JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 1993, p. 2192–2194
0095-1137/93/082192-03$02.00/0

Vol. 31, No. 8

NOTES

Use of Polyvalent Coagglutination Reagents for Serotyping of *Streptococcus suis*

M. GOTTSCHALK, R. HIGGINS,* AND M. BOUDREAU

Groupe de Recherche sur les Maladies Infectieuses du Porc, Faculté de Médecine Vétérinaire, Université de Montréal, C.P. 5000, St.-Hyacinthe, Québec, Canada J2S 7C6

Received 19 March 1993/Accepted 29 April 1993

Polyvalent coagglutination reagents (PRs) have been evaluated for the serotyping of *Streptococcus suis*. Monovalent antisera produced against 28 *S. suis* reference strains have been grouped to obtain five different pools. A total of 249 field isolates previously identified and belonging to different serotypes were tested with PRs prepared by two different procedures: (i) monovalent coagglutination reagents were individually prepared and mixed in equal proportions, and (ii) antisera were mixed in equal proportions before the addition of the *Staphylococcus aureus* suspension. Only antisera tested by a tube agglutination test with 2-mercaptoethanol and presenting titers of 1:32 or higher were used. Results obtained with PRs prepared by both procedures were similar, and there was a very good correlation between the capsular type of the isolate and the reaction obtained with PRs. Thus, from a practical viewpoint, it is suggested that PRs be prepared by the first procedure. To validate this methodology, a total of 577 *S. suis* field isolates, consisting of 486 typeable and 91 untypeable isolates, were tested in parallel with both the PRs and the monovalent coagglutination reagents over a 1-year period. Ninety-nine percent of the typeable and all of the untypeable isolates were correctly identified. Serotyping with PRs is suggested to be a very useful and reliable screening procedure, particularly when a large number of *S. suis* isolates have to be serotyped. In addition, the choice of antisera to be included in a given pool is facultative and should be oriented to the needs of a region or a country.

Streptococcus suis is a worldwide cause of a variety of porcine infections (8, 9). To date, 29 capsular types of *S. suis* have been described (5, 6, 14). *S. suis* has also been described as a pathogen for humans (17). All capsular types have been isolated in Canada, where capsular types 2, 1/2, 3, 4, and 8 are the most prevalent in diseased pigs. Capsular types 17, 18, 19, and 21 are frequently recovered from the nasal cavities of clinically healthy pigs (4, 8, 9, 12).

The serotyping system for *S. suis* is based on antigenic differences in the capsular material, which is mainly composed of carbohydrates. To identify the serotype-specific antigen of *S. suis*, different techniques have been described. The classical Lancefield method was the first used (2, 14). However, it is a laborious and time-consuming method, and, in addition, different concentrations of hydrochloric acid are required depending on the capsular type (6, 14). Other serotyping methods have been used for *S. suis*, such as, for example, the slide agglutination and immunodiffusion tests, but they did not appear to be very specific (10, 15). In recent years, the coagglutination and capsular reaction tests have been reported as the most reliable screening and confirmatory tests, respectively, for the serotyping of *S. suis* isolates (7). Recently, a sandwich enzyme-linked immunosorbent assay for the detection of some capsular types of *S. suis* has been developed (16).

Coagglutination is a very suitable test, and it is accessible at any diagnostic laboratory. Nonetheless, since the number of capsular types is presently 29, serotyping becomes more difficult and is actually limited to reference laboratories. In addition, biochemical identification alone can be misleading. Thus, veterinary diagnostic laboratories face a difficult situation. Each *S. suis* isolate must be tested with 28 different monovalent coagglutination reagents. It becomes an expensive and time-consuming methodology. The aim of this study was to evaluate the use of polyvalent coagglutination reagents (PRs) as a screening procedure for serotyping *S. suis* isolates.

