

Original article

**Production of Muraminidase-Released Protein (MRP),
Extracellular Factor (EF) and Suilysin
by field isolates of *Streptococcus suis* capsular
types 2, 1/2, 9, 7 and 3 isolated from swine in France**

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Abstract – A total of 323 isolates of *Streptococcus suis* recovered from diseased or healthy pigs in France were serotyped. The presence of virulence-related proteins, Muraminidase-Released Protein (MRP), Extracellular Factor (EF) and Suilysin was also studied in 122 isolates of capsular types 2, 1/2, 9, 7 and 3 to evaluate their implication in virulence of *S. suis*. Capsular types 2, 1/2, 9, 7 and 3 were the most frequently detected (93%), with 69% for the capsular type 2 alone. Capsular types 2, 1/2, 9, 7, 3, 1, 4, 8, 18, 10 and 12 were isolated from diseased pigs, whereas types 2, 7, 9, 1/2, and 3 originated from the nasal cavities or tonsils of healthy animals. Most of the *S. suis* type 2 isolates recovered from diseased pigs carried MRP+ EF- Suilysin- (46%) or MRP+ EF+ Suilysin+ (28%) phenotypes. The MRP+ EF- Suilysin- phenotype was also detected in 67% of *S. suis* type 2 strains isolated from healthy pigs. The production of the virulence-related proteins was less frequently found in *S. suis* types 1/2, 9, 7 and 3 recovered either from diseased or healthy pigs. In this study, all the capsular type 1/2 strains were MRP+ EF- Suilysin- and all the *S. suis* type 7 harboured an MRP- EF- Suilysin- phenotype. The MRP- EF- Suilysin- phenotype was found in *S. suis* types 2, 3, 7 and 9 isolated from septicæmia, meningitis, pneumonia, and pleurisy. These results suggest that the presence of these proteins should not be used as a single condition for classifying the virulence of a field isolate in France.

Streptococcus suis / capsular type / virulence / swine

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Résumé – Production de la protéine libérée par la muraminidase (MRP), du facteur protéique extracellulaire (EF) et de la suilysine par des souches de *Streptococcus suis*, sérotypes 2, 1/2, 9, 7 et 3, isolées dans les élevages de porcs en France. Un total de 323 souches de *Streptococcus suis* collecté en France chez des porcs malades ou cliniquement sains a été sérotypé. La production des facteurs de virulence, la protéine libérée par la muraminidase (MRP), le facteur protéique extracellulaire (EF) et la suilysine (hémolysine de *S. suis*) a été évaluée chez 122 souches appartenant aux sérotypes 2, 1/2, 9, 7 et 3 afin de déterminer leur implication dans la virulence de *S. suis*. Les sérotypes 2, 1/2, 9, 7 et 3 ont été les plus fréquemment isolés (93%), le sérotype 2 étant majoritaire (69%). Les sérotypes 2, 1/2, 9, 7, 3, 1, 4, 8, 18, 10 et 12 ont été isolés chez des porcs malades et les sérotypes 2, 7, 9, 1/2, et 3 ont été mis en évidence dans les cavités nasales ou les amygdales des porcs cliniquement sains. La plupart des souches de sérotype 2, isolées de porcs malades, présentaient les phénotypes MRP+ EF- Suilysin- (46%) et MRP+ EF+ Suilysin+ (28%). Le phénotype MRP+ EF- Suilysin- a également été détecté chez 67% des souches de sérotype 2 provenant de porcs cliniquement sains. Peu de souches de sérotypes 1/2, 3, 7 et 9 produisaient les protéines de virulence et cette production était analogue chez les animaux malades ou cliniquement sains. Dans cette étude, toutes les souches de sérotype 1/2 avaient le phénotype MRP+ EF- Suilysin- et toutes les souches de sérotype 7 présentaient le phénotype MRP- EF- Suilysin-. Le phénotype MRP- EF- Suilysin- a été détecté chez les souches de *S. suis* de sérotypes 2, 3, 7 et 9, isolées de septicémie, méningite, pneumonie et de pleurésie. Les résultats suggèrent que ces protéines ne sont pas suffisantes pour expliquer la virulence des souches françaises de *S. suis*.

