MOLECULAR CHARACTERIZATION OF *STREPTOCOCCUS SUIS* STRAINS BY PCR-RIBOTYPING ASSOCIATED WITH RFLP ANALYSIS

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Introduction

Streptococcus suis is an important pathogen of swine, causing meningitis, arthritis, pericarditis, polyserositis, septicemia and sudden death of weaning piglets as well as growing pigs. In addition, *S. suis* is a zoonotic agent (1). To date, 35 serotypes have been described (1, 2, 1/2, 3 to 34), and among them, serotype 2 has always been considered as the most virulent and prevalent type isolated from diseased pigs (2, 3, 4).

In the present study, we report the development of a molecular typing method based on PCR amplification of a larger fragment of rRNA genes, including a part of 16S and 23S genes and the 16S-23S rDNA intergenic spacer region (PCR-Ribotyping), followed by RFLP analysis with *Rsa*I endonuclease. The genetic relationships between 138 *S. suis* strains belonging to various serotypes, isolated from swine or human cases, were also performed.

Material and Methods

One hundred and thirty eight strains of *S. suis*, epidemiologically unrelated, isolated from diseased pigs suffering from meningitis, septicemia, or arthritis (84 strains), from nasal cavities or tonsils of clinically healthy pigs (22 strains) and from human meningitis cases (29 strains) were studied. Among these strains, capsular typing using the coagglutination test has revealed serotype 1 (1 strain), serotype 2 (98 strains), serotype 1/2 (7 strains), serotype 3 (6 strains), serotype 4 (1 strain), serotype 5 (1 strain), serotype 7 (12 strains) and serotype 9 (10 strains). Two strains were autoagglutinable.

The template DNA of each strain was amplified with the forward primer 16S-489(f) complementary to the 3' end of the 16S rRNA gene and the reverse primer 23S-206(r) complementary to the 5' end of the 23S rRNA gene (5). The PCR-Ribotyping products were digested with *Rsa*I endonuclease (5).

The patterns were digitized and analyzed by using the Biogene package (confidence interval of 8%). The numerical index of discrimination was calculated. The relationships between patterns of strains isolated from pathological cases or from clinically healthy pigs, including serotype, and origin of the strains (pig or human) were analysed by using the Fisher exact test ($n \leq 5$) or the Chi-square test ($n > 5$). Differences were estimated significant when $P < 0.05$.

Results

In our conditions, the *in vitro* stability of patterns and the reproducibility of the method were 100 % because a similar pattern was shown for each strain after the two independent DNA extractions. The index of discrimination was superior to 0.95, the threshold value for interpreting typing results with confidence.

The patterns were composed of 6 to 21 fragments of 134 to 925 bp. Fourty two patterns were identified among the 138 *S. suis* strains, analyzed. These strains diverged by up to 33 % (67% homology). At a level of 72 % homology, five groups A to E were identified. At 75 % homology, the group A was divided in two subgroups a and b.

Among the 113 strains isolated from diseased pigs and humans, 38 patterns were identified. The majority of strains (78 of 113) were in group A. No significant association between the strain origins and the groups was observed ($P > 0.05$). However, the pattern R17 were significantly associated with strains isolated from clinically health pigs ($P = 0.011$).

Among the 98 strains of serotype 2, 30 patterns were identified. 54 percent (53 / 98) and 24 percent (24 / 98) of *S. suis* serotype 2 strains were included in subgroup a ($P = 0.021$) and group C ($P = 0.002$) respectively. The patterns R6 was significantly associated with *S. suis* serotype 2 strains ($P = 0.019$). The *S. suis* capsular types 1, 1/2, 3, 4, 5, 7 and 9 were clustered in the subgroup b ($P < 0.001$). Significant associations were observed between these strains and the patterns R26 ($P < 0.001$) and R29 ($P = 0.002$).

Nine patterns were identified among the 29 strains isolated from humans, whereas 38 patterns were obtained from 109 strains isolated from pigs. Twelve of the 29 (41%) strains, isolated from humans, clustered in group C, whereas 96 of 109 (88%) strains were distributed among the other groups ($P < 0.001$). The patterns R6, R36, R11 and R38 were significantly associated with strains isolated from humans ($P < 0.05$), because 22 (76%) strains had these types.

Discussion-Conclusion

The PCR-Ribotyping followed by RFLP analysis with *Rsa*I endonuclease, because of its reproducibility and discriminatory power, can be used to characterize *S. suis* strains. Pulsed-field gel electrophoresis (PFGE) was previously described (6). However, PFGE is laborious and time-consuming technique whereas the PCR-Ribotyping offer the advantages of simplicity and rapidity conferred by the PCR procedure. The typing of 138 *S. suis* strains confirmed that the strains isolated from humans was less genetically diverse than strains isolated from pigs. For the first time, one molecular pattern was significantly associated with *S. suis* serotype 2 strains (R6) and one pattern was associated with strains isolated from clinically health pigs (R17).

In conclusion, this genetic tool could be a valuable help in distinguish individual isolates of *S. suis* during further epidemiological investigations.

References

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