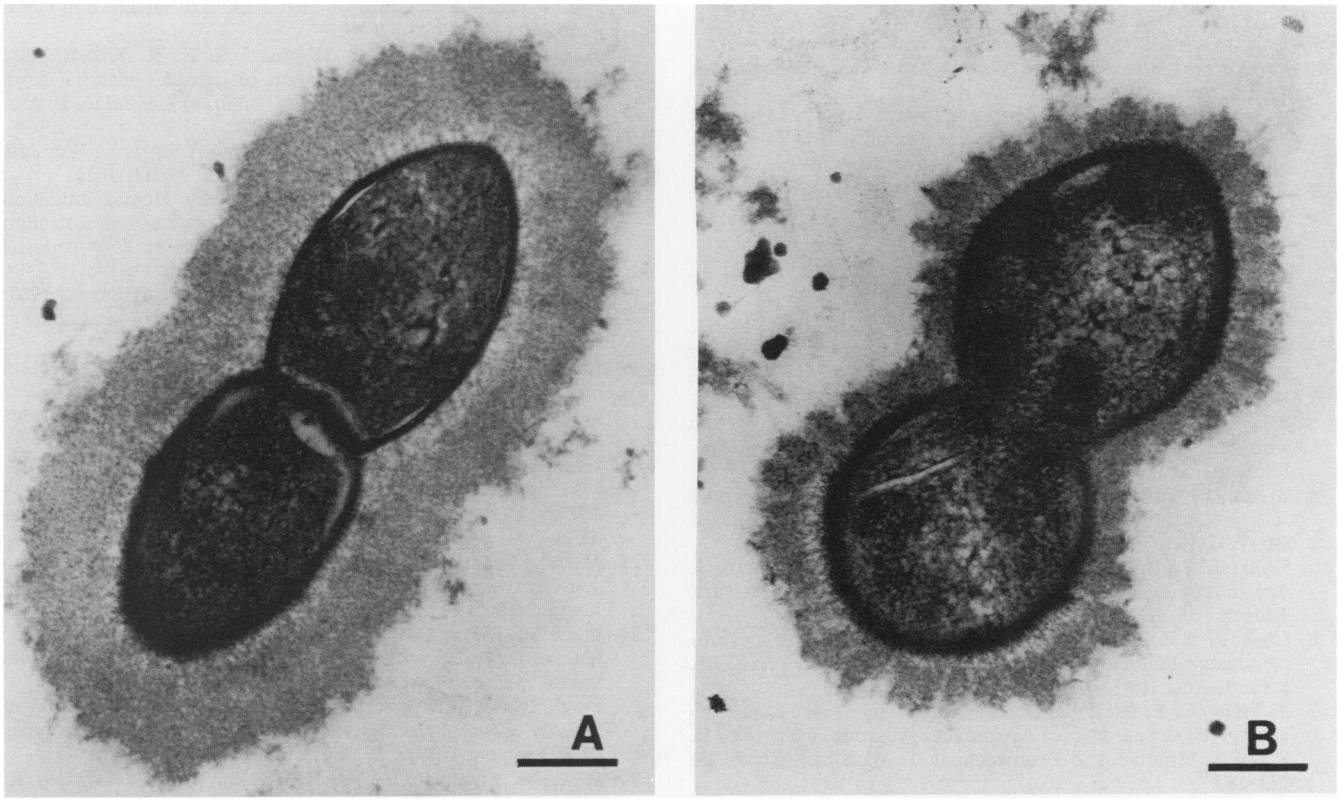

FIG. 1. Transmission electron micrographs of thin sections of cells of *S. suis* 88–1861 (type 22) exposed to homologous antiserum (A) or antiserum against type 2 (B) and stained with ruthenium red. Bar = 200 nm.

This might explain interlaboratory variations in the identification of type 2 strains (24). Immunoelectron microscopy showed stabilization of the capsular material of all cross-reacting capsular types with both homologous and cross-reacting heterologous antisera, confirming that capsular antigens are indeed involved in these cross-reactions (15).

