Prevalence of *Streptococcus suis* in Four to Eight Week Old Clinically Healthy Piglets

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ABSTRACT

The study was undertaken to determine the prevalence, in the nasal cavities, of *Streptococcus suis* in four to eight week old clinically healthy piglets. Streptococci biochemically compatible with *S. suis* were isolated from 94% of piglets and 98% of farms. Of the 782 isolates submitted to serotyping, only 164 (21%) were included in the nine official serotypes. These 164 typable isolates originated from 121 (31%) of the 388 piglets and from 36 (73%) of the 49 farms included in the study. The most frequent serotypes found in piglets were, in decreasing order, 3, 4, 8 and 2. Serotype 1 was not detected in this survey. As many as 32% of piglets were found positive for two different serotypes and three different serotypes were found in 1%.

RÉSUMÉ

Cette étude avait pour but de déterminer la prévalence, au niveau nasal, de *Streptococcus suis* chez des porcelets cliniquement sains et âgés entre quatre et huit semaines. Des streptocoques biochimiquement compatibles avec *S. suis* ont été retrouvés chez 94% des porcelets et 98% des fermes. Des 782 isolats soumis à la sérotypie, seulement 164 (21%) ont pu être associés aux neuf sérotypes officiels de *S. suis*. Ces 164 isolats sérotypables provenaient de 121 (31%) des 388 porcelets et de 36 (73%) des 49 fermes incluses dans cette étude. Les sérotypes retrouvés le plus fréquemment chez les porcelets ont été, par ordre décroissant, le 3, le 4, le 8 et le 2. Le sérotype 1 n'a pas été isolé au cours de cette étude. Un total de 32% des porcelets se sont avérés porteurs de deux sérotypes différents et trois sérotypes différents ont été retrouvés chez 1% des sujets.

INTRODUCTION

Streptococcus suis is an important pathogen in swine production throughout the world (1-7). Up to the present, nine serotypes have been described (8-10), but the high number of untypable *S. suis* isolates suggests that the number of serotypes is much higher (11,12).

The prevalence of carriers of *S. suis* in the swine population appears to vary considerably. Moreover, data must be considered carefully since most of the studies were conducted to search for *S. suis* serotype 2 only. Indeed, a carrier rate of 0 to 80% was found in Great Britain in infected farms in which pigs had already manifested clinical signs of meningitis (13,14). The same group reported that 20 to 90% of pigs at slaughter were infected with serotype 2 of *S. suis*. These animals also originated from herds with clinical signs compatible with *S. suis* infection (15). More recently, a study conducted in Ontario demonstrated a carrier rate of 43.6% in pigs at slaughter; this included *S. suis* isolates identified biochemically and serotype 2 isolates confirmed with the slide agglutination test (16).

Tonsils have been considered the main carrier site of *S. suis* (17), but nasal cavities are also recognized as a good site for the recovery of this microorganism (18-20). Finally, a higher carrier rate of *S. suis* was found in piglets between four and ten weeks of age (14,18).

The purpose of this study was to determine the prevalence of the different serotypes of *S. suis* in four to eight week old clinically healthy piglets in one of the main agricultural regions in Quebec. Also, it was intended to assess the prevalence of untypable isolates of this bacterium.

MATERIALS AND METHODS

ANIMALS

Region 06, one of the twelve agricultural regions of Quebec, was chosen because it represented about 25% of the swine population of the Province. Out of 661 farrow and farrow-to-finish operations and of an estimated number of 130,000 four to eight week old piglets in this region, it was statistically determined that a minimum of 385 animals were required in this survey (21). A total of 388 piglets, chosen at random from 49 different farms were sampled.

SPECIMEN COLLECTION

For practical reasons, nasal swabbing was preferred to tonsillar

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swabbing. Bacteriological samples were taken from nasal cavities, according to a previously described technique (11), with some modifications. Piglets were firmly restrained in a seated position by an assistant during the sampling. One external nare was cleaned with a sterile gauze soaked in alcohol and samples were collected with a sterile swab (Transet 1, Spectrum Diagnostic Inc., Glenwood, Illinois) twisted at a depth of 8 to 10 cm. Swabs were kept refrigerated until laboratory procedures were undertaken the same day.

