

## CHAPTER 2

# Distribution of capsular types and production of muramidase-released protein (MRP) and extracellular factor (EF) of *Streptococcus suis* strains isolated from diseased pigs in seven European countries

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## Abstract

*Streptococcus suis* strains (n=411), isolated from diseased pigs in seven European countries were serotyped using specific antisera against serotype 1 to 28, and were phenotyped on the basis of their muramidase-released protein (MRP) and extracellular-factor protein (EF) production. Overall, *S. suis* serotype 2 appeared to be most prevalent (32%), followed by serotype 9 (20%) and serotype 1 (12%). Serotype 2 was most frequently isolated in France, Italy and Spain, whereas serotype 9 was most frequently isolated in Belgium, the Netherlands and Germany. In the United Kingdom serotypes 1 and 14 were most frequently isolated. High percentages of *S. suis* serotype 1, 2, 1/2 and 14 strains, isolated from tissues associated with *S. suis* infections such as brain, serosae, joint, heart and organs expressed the EF-protein, indicating that in these serotypes expression of EF is likely to be associated with virulence. In contrast, strains belonging to serotype 7 and 9, isolated from tissues associated with *S. suis* infections did not produce EF. These results strongly suggest that in the serotypes 7 and 9 EF expression is not related to virulence. More than 80% of the *S. suis* serotype 9 strains produced an MRP\* protein, a high molecular variant of the 136-kDa MRP. Expression of MRP\* in serotype 9 strains is possibly associated with virulence.

## 1. Introduction

*Streptococcus suis* is a pathogen responsible for a variety of infections in pigs such as meningitis, arthritis, endocarditis, septicaemia and bronchopneumonia (Higgins et al. 1992; Kataoka et al. 1993; Reams et al., 1994; Vecht et al., 1985). The economic impact of *S. suis* infections for the swine industry is substantial (Chengappa et al., 1990). The prophylactic use of antibiotics in food and drinking water has been unsuccessful in controlling the disease. Antibiotics are becoming less effective because of an increase in resistance among *S. suis* isolates (Aarestrup et al., 1998b) and are less accepted because of the public awareness of antimicrobial residues. In addition, the development of effective vaccines is hindered by the number of virulent serotypes, by the lack of knowledge of virulence factors and by differences in virulence not only among serotypes but also within serotypes of *S. suis*.

So far, 35 serotypes of *S. suis* based on capsular antigens are described (Perch et al., 1983, Gottschalk et al., 1989, 1991, Higgins et al., 1995). Worldwide *S. suis* serotype 2 is the most frequently isolated serotype. However, the distribution varies with region and can change over time. In Australia and the Netherlands, *S. suis* serotype 9 is most frequently isolated from diseased pigs, whereas serotype 7 is the most prevalent serotype in Finland (Sihovenen et al., 1988; Gogolewski et al., 1990; Jacobs et al., 1995). In Denmark, serotype 7 was 15 years ago the most common serotype causing infections among diseased pigs. Recently, serotype 2 was most frequently isolated in this country (Perch et al., 1983; Aarestrup et al., 1998a). In Scotland, serotype 14 was most frequently isolated from diseased pigs (MacLennan et al., 1996).

Virulence can differ among various strains of *S. suis*. In our earlier work we showed that in *S. suis* serotype 2 a correlation exists between the production of muramidase-released protein (MRP) and extracellular-factor protein (EF) and virulence for pigs (Vecht et al., 1992). MRP<sup>+</sup>EF<sup>+</sup> serotype 2 strains were mainly isolated from diseased pigs and are virulent for pigs. In contrast, MRP<sup>-</sup>EF<sup>-</sup> serotype 2 strains were mainly isolated from the tonsils of healthy pigs and are nonvirulent. MRP<sup>+</sup>EF<sup>\*</sup> serotype 2 strains produce high molecular weight variants of EF and are weakly virulent for young pigs.

Serotype 1 strains can also produce MRP and EF. Highly virulent *S. suis* serotype 1 strains produced a 120 kDa-variant of MRP (MRP<sup>s</sup>) as well as EF. Serotype 1 strains that do not produce MRP and EF appeared to be less virulent for young piglets, but were still capable of inducing illness with the specific symptoms (Stockhofe-Zurwieden et al., 1996).