S. suis reference strains representing capsular types 1 to 28 were used for the production of antisera. Antigens for immunization from all capsular types, with the exception of capsular type 1/2, were prepared as previously described (3), with some modifications. Briefly, after the antigens were cultured for 18 h on blood agar plates at 37°C in an aerobic atmosphere as a purity control, a first 18-h subculture in Todd-Hewitt broth (Difco Laboratories, Detroit, Mich.) was performed. Then, three passages, each consisting of 6 h of incubation in 10 ml of broth, were done; after each one of the first two 6-h passages, cultures were kept at 4°C overnight. Finally, 5 ml of the last 6-h subculture was inoculated into 50 ml of a prewarmed broth, which was incubated for only 2 h. Formaldehyde was added to a final concentration of 0.3%, and the culture was allowed to stand overnight at room temperature. After being washed, cells were resuspended in phosphate-buffered saline (PBS)-formaldehyde (0.3%). For the production of antisera, rabbits weighing 3 kg were given three injections per week of increasing concentrations of bacteria for 4 weeks as follows: Week 1, 2×10^9 bacteria; weeks 2 to 4, 8×10^9 bacteria. Ten days after the last injection, blood samples were taken, and the serological response was evaluated with the capsular reaction test (7). If

* Corresponding author.

necessary, inoculations were continued for 1 or 2 weeks until a clear and strong reaction was obtained.

Antisera were titrated by using the tube agglutination test with 2-mercaptoethanol (11), and the antigens were the same as those used for immunization of rabbits; this test was preferred to the simple agglutination test since it detects mostly immunoglobulin G fractions, which react with the protein A of *S. aureus* in the coagglutination test. After heat inactivation at 56°C for 30 min, serial dilutions of sera from 1:2 to 1:128 were prepared in saline solution with 2-mercaptoethanol. Equal volumes of antigen and serum were used in each tube. Tubes were incubated at 37°C for 24 h. Most of the sera presented titers between 1/32 and 1/64. Only sera raised against capsular types 1, 7, 12, and 19 had lower titers, and they were concentrated with a SpeedVac concentrator (SVS 200H, Savant). Only sera with titers of 1:32 or higher were used in this study.

Monovalent coagglutination reagents were prepared by the procedure described by Christensen et al. (1), with some modifications. Briefly, *S. aureus* Cowan I was cultured onto tryptic soy agar (Difco) for 18 h at 37°C, and cells were collected in PBS (pH 7.4) containing 0.15 M NaCl, 0.01 M Na_2HPO_4, and 0.01 M KH_2PO_4 and then washed twice. Bacteria were suspended in 0.5% PBS-formaldehyde, kept for 3 h at room temperature, washed once in PBS, and adjusted to a concentration of 10% (vol/vol). The suspension was further heated at 80°C for 5 min and immediately cooled with tap water and ice. To 1 ml of this suspension, 50 μl of serum, with a titer of at least 1/32, was added. For monovalent coagglutination reagents, antisera presenting cross-reactions (serotypes 2 and 22, and serotypes 6 and 16) were absorbed as previously described (6). After being mixed, the suspension was allowed to stand at room temperature for 60 min, with gentle mixing each 15 min, and then was washed twice with PBS. Finally, the bacteria were resuspended to a concentration of 10% (vol/vol) in PBS containing 0.05% sodium azide and 0.1% bovine serum albumin (Sigma Chemical Co., St. Louis, Mo.).

Preparation of PRs was carried out by two different procedures: (i) monovalent coagglutination reagents were individually prepared and mixed in equal proportions; and (ii) antisera corresponding to each PR were mixed in equal proportions before the addition of *S. aureus* cells.

A total of five different PRs were produced: PR 1 contained the five capsular types more frequently found in Canada (1/2, 2, 3, 4, and 8) and capsular type 22, which cross-reacts with capsular type 2; PR 2 contained capsular types 17, 18, 19, and 21, which are frequently isolated from clinically healthy animals, along with capsular types 23 and 24; PR 3 contained two cross-reacting capsular types (types 6 and 16) as well as capsular types 5, 7, 9, and 20; PRs 4 and 5 were composed of capsular types rarely isolated in Canada, namely, types 10, 11, 12, 13, 14, and 15, and types 25, 26, 27, and 28, respectively.

Results of the coagglutination test obtained with PRs were recorded at 30 s and 1, 2, and 3 min. During the whole study, all tests were performed by the same person and interpreted in the same way. The capsular reaction test was used as the reference test (7).