Of the three techniques used to identify *S. suis*, the coagglutination test is the most frequently used in North America (4, 10, 24, 25). However, this study showed that the cross-reactions detected with the coagglutination technique among the 14 new types were not all due to cross-reacting capsular material. Agglutinations due to cell components other than the capsule may play a role. Surface fibrillar structures (M. Jacques et al., submitted for publication) as well as hemagglutinins (17) of *S. suis* have recently been described and would support this theory. Also, nonspecific agglutinations among different streptococci were described earlier (11, 12). Not only may the nonspecific agglutination described here with reference strains occur, but other agglutinations among field isolates (unpublished observations) which would make absorption of antisera useless may also occur. Our results indicate that the coagglutination test for identification of *S. suis* should not be used alone. Weak or multiple positive reactions must be confirmed by the capsular reaction test or the capillary precipitation test.

TABLE 3. Cross-reactions not due to the capsule among different *S. suis* types detected by the coagglutination test

S. suis capsular type[a]	Antiserum[b] (reaction strength[c])
1	14 (4+)
4	15 (4+), 17 (4+), 19 (4+)
9	19 (3+)
10	16 (2+)
11	12 (3+)
15	11 (3+), 16 (2+)
17	16 (3+), 18 (4+), 19 (4+)
18	16 (2+), 17 (4+), 19 (4+)
19	16 (2+), 17 (4+)
21	9 (2+)
All	22 (3+)

[a] Determined by the capsular reaction and capillary precipitation tests.
[b] Capsular types of strains against which the antisera were raised.
[c] With a 3-min reading. Only reactions measuring 2+ or stronger were noted. *E. faecalis* had a cross-reaction of strength 3+ with antiserum against *S. suis* capsular type 22.

S. suis type 22 presented characteristics different from those of other types. Although isolates belonging to this type were well encapsulated, as shown by electron microscopy, antibodies raised in rabbits against five of these isolates were directed against both cell wall antigens and capsular material. As a consequence, the use of the coagglutination test to identify this type requires absorption of serum with group D antigens, whereas the other tests are not affected by this particularity of type 22.

It is probable that other new types of *S. suis* will be found in the near future. It is important to continue work with the *S. suis* typing system, as incomplete epidemiologic studies could impair further understanding of the pathogenesis and control of the infection.

