Serology of Capsulated Streptococci Pathogenic for Pigs: Six New Serotypes of *Streptococcus suis*

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A total of 138 capsulated strains of streptococci were isolated from pigs with various infections, notably septicemia and bronchopneumonia. Biochemically all strains were similar, and 31 strains were serologically identical to the previously described groups R, S, and RS streptococci, provisionally designated *Streptococcus suis* types 1, 2, and 1/2 by Elliot. The remaining 107 strains could be divided into six new capsular serotypes of *S. suis*.

From cases of bacteremia and meningitis in young pigs, Elliott (1) and Windsor and Elliott (14) isolated streptococci that apparently belonged to the same taxa as the group R and S streptococci of de Moor (9). They designated their strains *Streptococcus suis* type 1 and type 2 because they could demonstrate the presence of a common cell wall antigen and either of two different capsular polysaccharide-type antigens in these strains. The cell wall antigen, furthermore, was shown to be a lipid-bound teichoic acid closely related to the streptococcal group D antigen (1, 2).

So-called nongroupable streptococci, morphologically and biochemically similar to *S. suis*, have repeatedly been isolated from pigs with various kinds of infections, including septicemia, meningitis, arthritis, and bronchopneumonia (3, 5, 6, 9, 10).

The hemolysis of these nongroupable streptococci isolated from pigs varies with the kind of erythrocytes used. For most strains, de Moor (9) found a narrow partial β -hemolysis slowly becoming complete on horse blood agar, but some strains were nonhemolytic and on sheep blood agar all strains were either nonhemolytic or α -hemolytic. Also using sheep blood agar, Koehne et al. (6) found their strains to be α -hemolytic. Variable hemolysis was reported by Engel et al. (3) and Jones (5) to occur on ox and horse blood agar, respectively; Windsor and Elliott (14) reported hemolysis to be complete in 18 h on horse blood agar but incomplete on sheep blood agar.

We here describe the results of a study of the serology of 138 capsulated porcine strains biochemically behaving like *S. suis*. The strains represent nine different serotypes, six of which have not been described before.

MATERIALS AND METHODS

Bacterial strains were isolated from deceased pigs received during a period of approximately 1.5 years (from late 1980 to early 1982) for examination at The State Veterinary Serum Laboratory in Copenhagen, Denmark. Liver, spleen, and jejunum were routinely cultured, and additional cultures were performed from organs exhibiting signs of infection. The primary isolation was made on meat infusion peptone agar with 5% citrate-stabilized calf blood; the plates were incubated aerobically at 37°C for 48 h. All strains exhibited α -hemolysis on this calf blood agar medium. Most strains grew as pure cultures on primary isolation (Table 1). Table 1 also lists all the strains studied as well as their origin.

Strains were chosen for inclusion in this study when, besides being judged to be pathogenic for pigs and α -hemolytic on calf blood agar, they were found to be morphologically and biochemically similar to *S. suis* (12).

Of the four type 6 strains, one (strain A227) was received from S. D. Elliott, Cambridge, England, and two (strains V-386 and V-441) were received from W. Sonnbend, St. Gallen, Switzerland.

For the production of antisera against types 1 and 2, the group S and group R reference strains, NCTC 10237 and NCTC 10234, from the National Collection of Type Cultures, Central Public Health Laboratory, London, England, were used.

Antisera against capsulated strains were prepared as described elsewhere (11). In the beginning, we had antisera against types 1 and 2. Thereafter, antisera against the other types, types 3 through 8, were prepared successively by immunizing rabbits with strains that did not give a capsular reaction with any of the antisera available at the time. In this way, we prepared antisera against two type 3 strains (and we received one from S. D. Elliott), six type 4 strains, two type 5 strains, one type 6 strain, two type 7 strains, and one type 8 strain.