BACTERIOLOGICAL STUDIES

Culture of swabs was carried out on bovine blood agar plates containing *Streptococcus* selective supplement SR-74 (Oxoid, Basingstoke, England: nalidixic acid, neomycin and polymyxin B). After incubation at 37°C for 24 h two or three different morphological types of alpha-hemolytic colonies were picked up from each plate in order to have a better chance of detecting *S. suis* but also to establish if more than one serotype could be present in the same animal. Each colony was streaked onto a blood agar plate and incubated as previously mentioned.

All isolates seen as gram-positive cocci and catalase negative were submitted to eight biochemical tests. The tests were chosen on the basis of their low variability, on data by some of us (12) and of other authors (6,7,19,22). They were: 6.5% NaCl, arginine dihydrolase, glycerol, inulin, lactose, salicin, sorbitol and trehalose. The arginine dihydrolase test was carried out with the Møller decarboxylase medium (Difco, Detroit, Michigan). Carbohydrates were tested in Phenol Red broths (Difco). Readings were carried out after 48 h of incubation at 37°C in aerobic conditions. Because of possible variations in test reactions, an isolate biochemically compatible with *S. suis* was defined as follows: absence of growth in the presence of NaCl, arginine dihydrolase positive and four of the six carbohydrates with a reaction as expected (12). In the majority of the cases, biochemical data were not available before serotyping was undertaken. For this reason, 782 of the 820 isolates were submitted to serotyping.

TABLE I. Comparison of percentage positivity obtained with eight biochemical tests carried out on 820 isolates of alpha-hemolytic streptococci and that obtained only with the 164 typable isolates

Test	Percentage positivity: Alpha-hemolytic streptococci (820)	Percentage positivity: Typable isolates (164)
6.5% NaCl	1	2
Arginine dihydrolase	86	84
Glycerol	1	0
Inulin	73	82
Lactose	71	94
Salicin	74	96
Sorbitol	0.2	1
Trehalose	87	92

SEROTYPING OF *S. SUIS* ISOLATES

Serotyping was carried out at the same time as the biochemical testing with the use of the same agar plate as source of bacterial suspension or antigens. The isolates were tested by slide agglutination (23) with antisera prepared in rabbits against reference strains of serotypes 1 to 8, kindly supplied by Dr. Jorgen Henrichsen of the Statens Seruminstitut in Denmark. Auto-agglutinable isolates were submitted to an immunodiffusion test using a hypersaline medium described by Le Menec *et al* (19). Antigens were extracted according to a technique previously described (24). Finally, 45 of our isolates biochemically compatible with *S. suis* and untypable with antisera against official serotypes were sent to Dr. Henrichsen who confirmed that they were untypable *S. suis*.

RESULTS

A total of 827 isolates visualized as alpha-hemolytic streptococci were taken from the primary plating of nasal swabs from the 388 piglets. Out of these isolates, seven were eliminated with the Gram or the catalase test, or lost, and 820 were submitted to the eight biochemical tests. Of this number, 716 (87%) were considered biochemically compatible with *S. suis*.

Only 164 (21%) of the 782 isolates appeared to be typable with the eight antisera. These included two isolates (serotypes 4 and 6) detected by immunodiffusion among 14 auto-agglutinable isolates. Serotype 1 was positivity obtained with the 820 isolates submitted to biochemical testing compared to that of the 164 typable isolates. The distribution of isolates according to serotype is presented in Table II. Because serotyping results were often available before biochemical data, nine isolates which would have been rejected after the biochemical testing were included in the group of typable isolates. Five of them had at least three divergent tests among carbohydrates; three reacted with serotype 6 and two with serotype 8. The four other isolates were able to grow in the presence of 6.5% NaCl; one of them was associated with serotype 1/2, two with serotype 3 and one with serotype 4.