To evaluate PRs, a total of 826 field isolates of *S. suis* were tested in two series. First, a total of 249 isolates, which had previously been identified by the usual procedure, were tested with both preparations of reagents. Results obtained after readings at 30 and 60 s are shown in Table 1. There was a very good correlation between the capsular type of the isolate and the result obtained with the PR. There was no difference in sensitivity between the reaction recorded at 30 s and that recorded at 60 s; however, the specificity was lower at 60 s. After 60 s, the number of nonspecific reactions increased dramatically. For example, after 2 min, the heterologous reaction varied from 25 to 64% for the different PRs, and at 3 min, most of the isolates agglutinated with more than one PR. A similar phenomenon is observed when monovalent reagents are used; in this case, reactions recorded at 60 s are generally specific, whereas nonspecific reactions are observed after 60 s. Although PRs 4 and 5 presented a high number of nonspecific reactions at 60 s, these reactions are not more commonly observed with any of the monovalent reagents included in those PRs. As a conclusion of the first part of this study, the preparation of PRs from reagents routinely prepared as the monovalent coagglutination reagents appeared very suitable; they are easier to prepare and the capsular types detected with each PR can be modified depending on the most prevalent capsular types detected in different geographic regions. Finally, it was shown that most of specific reactions were seen within 30 s.

TABLE 1. Reactions obtained with the PRs by using 249 previously serotyped field isolates of *S. suis*

PR	No. of homologous isolates tested[a]	No. of heterologous isolates tested[b]	Homologous reaction (%)[c]		Heterologous reaction (%)[d]	
			30 s	60 s	30 s	60 s
1	109	140	94	95	1	3
2	61	188	92	92	0	2
3	41	208	100	100	1	4
4	21	228	81	81	8	29
5	17	232	94	94	5	30

[a] Isolates whose capsular types corresponded to those included in each PR.
[b] Isolates whose capsular types were different than those included in each PR.
[c] Percent with homologous reaction = (number of positive isolates × 100)/number of homologous isolates tested.
[d] Percent with heterologous reaction = (number of positive isolates × 100)/number of heterologous isolates tested.

To validate the previous work, all *S. suis* isolates recovered at the clinical bacteriology laboratory of the Faculty of Veterinary Medicine of the University of Montreal along with isolates received from other Canadian laboratories were serotyped by using in parallel both monovalent reagents and PRs. From February 1992 to December 1992, 577 *S. suis* isolates were studied. Readings were carried out only at 30 and 60 s. In this second part of the study, 99% of the 486 *S. suis* typable isolates were correctly identified. The five isolates not recognized by any of the PRs belonged to capsular types 21 (two isolates), 11, 22, and 24. These isolates were weakly positive with the monovalent reagents but negative with the capsular reaction test. It has previously been reported that some isolates could present a certain degree of capsular degradation, which impedes correct serological identification (10, 13). All 91 untypeable isolates were negative with the PRs at a 30-s reading. At 60 s, two untypeable isolates reacted with PR 3, one isolate reacted with PR 4, and one isolate reacted with PR 5; all four isolates were negative when tested with their respective monovalent reagents.

The numerical distribution of capsular types of the 486 typeable isolates of *S. suis* tested in this 1-year period is shown in Table 2. Capsular type 2 was the most frequently found (24%), followed by capsular types 3, 1/2, and 8 (14, 12, and 8%, respectively). Only capsular types 14 and 26 were not represented by any isolate.

TABLE 2. Numerical distribution of capsular types of 486 typeable isolates of *S. suis* tested in parallel with the monovalent reagents and the PRs

Capsular type	No. of isolates (%)
1	2 (<1)
1/2	58 (12)
2	118 (24)
3	69 (14)
4	24 (5)
5	15 (3)
6	2 (<1)
7	32 (7)
8	37 (8)
9	18 (4)
10	5 (1)
11	5 (1)
12	2 (<1)
13	1 (<1)
14	0 (0)
15	7 (1)
16	6 (1)
17	4 (<1)
18	14 (3)
19	5 (1)
20	1 (<1)
21	21 (4)
22	14 (3)
23	13 (3)
24	2 (<1)
25	6 (1)
26	0 (0)
27	1 (<1)
28	4 (<1)

As a conclusion, PRs, once standardized, appear to be a very useful and reliable screening method for the serotyping of *S. suis*, especially when a large number of isolates have to be tested; these reagents can be prepared directly by mixing the standardized monovalent reagents. The reaction observed with the coagglutination test should be recorded within 30 s.