Streptococcus suis / sérotype / virulence / porc

1. INTRODUCTION

Streptococcus suis is recognised as a very common and important swine pathogen [16, 28]. *S. suis* infections may be the cause of meningitis, polyarthritis, polyserositis, endocarditis, septicaemia and sudden death, creating significant economic losses in pig farms [3, 25, 28]. Healthy carrier pigs harbouring the bacteria in nasal cavities or in tonsils also play an important role in the epidemiology and transmission of the infection [2, 6, 22]. Moreover, *S. suis* is a zoonotic agent [1, 17, 29].

Thirty five capsular types of *S. suis* (types 1 to 34 and type 1/2) have been described. Type 2 is the serotype the most often associated with disease and the most commonly isolated [6, 14–16, 21, 23, 28]. Recently, some studies have indicated that other capsular types could also be isolated from diseased animals and that their distribution differs between countries [9, 21, 22, 25].

Most studies on virulence factors and the pathogenesis of the infection have been focused on capsular type 2 strains. Vecht

et al. [31] showed that virulent *S. suis* type 2 produce two proteins, the 136 kDa-Muraminidase-Released Protein (MRP) and the 110 kDa Extracellular Factor (EF). Variants of MRP and EF proteins showing different electrophoretic mobility, MRP* (molecular weight, MW > 136 kDa), MRPs (MW < 136 kDa) and EF* (MW > 110 kDa) have also been detected [31, 33]. Relationships between the virulence of *S. suis* type 2 and the presence of MRP and EF have been reported in different studies but the results are not conclusive. Vecht et al. [31] indicate that most of the *S. suis* type 2 isolated from diseased pigs presents the MRP+ EF+ phenotype while isolates from healthy carriers have the MRP- EF- profile. However, the presence of these virulence-related proteins was shown not to be associated with the virulence of *S. suis* Canadian strains isolated from diseased pigs [13]. Other phenotypes have been recorded and the relevance of MRP and EF in virulence of *S. suis* differs according to the origin of the isolates [8, 13, 22, 26, 31, 32].

More recently, a thiol-activated haemolysin, the Suilysin, was described as

a potential virulence factor of *S. suis* [7, 12, 18]. The production of this *S. suis* haemolysin was evidenced in strains collected from diseased pigs, but its role has not been determined [19, 27]. Moreover, other surface components may be implicated in the virulence of *S. suis* [4, 11, 20].

In this study, 323 *S. suis* field strains isolated in France were serotyped and 122 of them were analysed for MRP, EF and Suilysin production to study the implication of these 3 factors in *S. suis* virulence.

2. MATERIALS AND METHODS

2.1. Collection and identification of *S. suis* isolates

A total of 323 isolates of *S. suis* were collected from piglets and growing pigs by six veterinary diagnostic laboratories in France from 1996 to 1998. Most of these isolates ($n = 295$) were collected from diseased or dead animals. *S. suis* was also isolated from nasal swabs or tonsillar biopsies ($n = 28$) collected during control investigations on pig farms.

All the isolates received from the veterinary diagnostic laboratories were cloned three times on Columbia blood agar base (Oxoid, Dardilly, France) supplemented with 5% sheep blood, and grown overnight at 37 °C with 5% CO₂. After cloning, all the isolates were biochemically typed using the API 20 STREP test kit (Bio Mérieux, Marcy l'Etoile, France). In addition, the aesculin hydrolysis test was carried out. Capsular typing was also determined by slide coagglutination using a negative serum and the 34 different type-specific hyperimmune sera raised in rabbits [10]. French commercial specific sera against capsular types 1 to 12 (Laboratoire de Développement et d'Analyses des Côtes d'Armor, Ploufragan, France) were used. When strains belonged to other capsular types, they were serotyped

with Canadian sera (University of Montréal, St-Hyacinthe, Canada).

2.2. Determination of the presence of the proteins MRP, EF and Suilysin in *S. suis* isolates by electrophoresis and western blot

A total of 122 *S. suis* field strains belonging to capsular types 2, 1/2, 9, 7 and 3 and isolated from diseased or healthy pigs were tested for the presence of MRP, EF and Suilysin by immunoblot as previously described [13, 24]. Briefly, concentrated supernatant was mixed with solubilisation buffer and separated by sodium-dodecyl-sulfate polyacrylamide vertical slab gels: 5% stacking gel and 8% separating gel. Separated proteins were further transferred to nitrocellulose membrane and after blocking unreacted sites with milk (2%), blots were incubated with a monoclonal antibody against MRP (kindly provided by H.J. Wisselink, ID-DLO, Lelystad, The Netherlands) or with monospecific polyclonal antibodies against EF and Suilysin, respectively, which were produced as previously described [5, 13]. Peroxydase-labeled anti-mouse or anti-rabbit immunoglobulins were added and revelation of bound antibodies was visualised after the addition of 4-chloro-1-naphtol (Sigma-Aldrich, St Quentin Fallavier, France). MRP⁺ EF⁺, MRP⁺ EF^{*} and MRP⁻ EF⁻ phenotypes were controlled with D-282, S735 and T15 strains respectively [13, 31]. The S735 strain was also used to control the production of Suilysin.