Different variants of the MRP-protein in *S. suis* serotype 2 strains were described (Vecht et al.,

1991; Galina et al., 1996). Enlarged or reduced forms of MRP, respectively called MRP<sup>\*</sup> (MW > 136kDa) and MRP<sup>s</sup> (MW < 136kDa) can compose phenotypes as MRP<sup>\*</sup>EF<sup>-</sup>, MRP<sup>s</sup>EF<sup>-</sup>, MRP<sup>-</sup>EF<sup>\*</sup> and MRP<sup>+</sup>EF<sup>\*</sup>. So far, the virulence of these strains has not been tested in animal models.

Although there is a strong correlation between MRP and EF and virulence, these proteins are dispensable for causing disease. Isogenic mutants of *S. suis* serotype 1 and 2 impaired in expression of MRP and EF were as virulent for pigs as the parent strains (Smith et al., 1996). Moreover, most of the *S. suis* serotype 2 strains, isolated from diseased pigs in Canada, do not produce MRP and EF (Gottschalk et al., 1998). Nevertheless, most of the *S. suis* serotype 2 strains isolated in America, Austria, Germany and Spain showed the MRP<sup>+</sup>EF<sup>+</sup> phenotype. (Awad-Masalmeh et al., 1999; Galina et al., 1996; Luque et al., 1999; Salasia and Lämmler, 1995).

In serotypes other than serotypes 1 and 2 the production of MRP and EF, and a possible correlation of their production with virulence, is unknown. Therefore, we performed serotyping and we determined the production of MRP and EF of *S. suis* strains isolated from diseased pigs in seven European countries. It has been suggested that *S. suis* is not a primary cause of pneumonia and strains isolated from lungs may be less virulent than strains isolated from organs as brains, serosae, heart and joints (Reams et al., 1995; Hoefling et al., 1998). Therefore, we compared the relationship between MRP/EF phenotypes, serotypes of strains and their sites of isolation.

## 2. Materials and methods

### 2.1 *S. suis* isolates.

Four hundred and eleven strains of *S. suis* were obtained from seven European countries: Belgium, UK, France, Italy, Germany, Spain and the Netherlands (Table 1). Strains were isolated in the course of routine diagnostic procedures from tissues of diseased pigs.

Strains were kindly provided by Ing. J. Hommez, Provinciaal Verbond voor Dierenziektenbestrijding van West-Vlaanderen, Belgium; Dr. P. Heath, Veterinary Investigation Centre, Suffolk, UK; Dr. H. Morvan, Laboratoire de Développement et d'Analyses, Ploufragan, France; Prof. Dr. V. Sala, Institute of Infectious Diseases, Milan, Italy; Prof. Dr. G. Amtsberg, Institut für Mikrobiologie und Tierseuchen, Tierärztliche Hochschule Hannover, Germany; Dr. M. Ganter, Außenstelle für Epidemiologie der Tierärztliche Hochschule Hannover, Bakum, Germany; Prof. Dr. Ch. Lämmler, Justus-Liebig Universität Giessen, Germany; Carmen Terradas Iglesias, Facultad de Veterinaria,

Universidad de Cordoba, Spain. Dutch *S. suis* strains were isolated from diseased pigs at the Animal Health Service, Boxtel. If known, the site of isolation and the age of the pigs were recorded.

Table 1

*S. suis* strains isolated from diseased pigs

| Country        | Number of strains | Period of isolation |
|----------------|-------------------|---------------------|
| Belgium        | 60                | 1994–1996           |
| France         | 51                | 1994–1997           |
| Germany        | 48                | 1997                |
| Italy          | 48                | unknown             |
| Netherlands    | 78                | 1996                |
| Spain          | 47                | 1991–1995           |
| United Kingdom | 79                | 1987–1996           |

## *2.2 Culture conditions and typing*

A 1-day-old colony of each strain, grown on Columbia blood agar base (code CM 331; Oxoid, Ltd., Inc., Columbia, Md) containing 6% horse blood, was incubated overnight at 37°C in Todd Hewitt broth (code CM 189; Oxoid). Cultures were centrifuged at 4,000 g for 15 min. Aliquots of the supernatants were stored at –20°C until use. All strains were typed as *S. suis* by biochemical methods (Devriese et al., 1991). Serotyping was performed by slide agglutination with specific rabbit antisera (ID-Lelystad) against the reference strains of serotypes 1 to 28 as described by Gottschalk et al. (1993).

