

BRIEF COMMUNICATION

SEROTYPING AND EVALUATION OF THE VIRULENCE IN MICE OF *Streptococcus suis* STRAINS ISOLATED FROM DISEASED PIGS

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SUMMARY

A total of 110 strains of *Streptococcus suis*, isolated from diseased pigs in Brazil were serotyped and analyzed for virulence. Serotyping of the strains resulted in the following classification: 42 strains of serotype 2 (38.2%), 10 strains of serotype 14 (9.1%), seven strains of serotype 9 (6.4%), three strains each of serotype 7 and 11 (2.7%), two strains each of serotype 1 and 8 (1.8%) and one strain each of serotypes ½, 3, 5, 6 and 10 (0.9%). Cross reactions among serotypes 1, 14 and 7 were observed in 21 strains (19.1%). Only 41.9% of the strains were lethal for mice using the pathogenicity test.

KEYWORDS: *Streptococcus suis*; Diseased pigs; Serotyping; Virulence in mice.

Streptococcus suis is an important pathogen for pigs worldwide. This microorganism is associated with meningitis, arthritis, endocarditis, septicemia, pneumonia and sudden death in pigs during post-weaning and growing. *S. suis* is also associated with human infections, and is considered an occupational hazard of abattoir workers, meat workers and veterinarians¹⁰. Thirty-five serotypes of this microorganism have been described, with the serotype 2 being the most prevalent worldwide. In Canada the most prevalent serotypes are: 2, ½, 3, 4 and 8 (GOTTSCHALK *et al.*²). In Italy, the serotype 2 is most prevalent followed by the serotypes 1, 9, ½, 3, 7, 4 and 8 (SALA *et al.*⁸). In Holland and France the most common serotypes are 2 and 9 (JACOBS *et al.*³). In Brazil serotyping of *S. suis* was performed by MADUREIRA JÚNIOR & SONCINI⁵, SANTOS *et al.*⁹ and PAGNANI *et al.*⁶. Serotype 2 proved to be the most prevalent in all studies. However in all the mentioned studies, virulence of the serotypes was not evaluated.

The objective of the present work was to serotype and evaluate the virulence of serotypes of *S. suis* isolated from diseased pigs in Brazil.

One hundred and ten strains of *S. suis* isolated from the brain (70%), lungs (16.4%), cerebro-spinal fluid (7.4%), joints (2.7%), ascitis fluid (0.9%), heart (0.9%) and blood (1.8%) from diseased pigs in Brazil were available. The identification of *S. suis* was based on hemolysis on sheep blood agar, absence of growth in media containing NaCl and the conventional biochemical tests of catalase, amylase and acetoin. The strains were maintained in sheep blood agar at 4 °C in the laboratory

following their isolation, for a period of approximately three years, until processed for serotyping and virulence evaluation.

The antigens for serotyping were prepared using a slight modification of the technique described by GOTTSCHALK *et al.*². The strains of *S. suis* were inoculated onto sheep blood agar and incubated for 18 hours at 37 °C, with 5% CO₂. A subculture was made in tubes containing 5 mL of Todd Hewitt Broth (Difco Laboratories, Detroit, MI) and incubated for 12 hours under the same conditions. Two drops of (37%) formalin were then added, and the cultures incubated for two hours at 37 °C in aerobiosis with continuous stirring. The inactivated cultures were centrifuged at 2000 x g for 10 min at room temperature. The supernatant was discarded and the sediment resuspended in buffered saline containing 0.5% of formalin to a concentration of approximately 3.0 x 10⁹ cells/mL, standardized according to tube 10 of Mc Farland's Scale. The antigens were submitted to the coagglutination test, using a Kit purchased from Laboratory Biovet (Biovét, St Anthony, USA) according to the manufacturer's instructions. The strains that showed cross-reactions were subcultured again and the coagglutination test was repeated. A suspension of *Staphylococcus aureus* and normal rabbit serum were used as negative controls. The serotyped isolates of *S. suis* were submitted to a pathogenicity test using five mice per serotype by intraperitoneal inoculation of 0.2 mL of a standardized suspension of *S. suis*. The serotype was considered pathogenic when at least two mice in the group died, and not pathogenic when all the mice survived after a period of seven days.