In addition, we used type-specific diagnostic pneumococcal antisera prepared and routinely in use at

TABLE 1. Strains of *S. suis* isolated from pigs in Denmark, 1980 to 1982

Designation of representative strain	Sero-type	No. of strains	Isolates from cases of:							Strains in primary pure culture
			Septi-cemia	Menin-gitis	Arthri-tis	Broncho-pneumonia	Fibrinous polysero-sitis	Endocar-ditis	No in-formation	
428	1	17	13	2	2					17
735	2	9	1		1	6		1		6
2651	1/2	5				4			1	4
4961	3	17	1			16				11
6407	4	12	1			10			1	10
11538	5	5	4			1				5
2524	6	4	3						1	3
8074	7	49	20 ^a	1	3	21	2		2	41
14636	8	20	1			18			1	12

^a Three of the animals also had polyarthritis, and two had bronchopneumonia.

Statens Seruminstitut, Copenhagen, Denmark (8), and an anti-group D streptococcus antiserum for coagglutination (Phadebact; Pharmacia Diagnostics, Uppsala, Sweden).

The capsular reaction test was carried out as described before (8). The presence or absence of capsules was checked by microscopy of wet mounts with India ink added.

The capillary precipitation test was performed with antigens extracted with hydrochloric acid (0.2 N and 0.066 N), with formamide (4) and with saline (1).

The coagglutination test with anti-group D antiserum from Pharmacia Diagnostics was carried out according to instructions given by the manufacturer with enzyme-treated cultures or (in case of a negative reaction) heavy cell suspensions exposed to a pressure of 12,000 lb/in² in a French pressure cell (American Instrument Co., Silver Spring, Md.) with a piston diameter of 1 in. (2.5 cm). The latter procedure was also applied, for control purposes, to one strain of each of the following bacteria from the collection of The Streptococcus Department, Statens Seruminstitut: Groups A, B, C, and G streptococcus, *S. bovis*, *S. mitior*, and *S. pneumoniae* type 19F.

Biochemical reactions and bile solubility were tested as described previously (7, 11).

RESULTS

A total of 138 strains of *S. suis* were isolated from pigs. The pigs with septicemia, meningitis, arthritis, and fibrinous polyserositis were 1 to 5 weeks old; those with bronchopneumonia were 6 weeks old on the average (1 to 20 weeks); the pig with endocarditis was 12 weeks old. All strains exhibited α -hemolysis on calf blood agar; on 5% horse blood agar, their hemolysis varied from α -hemolysis through uncharacteristic and ill-defined β -hemolysis to classical but narrow β -hemolysis.

The colony morphology of the different types was much alike; type 7, however, grew with larger and more watery colonies than the other types.

With a few exceptions, the biochemical reactions were not related to particular serotypes. However, all but 1 of the 69 strains of types 7

and 8 produced hyaluronidase. Some 6 out of 17 strains of type 3 and 1 out of 12 strains of type 4 hydrolyzed sodium hippurate, which none of the other strains did (Table 2). The strains were not soluble in bile.

By means of eight different antisera, six of which were produced successively by immunizing rabbits with capsulated strains that did not react with any of the typing antisera at our disposal at the time, we succeeded in typing all strains isolated by means of both the capsular reaction test and the capillary precipitation test with saline extracts.

The type antigen of types 7 and 8, in contrast to that of types 1, 3, 4, 5, and 6, was destroyed by both 0.2 N HCl extraction and the formamide extraction procedure of Fuller (4) but not by 0.066 N HCl extraction. The type 2 antigen could be extracted both with formamide (4) and with 0.066 N HCl but was destroyed by heating

TABLE 2. Variable biochemical reactions of *S. suis* and resistance of type antigens to two common extraction procedures

Sero-type ^a	Pro-duction of hy-alu-roni-dase ^b	Hy-drol-ysis of hip-pu-rate ^b	Resistance to extraction			
			HCl ^c		Formamide	
			0.2 N	0.066 N	Etha-nol	Ace-tone
1	0/17	0/17	+	+	+	-
2	1/9	0/9	-	+	+	-
1/2	2/5	0/5				
3	9/17	6/17	+	ND ^d	+	+
4	5/12	1/12	+	ND	+	+
5	0/5	0/5	+	ND	+	+
6	0/4	0/4	+	ND	+	-
7	48/49	0/49	-	+	-	-
8	20/20	0/20	-	+	-	-

^a Determined by the capsular reaction test.