The 164 typable isolates originated from 121 (31%) of the 388 piglets included in the study. The most frequent serotypes found in piglets were, in decreasing order, 3, 4, 8 and 2. Positive animals were found in 36 (73%) of the 49 farms. Table III presents the distribution of piglets and farms according to serotyping results. However, taking into account typable and nontypable isolates of *S. suis* it was determined that 94% of piglets and 98% of farms were positive for the presence of this microorganism. It should be noted that 10% of piglets were found positive for two different serotypes and that three different serotypes were found in 1% of them.

DISCUSSION

Isolation of streptococci biochemically compatible with *S. suis* from 94% of piglets and 98% of farms is indicative of the high prevalence of this microorganism in clinically

TABLE II. Distribution of the 164 isolates of *Streptococcus suis* according to their serotype

Serotype	Number of isolates	%
1	0	0
1/2	9	5
2	15	9
3	44	27
4	41	25
5	3	2
6	4	3
7	5	3
8	43	26

TABLE III. Distribution of piglets and farms according to serotyping results

Serotype	No. of positive piglets (388)[a]	%	No. of positive farms (49)[b]	%
1	0	0	0	0
1/2	7	2	4	8
2	14	4	6	12
3	36	9	15	31
4	36	9	17	35
5	3	1	3	6
6	4	1	4	8
7	4	1	3	6
8	29	7	18	37

[a]Number of piglets sampled
[b]Number of farms visited

healthy piglets. These percentages could have been higher if we consider that among the isolates that did not correspond to our definition of an isolate biochemically compatible with *S. suis*, nine belonged to one of the nine official serotypes, and that others will eventually be grouped into new serotypes. Indeed, recent studies carried out on all the untypable isolates collected during this study show that more than 90% of them have been grouped into four new serotypes of *S. suis*, and work is in progress (M. Gottschalk, personal communication, 1989). In addition, they have all been found negative to the Voges-Proskauer test and this eliminated the possibility that arginine dihydrolase negative isolates could have been *Streptococcus bovis* strains (M. Gottschalk, personal communication, 1989; J. Henrichsen, personal communication, 1986).

Our data show that biochemical identification of *S. suis* can be misleading if the number of tests is low or their choice inaccurate. In this study, four typable isolates were found positive for the NaCl test. This finding has also been reported earlier (12), and this is only one example of the fact that many *S. suis* isolates can be missed by relying only on a limited number of tests. This is particularly true when isolates with an unusual biochemical pattern are isolated in large numbers or when they appear to be virulent. They should be submitted to serotyping.

Data obtained by others in live animals indicate a high prevalence of *S. suis* serotype 2 but contrary to our findings, animals originated from farms with a history of clinical signs of *S. suis* infection (13,14). This figure could have varied if we consider that the carrier state is variable and that a given animal, tested twice, could have been found positive upon a second testing (13).

It has been suggested that tonsils would have been a better site than nasal cavities for testing (13,14,25), but other authors obtained better results with nasal swabs (19). Previous work by some of us demonstrated that nasal and tonsillar samples respectively revealed 55.3% and 65% of all *S. suis* serotype 2 isolates from 103 carrier piglets, and that the microorganism was found in both sites in only 20.4% of these animals (26). This suggests that greater sensitivity is obtained by sampling both sites.

It is noteworthy that most of the prevalence studies on carrier rates of *S. suis* were concentrated on serotype 2 (14,17,23,25,27-29) and that considerable differences could have been noted if *S. suis per se* would have been detected in lieu of serotype 2 only. In the present study, serotype 2 of *S. suis* represented only 2% of all isolates whereas it represented 9% of the typable isolates, and this serotype was present in 4% of all the piglets. This is in agreement with another Canadian study in which this serotype was found in 7% of the pigs at slaughter (16), and also with our previous study in which 6% of 1716 piglets were positive for the presence of *S. suis* serotype 2 (26). This is also in agreement with a British study which demonstrated that in four farms considered free of infection and without any history of clinical signs, two were negative for *S. suis* serotype 2 and of the two others, one had a prevalance of 1.5% for this serotype and the other, 20% (15). It appears that in piglets or in clinically healthy animals, serotype 2 is less prevalent than serotypes 3, 4 and 8, and that in diseased animals, serotype 2 is by far the most prevalent serotype (12). None of the isolates in this study could be associated with serotype 1 of *S. suis*. It is possible that this particular serotype, which causes problems mostly in preweaned piglets, could have been absent or undetectable in the nasal cavities of the sampled animals. It is accepted that sows can carry this agent and transmit it to suckling piglets (30,31).