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The serotype 2 was the most frequent (38.2%), followed by the serotypes 14 (9.1%), 9 (6.4%), 7 (2.7%), 11 (2.7%), 8 (1.8%), 1 (1.8%), 1/2 (0.9%), 3 (0.9%), 5 (0.9%), 6 (0.9%), and 10 (0.9%), respectively. The high frequency of the serotype 2 found in this work is in agreement with previous reports from Brazil and elsewhere^{5,6,8,9,12}.

In this work, 9.1% of the strains studied were identified as belonging to serotype 14. These results differ from a previous study (PAGNANI *et al.*⁶) in which a much lower prevalence (1.96%) of this serotype was found. One possible reason for this is the lower number of strains of *S. suis* studied by PAGNANI *et al.*⁶. The serotype 14 was not evaluated by MADUREIRA JÚNIOR & SONCINI⁵ or by SANTOS *et al.*⁹.

Cross reactions were observed among the serotypes 1, 14 and 7 in 21 (19.1%) of the strains. Cross reactions among the serotypes 1 and 7 are not common (GOTTSCHALK, M. Personal communication). However cross reactions are frequent among the serotypes 1 and 14, these cross-reacting strains being considered serotype 1/14 (DONE *et al.*¹).

In this study, 15 strains (13.7%) did not show reactions to the coagglutination test, probably due to the fact that only 16 serotypes out of 35 known *S. suis* serotypes were investigated. We can not rule out, however, loss of capsular antigens in our strains. This phenomenon has already been reported by STAATS *et al.*¹⁰.

No coagglutination was observed when the suspension of *Staphylococcus aureus* and normal rabbit sera were used as negative controls in all the tests performed.

Of the 110 strains isolated, 105 were tested because five were lost after primary isolation. Forty-four strains (41.9%) were pathogenic to mice. The strains belonging to serotype 2 and those that had cross reactions with the serotypes 1, 14 and 7, presented virulence, respectively, of 46.15% and 33.3%. Five (50%) out of ten strains belonging to serotype 14, were considered virulent to mice. Forty percent of the 15 strains not serotyped were pathogenic for mice.

Of the 18 serotype pathogenic strains, 17 (94.4%) were isolated from the brain, suggesting that strains isolated from this organ are more virulent than serotype 2 strains isolated from other organs. The high number of non pathogenic strains suggests that some might have lost virulence factors as a consequence of storage between 2 °C and 8 °C for a prolonged period between the isolation and the evaluation of the virulence. This has been reported before¹¹. Also, in this work, the evidence of virulence of *S. suis* isolated from organs other than the brain (i.e., serotype 5 from the lungs); shows that serotypes of *S. suis* isolated from other organs can also be potentially pathogenic.

Experimental models for evaluation of virulence of *S. suis* isolates have been developed. WILLIAMS *et al.*¹⁴ conducted several experiments to determine whether mice might provide a suitable model for studies on the pathogenesis of *S. suis* type 2 meningitis in pigs. Their work showed that mice were infected experimentally with isolates of *S. suis* and the organisms produced disease similar to pig diseases. Many different immunity studies have been done using mice as experimental models also. QUESSY *et al.*⁷ reported the protective effect against

heterologous challenge to mice with virulent strains of *S. suis* type 2. JACOBS *et al.*⁴ demonstrated that mice immunized with a vaccine containing purified hemolysin were completely protected against challenge with lethal *S. suis* type 2. The same results were obtained with pigs. Results of experimental infections in the natural host confirmed the usefulness of the mouse model in the study of *S. suis* capsular type 2 infection. However a study by VECHE *et al.*¹³ demonstrated that the resulting pathogenicity of *S. suis* type 2 for mice and pigs is incomparable and that when dealing with virulence, the different animal species should be specified.

RESUMO

Serotipagem e avaliação de virulência em camundongos, de cepas de *Streptococcus suis* isoladas de suínos doentes

Um total de 110 amostras de *Streptococcus suis* isoladas de suínos doentes, no Brasil foram sorotipificadas e analisadas para a virulência. Sorotipificação das amostras resultou na seguinte classificação: 42 amostras do sorotipo 2 (38,2%), 10 amostras do sorotipo 14 (9,1%), sete amostras do sorotipo 9 (6,4%), três amostras de cada sorotipo, 7 e 11 (2,7%), duas amostras de cada sorotipo, 1 e 8 (1,8%) e uma amostra de cada um dos sorotipos, 1/2, 3, 5, 6 e 10 (0,9%). Reações cruzadas entre os sorotipos 1, 14 e 7 foram observadas em 21 amostras (19,1%). Somente 41,9% das amostras foram patogênicas para camundongos.

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