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LITERATURE CITED

1. **Arends, J. P., and H. C. Zanen.** 1988. Meningitis caused by *Streptococcus suis* in humans. Rev. Infect. Dis. **10**:131–137.
2. **Azuma, R., F. Mara, Y. Oonuma, and C. Sugimoto.** 1983. *Streptococcus* R (*Streptococcus suis* type II) infection in pigs in Japan. Natl. Inst. Anim. Health Q. **23**:117–126.
3. **Boetner, A. G., M. Ginder, and V. Bille-Hansen.** 1987. *Streptococcus suis* infections in Danish pigs and experimental infection with *Streptococcus suis* serotype 7. Acta Pathol. Microbiol. Immunol. Scand. Sect. B **95**:233–239.
4. **Breton, J., W. R. Mitchell, and S. Rosendal.** 1986. *Streptococcus suis* in slaughter pigs and abattoir workers. Can. J. Vet. Res. **50**:338–341.
5. **Castryck, F., J. Hommez, L. Devriese, and P. Cassimon.** 1987. *Streptococcus suis* infections in pigs. Vlaams Diergeneeskd. Tijdschr. **56**:231–237.
6. **Chau, P. Y., C. Y. Huang, and R. Kay.** 1983. *Streptococcus suis* meningitis: an important underdiagnosed disease in Hong Kong. Med. J. Aust. **1**:414–417.
7. **de Moor, C. E.** 1963. Septicaemic infections in pigs caused by haemolytic streptococci of new Lancefield groups designated R, S and T. Antonie van Leeuwenhoek J. Microbiol. Serol. **29**: 272–280.
8. **Elliott, S. D.** 1966. Streptococcal infections in young pigs. I. An immunochemical study of the causative agent (PM *Streptococcus*). J. Hyg. **64**:205–212.
9. **Elliott, S. D., and J. Y. Tai.** 1978. The type-specific polysaccharides of *Streptococcus suis*. J. Exp. Med. **148**:1699–1704.
10. **Erickson, E. D., A. R. Doster, and T. S. Pokorny.** 1984. Isolation of *Streptococcus suis* from swine in Nebraska. J. Am. Vet. Med. Assoc. **185**:666–668.
11. **Finch, R. G., and I. Phillips.** 1977. Serological grouping of streptococci by a slide coagglutination method. J. Clin. Pathol. **39**:168–170.
12. **Giunta, S., G. Sampaoli, L. Galeazzi, L. Bilei, R. Rinaldi, and G. Groppa.** 1983. Inhibition of nonspecific streptococcal coagglutination reactions. J. Clin. Microbiol. **17**:192–194.
13. **Hommez, L., L. A. Devriese, J. Henrichsen, and F. Castryck.** 1986. Identification and characterization of *Streptococcus suis*. Vet. Microbiol. **11**:349–355.
14. **Hommez, L., J. Wullepit, P. Cassimon, F. Castryck, F. Ceyssens, and L. A. Devriese.** 1988. *Streptococcus suis* and other streptococcal species as a cause of extramammary infection in ruminants. Vet. Rec. **123**:626–627.
15. **Jacques, M., B. Foiry, R. Higgins, and K. R. Mittal.** 1988. Electron microscopic examination of capsular material from various serotypes of *Actinobacillus pleuropneumoniae*. J. Bacteriol. **170**:3314–3318.
16. **Kilpper-Balz, R., and K. H. Schleifer.** 1987. *Streptococcus suis* sp. nov., nom. rev. Int. J. Syst. Bacteriol. **37**:160–162.
17. **Kurl, D. N., S. Haataja, and J. Finne.** 1989. Hemagglutination activities of group B, C, D, and G streptococci: demonstration of novel sugar-specific cell-binding activities in *Streptococcus suis*. Infect. Immun. **57**:384–389.
18. **Lamont, M. H., P. E. Edwards, and R. S. Windsor.** 1980. Streptococcal meningitis in pigs: results of a five-year survey. Vet. Rec. **107**:467–469.
19. **LeMenec, M., M. Kobisch, and P. Sanders.** 1985. Infection porcine à *Streptococcus suis* dans le département des Côtes-du-Nord. Méthodes d'identification et répartition des différents sérotypes recontrés. Recl. Méd. Vét. Éc. Alfort **161**:353–359.
20. **Lund, E., and J. Henrichsen.** 1978. Laboratory diagnosis, serology and epidemiology of *Streptococcus pneumoniae*, p. 241–262. *In* T. Bergan and J. R. Norris (ed.), Methods in microbiology, vol. 12. Academic Press, Inc., New York.
21. **Mittal, K. R., R. Higgins, and S. Larivière.** 1983. Identification and serotyping of *Haemophilus pleuropneumoniae* by coagglutination test. J. Clin. Microbiol. **18**:1351–1354.
22. **Perch, B., E. Kjems, P. Slot, and B. Pedersen.** 1981. Biochemical and serological properties of R, S and RS streptococci. Acta Pathol. Microbiol. Scand. Sect. B **89**:167–171.
23. **Perch, B., K. B. Pedersen, and J. Henrichsen.** 1983. Serology of capsulated streptococci pathogenic for pigs: six new serotypes of *Streptococcus suis*. J. Clin. Microbiol. **17**:993–996.
24. **Rosendal, S., J. Breton, J. Henrichsen, L. Hilt, and W. R. Mitchell.** 1986. Isolation of *Streptococcus suis* using a selective medium. Can. J. Vet. Res. **50**:537–539.
25. **Sanford, E. S.** 1987. Gross and histopathological findings in unusual lesions caused by *Streptococcus suis* in pigs. I. Cardiac lesions. Can. J. Vet. Res. **51**:481–485.
26. **Sihvonen, L., D. N. Kurl, and J. Henrichsen.** 1988. *Streptococcus suis* isolated from pigs in Finland. Acta Vet. Scand. **29**:9–13.
27. **Sørensen, U. B. S., J. Blom, A. Birch-Andersen, and J. Henrichsen.** 1988. Ultrastructural localization of capsules, cell wall polysaccharide, cell wall proteins, and F antigen in pneumococci. Infect. Immun. **56**:1890–1896.
28. **Touil, F., R. Higgins, and M. Nadeau.** 1988. Isolation of *Streptococcus suis* from diseased pigs in Canada. Vet. Microbiol. **17**:171–177.
29. **Windsor, R. S., and S. D. Elliott.** 1975. Streptococcal infection in young pigs. IV. An outbreak of streptococcal meningitis in weaned pigs. J. Hyg. **75**:69–78.
30. **Zanen, H. C., and H. W. B. Engel.** 1975. Porcine streptococci causing meningitis and septicemia in man. Lancet **i**:1286–1288.