^b Number of positive strains/total number of strains.

^c Reactions in the capillary precipitation test.

^d ND, Not done.

with 0.2 N HCl. With the Fuller extraction procedure, the type antigen of types 1, 2, and 6 was only precipitated by ethanol; that of types 3, 4, and 5 was precipitated by both ethanol and acetone (Table 2).

There were no cross-reactions between types, but there were cross-reactions among the five strains that possess both type 1 and type 2 antigenic determinants. Cross-absorptions did not reduce homologous titers. However, capsular reaction tests with pneumococcal antisera disclosed pronounced cross-reactions between types 6, 7, and 8, on the one hand and the antisera of pneumococcal type 2 and of group 6 and 19, respectively on the other. (In a separate study we have been able to demonstrate that the capsular polysaccharides of *S. suis* type 8 and *S. pneumoniae* type 19F, in actual fact, are identical [Lee et al., manuscript in preparation]). None of the strains studied reacted with an antiserum raised against the C-carbohydrate antigen common to all pneumococci (Henrichsen and Sørensen, unpublished observation).

Using the anti-group D streptococcus coagglutination reagent, we were able to demonstrate the presence of an antigen closely similar to the group D antigen in strains of all eight types. In strains of types 7 and 8, however, this was only possible by using bacterial suspensions treated in the French pressure cell with the *S. bovis* strain as a positive control and the strains of groups A, B, C, and G streptococci, *S. mitior*, and *S. pneumoniae* type 19F as negative controls.

Likewise, we prepared an antiserum against a type 8 strain that had lost its capsule after 19 passages in broth containing anti-type 8 antiserum. The antiserum thus produced reacted with saline extracts of all the other types of *S. suis* as well as with extracts of *S. faecalis*.

DISCUSSION

In his original description of streptococci of groups R and S, de Moor (9) conceived the (capsular) polysaccharide antigens as new (cell wall) group antigens, most likely because he did not realize that the bacteria were capsulated, but probably also because he construed the chemical analysis of the antigens carried out by Roberts (13) as an indication of a structural similarity to other streptococcal group antigens. The saccharide moieties found, however, are also common elements of capsular polysaccharides. de Moor's reactions of extracts of group D streptococci with his hyperimmune R and S antisera were considered to be merely nonspecific cross-reactions. Elliott in 1966 (1) described the isolation from young pigs of capsulated streptococci, identical with de Moor's group S strains, with an intracellular group D teichoic acid. Accordingly,

he regarded his isolates as a new species within group D, and he suggested the designation *S. suis*.

We typed our strains by means of the capsular reaction test. Because hydrochloric acid and formamide extracts are commonly used for typing in the capillary precipitation test, we examined the resistance of the type antigens to these extraction procedures and found that some of the antigens were actually destroyed (Table 2).

Elliott et al. suggested that for the group identification of these streptococci, physical disruption of the cells might be an idea (2). Our findings with types 7 and 8 confirm this.

Because all eight capsular serotypes of the porcine streptococci dealt with in the present study possess a common antigen that reacts with anti-group D streptococcus serum, we find it logical, and in accordance with taxonomic principles, to regard these biochemically very similar streptococci as one species belonging to the group D streptococci. Similarly, as *S. bovis* has been considered a group D streptococcal species even though it possesses a teichoic acid antigen slightly different form that of *S. faecalis* (2). The name *S. suis* has never formally been proposed, but we intend to do so, in a separate publication, and to deposit a type strain.

Apparently *S. suis* may be isolated quite commonly from infectious processes in pigs, especially if strains with α -hemolysis and uncharacteristic β -hemolysis are not disregarded.

We believe that future work will result in the isolation, characterization, and description of further serotypes. For instance, we are presently examining a strain that cross-reacts with, but is different from, type 6 as well as 12 so far nontypable strains.

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