3. RESULTS

3.1. Distribution of *S. suis* capsular types in French swine herds

From 1996 to 1998, capsular type 2 was the most prevalent type (69.2%) isolated from diseased pigs (Tab. I). Capsular types

Table I. Distribution of capsular types of *Streptococcus suis* in diseased pigs in France from 1996 to 1998.

Capsular type	Year			Total (%)
	1996	1997	1998	
1	1	2	4	7 (2.4)
1/2	5	7	12	24 (8.1)
2	79	71	54	204 (69.2)
3	0	1	6	7 (2.4)
4	3	1	2	6 (2.1)
7	1	4	10	15 (5.1)
8	0	0	4	4 (1.4)
9	8	8	7	23 (7.8)
10	0	0	1	1 (0.3)
12	1	0	0	1 (0.3)
18	0	0	2	2 (0.6)
A	0	0	1	1 (0.3)
Total	98	94	103	295 (100.0)

A: Autoagglutinable isolates.

1/2, 9, 7, 3, 1, 4, 8, 18, 10 and 12 and one autoagglutinable strain were also recovered from diseased pigs. None of the isolates tested belonged to capsular types 19 to 34. Ninety three percent of *S. suis* isolates belonged to capsular types 2, 1/2, 9, 7 or 3 from 1996 to 1998 (Tab. I).

Most of the *S. suis* strains (36%) were isolated from septicaemic animals and they often belonged to capsular type 2, but other types (1, 1/2, 3, 4, 7, 8, 12) and one autoagglutinable isolate were also isolated from bloody supplied organs (data not shown). Capsular types 1/2, 2, 3, 7 and 9 were also found in cases of pleurisy or pneumonia (35%), meningitis (22%) and arthritis (7%) (Tab. II).

Twenty eight isolates of *S. suis* were collected from healthy carrier pigs during the present study. Twenty four of them belonged to capsular types also recovered from diseased animals: types 1/2, 2, 3, 7 and 9 (Tab. II). In addition, four autoagglutinable isolates from tonsils of healthy pigs were recorded (data not shown).

3.2. Production of MRP, EF and Suilysin in French *S. suis* strains

3.2.1. Isolates from diseased animals

Most of the *S. suis* capsular type 2 isolated from diseased animals, presented MRP+ EF- Suilysin- (46.3%) or MRP+ EF+ Suilysin+ (27.7%) phenotypes (Tab. II). MRP- EF- Suilysin- (9.2%), MRP+ EF* Suilysin+ (7.4%) and MRP+ EF+ Suilysin- (7.4%) phenotypes were also detected in *S. suis* type 2 isolated from diseased animals.

The production of Suilysin was detected in 37.0% of *S. suis* isolates and it was often associated with the presence of EF. The EF variant (EF*) was recovered from 5 strains. These strains were often isolated from lung disorders (4/5) and belonged only to capsular type 2 (Tab. II). EF* was always associated with Suilysin. The MRP variants were not detected.

A strong relationship with the capsular types and the phenotypes was observed for types 1/2 and 7: all the isolates of each type presented MRP+ EF- Suilysin- and MRP- EF- Suilysin- phenotypes, respectively (Tab. II).

3.2.2. Isolates from healthy animals

All *S. suis* type 2 isolates produced the MRP protein and MRP+ EF*, MRP+ EF+ and MRP+ EF- phenotypes were detected (Tab. II). Moreover, the Suilysin was not a marker of *S. suis* virulence, since some type 2 *S. suis* strains isolated from healthy animals produced this protein (Tab. II). Most of the isolates of type 2 from healthy pigs presented the MRP+ EF- Suilysin- phenotype (66.7%). Isolates belonging to other types (types 1/2, 3 and 9) carried this phenotype (40%) or the MRP- EF- Suilysin- phenotype (60%).