## *2.3 SDS-PAGE and Western blot analysis*

Supernatants were analysed by using sodium dodecyl sulfate-polyacrylamide gel electrophoresis as described by Laemmli (1970). Separating gels contained 6% polyacrylamide, stacking gels 4%. After electrophoresis proteins were transferred to nitrocellulose filters by using a Multiphor II Nova Blot system according to the recommendations of the manufacturer (Pharmacia LKB, Uppsala, Sweden). The blots were either incubated with a 1:200 dilution of monoclonal antibodies against MRP or EF (Vecht et al., 1992). After washing, bound mouse antibodies were visualized with a 1:1,000 dilution of rabbit-anti-mouse globulins conjugated to alkaline phosphatase (Zymed laboratories, Inc., San Francisco, Calif.) and bromochloroindolyl phosphate (Sigma, St. Louis, Mo) - Nitro Blue Tetrazolium (Merck, Darmstadt, Germany) in phosphatase buffer (100 mM NaCl, 5 mM

MgCl<sub>2</sub>, 100 mM diethanolamine [pH 9.5]) as substrate solution.

### 3. Results

#### 3.1 Serotypes

Overall, most of the strains belonged to the serotypes 1/2, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 14 (88%). Thirty-seven strains were non-typable, 13 of these strains were polyagglutinable, 14 autoagglutinable and 10 strains could not be classified with the 28 antisera used (Table 2).

Table 2  
Capsular serotypes of *S. suis* strains isolated from diseased pigs

| Serotype | No. (%) of strains isolated in |         |         |         |             |         |                | Total    |
|----------|--------------------------------|---------|---------|---------|-------------|---------|----------------|----------|
|          | Belgium                        | France  | Germany | Italy   | Netherlands | Spain   | United Kingdom |          |
| 1        | 6 (10)                         |         |         | 7 (15)  | 3 (4)       | 4 (9)   | 30 (38)        | 50 (12)  |
| 2        | 10 (16)                        | 30 (59) | 5 (10)  | 13 (27) | 22 (28)     | 24 (51) | 28 (35)        | 132 (32) |
| 1/2      | 2 (3)                          |         | 3 (6)   | 4 (8)   | 2 (3)       | 4 (9)   | 1 (1)          | 16 (4)   |
| 3        | 1 (2)                          |         | 8 (17)  | 2 (4)   |             | 2 (4)   |                | 13 (3)   |
| 4        |                                | 4 (8)   | 5 (10)  | 5 (10)  |             |         |                | 14 (3)   |
| 5        |                                |         | 3 (6)   |         |             | 1 (2)   |                | 4 (1)    |
| 7        | 5 (8)                          | 6 (12)  | 5 (10)  | 2 (4)   | 3 (4)       | 1 (2)   | 1 (1)          | 23 (6)   |
| 8        | 4 (7)                          | 4 (8)   | 1 (2)   | 1 (2)   |             | 1 (2)   |                | 11 (3)   |
| 9        | 14 (23)                        | 6 (12)  | 9 (19)  | 7 (15)  | 45 (58)     | 2 (4)   | 1 (1)          | 84 (20)  |
| 10       |                                |         |         |         |             | 1 (2)   | 1 (1)          | 2 (1)    |
| 12       | 1 (2)                          |         |         |         |             |         |                | 1 (0)    |
| 14       |                                |         |         |         |             |         | 13 (17)        | 13 (3)   |
| 15       |                                |         |         |         |             |         | 1 (1)          | 1 (0)    |
| 16       |                                |         | 1 (2)   |         |             |         |                | 1 (0)    |
| 22       | 2 (3)                          |         |         |         | 3 (4)       |         |                | 5 (1)    |
| 25       | 2 (3)                          |         | 1 (2)   | 1 (2)   |             |         |                | 4 (1)    |
| NT       | 13 (22)                        | 1 (2)   | 7 (14)  | 6 (13)  |             | 7 (15)  | 3 (4)          | 37 (9)   |
| Total    | 60                             | 51      | 48      | 48      | 78          | 47      | 79             | 411      |

NT: Not typable isolates

*S. suis* serotypes 2 (32%), 9 (20%) and 1 (12%) were most frequently isolated in the seven European countries (Table 2). *S. suis* serotype 2 was most prevalent in France, Italy and Spain, while serotype 9 was most prevalent in the Netherlands, Germany and Belgium. In the UK *S. suis* serotype 1 was most prevalent (38%) followed by serotype 2 (35%). *S. suis* serotype 14 strains were exclusively isolated in the UK.