We acknowledge the invaluable technical assistance of Nicole Morier.

This work was supported by a grant from Fonds pour la Formation de Chercheurs et l'Aide à la Recherche (89-EQ-4101), Langford Inc. (Guelph, Ontario, Canada), and the Ministère de l'Enseignement Supérieur et de la Science.

REFERENCES

1. **Christensen, P., G. Kahlmeter, S. Jonsson, and G. Kronvall.** 1973. New method for the serological grouping of streptococci with specific antibodies adsorbed to protein A-containing staphylococci. Infect. Immun. **7**:881–885.
2. **de Moor, C. E.** 1963. Septicaemic infections in pigs caused by haemolytic streptococci of new Lancefield groups designed R, S and T. Antonie van Leeuwenhoek J. Microbiol. Serol. **29**:272–280.
3. **Gottschalk, M., R. Higgins, and M. Jacques.** 1993. Production of capsular material by *Streptococcus suis* serotype 2 under different growth conditions. Can. J. Vet. Res. **57**:49–52.
4. **Gottschalk, M., R. Higgins, M. Jacques, M. Beaudoin, and J. Henrichsen.** 1991. Isolation and characterization of *Streptococcus suis* capsular types 9-22. J. Vet. Diagn. Invest. **3**:60–65.
5. **Gottschalk, M., R. Higgins, M. Jacques, M. Beaudoin, and J. Henrichsen.** 1991. Characterization of six new capsular types (23 through 28) of *Streptococcus suis*. J. Clin. Microbiol. **29**:2590–2594.
6. **Gottschalk, M., R. Higgins, M. Jacques, K. Mittal, and J. Henrichsen.** 1989. Description of 14 new capsular types of *Streptococcus suis*. J. Clin. Microbiol. **27**:2633–2636.
7. **Higgins, R., and M. Gottschalk.** 1990. An update on *Streptococcus suis* identification. J. Vet. Diagn. Invest. **2**:249–252.
8. **Higgins, R., M. Gottschalk, M. Beaudoin, and S. Rawluk.** 1992. Distribution of *Streptococcus suis* capsular types in Quebec and western Canada. Can. Vet. J. **33**:27–30.
9. **Higgins, R., M. Gottschalk, K. R. Mittal, and M. Beaudoin.** 1990. *Streptococcus suis* infections in swine. A 16-month study. Can. J. Vet. Res. **54**:170–173.
10. **Hommez, J., L. A. Devriese, J. Henrichsen, and F. Castrick.** 1986. Identification and characterization of *Streptococcus suis*. Vet. Microbiol. **16**:349–355.
11. **Mittal, K., R. Higgins, S. Larivière, and D. Leblanc.** 1984. A 2-mercaptoethanol tube agglutination test for diagnosis of *Haemophilus pleuropneumoniae* infection in pigs. Am. J. Vet. Res. **45**:715–719.
12. **Monter Flores, J. L., R. Higgins, S. D'Allaire, R. Charette, M. Boudreau, and M. Gottschalk.** 1993. Distribution of the different capsular types of *Streptococcus suis* in 19 swine nurseries. Can. Vet. J. **34**:170–171.
13. **Perch, B., E. Kjems, P. Slot, and K. B. Pedersen.** 1981. Biochemical and serological properties of R, S and RS streptococci. Acta Pathol. Microbiol. Scand. Sect. B **89**:167–171.
14. **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.
15. **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.
16. **Serhir, B., R. Higgins, D. Dubreuil, M. Gottschalk, and R. Lallier.** 1993. A sandwich enzyme-linked immunosorbent assay for the detection of *Streptococcus suis*. Can. J. Vet. Res. **57**:19–24.
17. **Trottier, S., R. Higgins, G. Brochu, and M. Gottschalk.** 1991. A case of human endocarditis due to *Streptococcus suis* in humans. Rev. Infect. Dis. **13**:1251–1252.