4. DISCUSSION

Capsular type 2 strains was the most prevalent type from diseased pigs and

Table II. Distribution of *Streptococcus suis* phenotypes in relation to serotypes isolated from pigs.

Phenotype	Capsular types isolated from diseased pigs (n = 98)																Capsular types isolated from healthy pigs (n = 24)								
	Septicaemia						Meningitis					Pleurisy or pneumonia					Arthritis			Nasal cavities- tonsils					
	1/2	2	3	7	9	T ^a	1/2	2	7	9	T	1/2	2	3	7	9	T	2	2	1/2	2	3	7	9	T
MRP+ EF* Suiysin+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	1
MRP+ EF+ Suiysin+	-	1	-	-	1	-	-	9	-	-	9	-	-	-	-	-	-	-	-	5	-	2	-	-	2
MRP+ EF+ Suiysin-	-	2	-	-	2	-	-	2	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MRP+ EF- Suiysin+	-	-	1	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MRP- EF- Suiysin-	-	4	3	5	2	14	-	-	3	4	7	-	1	2	4	1	8	-	-	-	-	1	8	-	9
MRP- EF* Suiysin+	-	1	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MRP+ EF- Suiysin-	3	10	-	-	2	15	1	2	-	1	4	10	11	1	-	-	22	2	2	2	6	1	-	3	12
Total	3	18	4	5	5	35	1	13	3	5	22	10	16	3	4	1	34	7	2	9	2	8	3	24	

^a Total number of *S. suis* strains isolated from septicaemia and presenting different phenotypes.

^b No strains presenting the MRP+ EF* Suiysin+ phenotype.

^c Four strains of *S. suis* capsular type 2 isolated from pleurisy or from pneumonia presenting the MRP+ EF* Suiysin+ phenotype.

healthy carriers. This result is an agreement with previous results [8, 14–16, 21, 30]. Virulence of *S. suis* did not differ according to capsular antigens, with most of the capsular types being isolated from damaged organs as well as from healthy pigs as previously reported [21].

Common phenotypes of *S. suis* type 2 isolated from diseased animals were recovered with various rates. Although the rates of MRP+ EF- Suilysin- or MRP+ EF+ Suilysin+ were higher or lower than previous results [13, 22, 31], they were similar to data reported by Galina et al. [9] and by Salasia and Laemmle [26].

Virulence of *S. suis* capsular type 2 has been associated with the presence of MRP and EF; however, isolates from tonsils did not produce any of these proteins [31]. It has also been suggested that virulence among *S. suis* isolates differs according to the tissue origins. Accordingly, *S. suis* isolated from lung is less virulent [32]. Our results were different. Indeed, the MRP-EF- phenotype was not observed with *S. suis* type 2 isolated from palatine tonsils or nasal cavities of healthy animals in contrast to previous results [13, 22, 31]. Most of the isolates of type 2 from healthy pigs presented the phenotype shown with virulent strains isolated from septicaemia, pneumonia and pleurisy. *S. suis* isolates from meningitis and arthritis often showed another phenotype (MRP+ EF+ Suilysin+), as previously described [9].

Virulence phenotypes of *S. suis* type 2 strains isolated from diseased animals differed according to the country, particularly with Suilysin and EF* productions. Suilysin was recorded in *S. suis* French strains which mostly belonged to the capsular type 2. Production of Suilysin was tested by western blot in our study. Negative strains for Suilysin were not tested again with a microtitre assay as previously carried out [18, 19] because the haemolysin-negative strains presented identical results using both methods [13]. Previous studies in Canada and the Netherlands have reported *S. suis*

isolates producing a lower or a higher rate of Suilysin [13, 19, 27]. As previously described with European *S. suis* isolates and in contrast to strains from North American continent, some French strains presented also EF* [9, 13, 22, 26]. As we observed, the production of the EF* protein has previously been associated with capsular type 2 and is less common with other types: 1, 1/2, 14 and 22 [22, 26]. Moreover, the three proteins, MRP, EF and Suilysin, were never observed at once in *S. suis* strains of capsular types 1/2, 3, 7 and 9, as shown by other authors [9, 22, 33].

Relationships between the studied components and virulence of *S. suis* strains were not established with the selected French isolates. These results suggest the implication of virulence factors other than capsular antigens, MRP, EF and Suilysin in *S. suis* infections.

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