### 3.2 Serotypes and sites of isolation

Sixty-six per cent of the strains was isolated from tissues typically affected by *S. suis* (brains, serosae, heart, joints and parenchymateous organs like liver, kidney or spleen) while twenty per cent of the strains was isolated from lungs (Table 3). Seven per cent of the strains was isolated from

Table 3

*S. suis* strains isolated from diseased pigs: serotypes and sites of isolation.

| Serotype | No. (%) of strains isolated from            |         |                    |         | Total |
|----------|---|---------|--------------------|---------|-------|
|          | Typical <i>S. suis</i> tissues <sup>a</sup> | Lung    | Other <sup>b</sup> | Unknown |       |
| 1        | 47 (94)                                     | 1 (2)   | 2 (4)              | 0 (0)   | 50    |
| 2        | 101 (77)                                    | 18 (14) | 6 (4)              | 7 (5)   | 132   |
| 1/2      | 8 (50)                                      | 8 (50)  | 0 (0)              | 0 (0)   | 16    |
| 3        | 1 (8)                                       | 12 (92) | 0 (0)              | 0 (0)   | 13    |
| 4        | 1 (7)                                       | 5 (36)  | 4 (29)             | 4 (29)  | 14    |
| 5        | 0 (0)                                       | 4 (100) | 0 (0)              | 0 (0)   | 4     |
| 7        | 6 (26)                                      | 7 (30)  | 3 (13)             | 7 (30)  | 23    |
| 8        | 1 (8)                                       | 4 (33)  | 2 (17)             | 5 (42)  | 12    |
| 9        | 62 (74)                                     | 9 (11)  | 8 (10)             | 5 (6)   | 84    |
| 10       | 1 (50)                                      | 1 (50)  | 0 (0)              | 0 (0)   | 2     |
| 12       | 1 (100)                                     | 0 (0)   | 0 (0)              | 0 (0)   | 1     |
| 14       | 13 (100)                                    | 0 (0)   | 0 (0)              | 0 (0)   | 13    |
| 15       | 1 (100)                                     | 0 (0)   | 0 (0)              | 0 (0)   | 1     |
| 16       | 0 (0)                                       | 1 (100) | 0 (0)              | 0 (0)   | 1     |
| 22       | 5 (100)                                     | 0 (0)   | 0 (0)              | 0 (0)   | 5     |
| 25       | 1 (25)                                      | 3 (75)  | 0 (0)              | 0 (0)   | 4     |
| NT       | 22 (61)                                     | 10 (28) | 4 (11)             | 0 (0)   | 36    |
| Total    | 271 (66)                                    | 83 (20) | 29 (7)             | 28 (7)  | 411   |

<sup>a</sup> brains, serosae, heart, joints, parenchymatous organs (liver, kidney, spleen)

<sup>b</sup> miscellaneous tissues as tonsils, lymph nodes, urinary tract, skin, trachea, nose, vagina, cervix or intestines

NT: Not typable isolates

miscellaneous tissues such as tonsils, lymph nodes, urinary tract, skin, trachea, nose, vagina, cervix and intestines. For seven per cent of the strains the site of isolation was unknown.

*S. suis* serotype 1, 2, 9, 14 and 22 strains were most frequently isolated from tissues commonly involved in clinical disease, while serotype 3, 4, 5, 8 and 25 strains were mostly isolated from lungs. Serotype 1/2 and 7 strains were isolated in similar proportions from both tissues typically affected by *S. suis* and lungs.

Most of the strains isolated from tissues typically affected by *S. suis* were from brains (58%), followed by joints (12%) and organs: liver, kidney or spleen (8%) (results not shown). Compared to serotype 2 and 9 strains, serotype 1 and 14 strains were more frequently isolated from the joints (22% and 29% for serotypes 1 and 14 compared to 8 and to 10% for serotypes 2 and 9, respectively). *S. suis* serotype 1/2 was as frequently isolated from brains as from organs (44% each).

### 3.3 Serotypes and age

The age of the pigs was known for 135 of the strains and varied between 0.5 and 20 weeks. Most pigs (92%) were less than 10 weeks old (Table 4).