The presence of two different serotypes of *S. suis* in the same animal, already mentioned by others (32), has been confirmed, and in some instances we isolated three different serotypes. This emphasizes the fact that the detection of a given serotype of *S. suis* in a swine herd is a difficult task without the use of special techniques: immunoprecipitation (26,33) or immunofluorescence (34).

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REFERENCES

1. **KOEHNE G, MADDUX RL, CORNELL WD.** Lancefield group R streptococci associated with pneumonia in swine. Am J Vet Res 1979; 40: 1640-1641.
2. **CLIFTON-HADLEY FA, ALEXANDER TJL.** Studies of *Streptococcus suis* type 2 infection. Proc Int Pig Vet Soc 1981; 8:8-17.
3. **KOBISCH M, LAGADIC M, LE MENEC M, VASSAIRE J.** Les infections à *Streptococcus suis* type 2 (groupe R): connaissances actuelles. Bull Lab Vet 1982; 12: 5-9.
4. **SANFORD E, TILKER AME.** *Streptococcus suis* type II. Associated diseases in swine: observations of a one-year study. J Am Vet Med Assoc 1982; 181: 673-676.
5. **ST JOHN VS, WILCOCK B, KIERSTEAD M.** *Streptococcus suis* type 2 infection in swine in Ontario: A review of clinical and pathological presentations. Can Vet J 1982; 23: 95-97.