*S. suis* serotype 1 strains were predominantly isolated from 3-week-old pigs, while serotype 2, 7, 9 and 14 strains were mainly isolated from 6- to 8-week-old pigs. Serotype 22 strains were mainly

Table 4

*S. suis* strains isolated from diseased pigs: serotypes and age of pigs

| Serotype | No. of strains | Age (no. of weeks) of pigs |                |                |         |
|----------|----------------|----------------------------|----------------|----------------|---------|
|          |                | Minimum                    | Mean $\pm$ SD  | Median         | Maximum |
| 1        | 29             | 0.5                        | 3.8 $\pm$ 5.1  | 3              | 21      |
| 2        | 42             | 2                          | 8.0 $\pm$ 3.6  | 8              | 18      |
| 1/2      | 2              | 7                          | 8.5 $\pm$ 2.1  | — <sup>a</sup> | 10      |
| 7        | 4              | 4                          | 8.0 $\pm$ 5.5  | 6              | 16      |
| 9        | 43             | 1                          | 6.3 $\pm$ 1.9  | 7              | 9       |
| 10       | 1              | 6                          | —              | —              | 6       |
| 14       | 6              | 2.5                        | 7.3 $\pm$ 4.3  | 6.5            | 14      |
| 22       | 4              | 1.5                        | 4.6 $\pm$ 2.8  | 5              | 7       |
| NT       | 4              | 5                          | 10.8 $\pm$ 6.5 | 9              | 20      |
| All      | 135            | 0.5                        | 6.7 $\pm$ 3.9  | 6              | 21      |

<sup>a</sup>Not enough observations

NT: Not typable isolates

isolated from 4.5-week-old pigs, serotype 1/2 strains from 8.5-week-old pigs and non-typable strains from 10.8-week-old pigs.

### 3.4 Serotypes and phenotypes

MRP and EF phenotypes of the *S. suis* strains were determined by Western blot analysis using monoclonal antibodies directed against MRP or EF. A high percentage of the serotype 1, 2 and 14 strains showed an EF-positive phenotype, either MRP<sup>+</sup>EF<sup>+</sup>, MRP<sup>s</sup>EF<sup>+</sup> or MRP<sup>-</sup>EF<sup>+</sup>. (Table 5). Among serotypes 1 and 1/2 EF\*-producing strains were found. EF-negative strains (MRP<sup>\*</sup>EF<sup>-</sup>, MRP<sup>s</sup>EF<sup>-</sup> or MRP<sup>-</sup>EF<sup>-</sup>) were found in nearly all serotypes. Variants of MRP (MRP\* or MRP<sup>s</sup>) were found in nearly all serotypes. A high percentage (81%) of the serotype 9 strains belonged to the MRP\*EF<sup>-</sup> phenotype (Table 5).

Table 5  
*S. suis* strains isolated from diseased pigs: serotypes and MRP/EF phenotypes

| Serotype | No. (%) of strains with phenotype  |  |  |                                  | Total |
|----------|--|--|--|----------------------------------|-------|
|          | MRP <sup>+</sup> EF <sup>+</sup><br>MRP <sup>s</sup> EF <sup>+</sup><br>MRP <sup>-</sup> EF <sup>+</sup> | MRP <sup>+</sup> EF <sup>*</sup><br>MRP <sup>s</sup> EF <sup>*</sup> | MRP <sup>*</sup> EF <sup>-</sup><br>MRP <sup>s</sup> EF <sup>-</sup> | MRP <sup>-</sup> EF <sup>-</sup> |       |
| 1        | 33 (66)  | 2 (4)  | 3 (6)  | 12 (24)                          | 50    |
| 2        | 91 (71)  | 0 (0)  | 32 (25)  | 6 (4)                            | 129   |
| 1/2      | 3 (20)   | 4 (27)   | 6 (40)   | 2 (13)                           | 15    |
| 3        | 0 (0)  | 0 (0)  | 4 (31)   | 9 (69)                           | 13    |
| 4        | 0 (0)  | 0 (0)  | 3 (21)   | 11 (79)                          | 14    |
| 5        | 0 (0)  | 0 (0)  | 1 (25)   | 3 (75)                           | 4     |
| 7        | 0 (0)  | 0 (0)  | 1 (4)  | 22 (96)                          | 23    |
| 8        | 0 (0)  | 0 (0)  | 1 (9)  | 10 (91)                          | 11    |
| 9        | 0 (0)  | 0 (0)  | 68 (81)  | 16 (19)                          | 84    |
| 10       | 0 (0)  | 0 (0)  | 1 (50)   | 1 (50)                           | 2     |
| 12       | 0 (0)  | 0 (0)  | 0 (0)  | 1 (100)                          | 1     |
| 14       | 11 (85)  | 0 (0)  | 1 (8)  | 1 (8)                            | 13    |
| 15       | 1 (100)  | 0 (0)  | 0 (0)  | 0 (0)                            | 1     |
| 16       | 0 (0)  | 0 (0)  | 1 (100)  | 0 (0)                            | 1     |
| 22       | 0 (0)  | 0 (0)  | 1 (20)   | 4 (80)                           | 5     |
| 25       | 0 (0)  | 0 (0)  | 0 (0)  | 4 (100)                          | 4     |
| NT       | 2 (5)  | 0 (0)  | 11 (30)  | 24 (65)                          | 37    |