6. **HOFFMAN LJ, HENDERSON LM.** The signification of *Streptococcus suis* in swine disease: clinical pathological and bacteriological data from a two year study. Proc Assoc Vet Lab Diagnost 1985: 201-210.
7. **HOMMEZ J, CASTRYCK F, DEVRIESE LA, HENRICHSEN J.** Prevalence of *Streptococcus suis* and methods of identification. Proc Int Pig Vet Soc 1984: 143.
8. **ELLIOTT SD.** Streptococcal infection in young pigs. I. An immunological study of the causative agent (PM *Streptococcus*). J Hyg (Camb) 1966; 64: 205-212.
9. **WINDSOR RS, ELLIOTT SD.** Streptococcal infection in young pigs. IV. An outbreak of streptococcal meningitis in weaned pigs. J Hyg (Camb) 1975; 75: 69-78.
10. **PERCH B, PEDERSEN KB, HENRICHSEN J.** Serology of capsulated streptococci pathogenic for pigs: Six new serotypes of *Streptococcus suis*. J Clin Microbiol 1983; 17: 993-996.
11. **ROSENDAL S, BRETON J, HENRICHSEN J, HILT L, MITCHELL WR.** Isolation of *Streptococcus suis* using a selective medium. Can J Vet Res 1986; 50: 537-539.
12. **TOUIL F, HIGGINS R, NADEAU M.** Isolation of *Streptococcus suis* from diseased pigs in Canada. Vet Microbiol 1988; 17: 171-177.
13. **CLIFTON-HADLEY FA, ALEXANDER TJL.** Further studies of *Streptococcus suis* type 2 infection. Proc Int Pig Vet Soc 1983; 10: 14-19.
14. **CLIFTON-HADLEY FA, ALEXANDER TJL, UPTON I, DUFFUS WPH.** Further studies in the subclinical carries state of *Streptococcus suis* type 2 in pigs. Vet Rec 1984; 114: 513-518.
15. **CLIFTON-HADLEY FA, ALEXANDER TJL, ENRIGHT MR, GUISE J.** Monitoring herds for *Streptococcus suis* type 2 by sampling tonsils of slaughter pigs. Vet Rec 1984; 115: 562-564.
16. **BRETON J, MITCHELL WR, ROSENDAL S.** *Streptococcus suis* in slaughter pigs and abattoir workers. Can J Vet Res 1986; 50: 338-341.
17. **CLIFTON-HADLEY FA, ALEXANDER TJL.** The carrier site and carrier rate of *Streptococcus suis* type 2 in pigs. Vet Rec 1980; 107: 40-41.
18. **VAISSAIRE J, LE MENEC M, LAROCHE M.** Streptococcie à streptocoque R chez le porc. Bull Acad Vét Fr 1983; 56: 331-338.
19. **LE MENEC M, KOBISCH M, SANDERS P.** Infection porcine à *Streptococcus suis* dans le département des Côtes-du-Nord. Méthodes d'identification et répartition des différents sérotypes rencontrés. Rec Méd Vét 1985; 161: 353-359.
20. **MADEC F, TILLON JP, GUILMOTO H, LE MENEC M.** Streptococcie R et mortalités subites en porcherie d'engraissement: résults d'une enquête. PointVét 1985; 17: 433-441.
21. **DANIEL WW.** Biostatistics: A Foundation for Analysis in the Health Sciences. 3rd ed. Wiley, 1983: 146-148.
22. **PERCH B, KJEMS E, SLOT P, PEDERSEN KB.** Biochemical and serological properties of R, S and RS streptococci. Acta Pathol Microbiol Scand 1981; 89: 167-171.
23. **CLIFTON-HADLEY FA, ALEXANDER TJ, ENRIGHT MR.** Diagnosis of *Streptococcus suis* type 2 infection in pigs. Proc Int Pig Vet Soc 1986; 14: 27-34.
24. **FACKLAM RR, CAREY RB.** Streptococci and Aerococci. In: Manual of Clinical Microbiology. 4th ed. Washington, DC: American Society for Microbiology, 1985: 170.
25. **CLIFTON-HADLEY FA.** Studies of *Streptococcus suis* type 2 infection in pigs. Vet Res Commun 1984; 8: 217-227.
26. **MOREAU A, HIGGINS R, BIGRASPOULIN M, NADEAU M.** Rapid detection of *Streptococcus suis* serotype 2 in weaned piglets. Am J Vet Res 1989; 50: 1667-1671.
27. **VAN LEENGOED LAMG, VECHT U, VERHEYEN ERM.** *Streptococcus suis* type 2 infection in pigs in The Netherlands (part two). Vet Q 1987; 9: 111-117.
28. **ROBERTSON ID, BLACKMORE DK.** The detection of pigs carrying *Streptococcus suis* type 2. NZ Vet J 1987; 35: 1-4.
29. **CLIFTON-HADLEY FA, ALEXANDER TJL, ENRIGHT MR.** Diagnosis of *Streptococcus suis* type 2 infection in pigs. Proc Int Pig Vet Soc 1985; 14: 27-34.
30. **NIELSEN NC, CHRISTENSEN K, BILLE N, LARSEN JL.** Preweaning mortality in pigs. 1. Herd investigations. Nord Vet Med 1974; 26: 137-150.
31. **WINDSOR RS, ELLIOTT SD.** Streptococcal infection in young pigs. IV. An outbreak of streptococcal meningitis in weaned pigs. J Hyg (Camb) 1975; 75: 69-78.
32. **SIHVONEN L, KURL DN, SALMELA P.** Infection with *Streptococcus suis* serotypes 1 and 2 in the same diseased pig. Acta Vet Scand 1986; 27: 626-628.
33. **ARENDS JP, HARTWIG N, RUDOLPHY M, ZANEN HC.** Carrier rate of *Streptococcus suis* capsular type 2 in palatine tonsils of slaughtered pigs. J Clin Microbiol 1984; 20: 945-947.
34. **ROBERTSON ID.** An indirect fluorescent antibody technique for the detection of *Streptococcus suis* type 2. NZ J Med Lab Technol 1985; 39: 171-172.