MRP<sup>+</sup> = 136 kDa MRP; MRP<sup>s</sup> = lower molecular weight variant of MRP; MRP<sup>\*</sup> = higher molecular weight variant of MRP; EF<sup>+</sup> = 110 kDa EF; EF<sup>\*</sup> = higher molecular weight variant of EF

NT: Not typable isolates

## Chapter 2

Table 6

*S. suis* strains isolated from diseased pigs: serotypes, MRP/EF phenotypes<sup>a</sup> and site of isolation

| Serotype   | No. of strains with phenotype  |  |  |                                  | Total |
|--|--|--|--|----------------------------------|-------|
|  | MRP <sup>+</sup> EF <sup>+</sup><br>MRP <sup>s</sup> EF <sup>+</sup><br>MRP <sup>-</sup> EF <sup>+</sup> | MRP <sup>+</sup> EF <sup>*</sup><br>MRP <sup>s</sup> EF <sup>*</sup> | MRP <sup>*</sup> EF <sup>-</sup><br>MRP <sup>s</sup> EF <sup>-</sup> | MRP <sup>-</sup> EF <sup>-</sup> |       |
| <i>S. suis</i> strains isolated from brains, serosae, heart, joints or organs: |  |  |  |                                  |       |
| 1  | 32 (70)  | 2 (4)  | 3 (7)  | 9 (20)                           | 46    |
| 2  | 76 (79)  | 0 (0)  | 15 (16)  | 5 (5)                            | 96    |
| 1/2  | 3 (43)   | 3 (43)   | 1 (14)   | 0 (0)                            | 7     |
| 3  | 0 (0)  | 0 (0)  | 1 (100)  | 0 (0)                            | 1     |
| 4  | 0 (0)  | 0 (0)  | 0 (0)  | 1 (100)                          | 1     |
| 7  | 0 (0)  | 0 (0)  | 0 (0)  | 6 (100)                          | 6     |
| 8  | 0 (0)  | 0 (0)  | 0 (0)  | 1 (100)                          | 1     |
| 9  | 0 (0)  | 0 (0)  | 50 (83)  | 10 (16)                          | 60    |
| 10   | 0 (0)  | 0 (0)  | 0 (0)  | 1 (100)                          | 1     |
| 12   | 0 (0)  | 0 (0)  | 0 (0)  | 1 (100)                          | 1     |
| 14   | 11 (85)  | 0 (0)  | 1 (8)  | 1 (8)                            | 13    |
| 15   | 1 (100)  | 0 (0)  | 0 (0)  | 0 (0)                            | 1     |
| 22   | 0 (0)  | 0 (0)  | 1 (20)   | 4 (80)                           | 5     |
| 25   | 0 (0)  | 0 (0)  | 0 (0)  | 1 (100)                          | 1     |
| NT   | 2 (9)  | 0 (0)  | 6 (27)   | 14 (64)                          | 22    |
| <i>S. suis</i> strains isolated from lungs:                                    |  |  |  |                                  |       |
| 1  | 0 (0)  | 0 (0)  | 0 (0)  | 1 (100)                          | 1     |
| 2  | 9 (45)   | 0 (0)  | 11 (55)  | 0 (0)                            | 20    |
| 1/2  | 0 (0)  | 1 (13)   | 5 (63)   | 2 (25)                           | 8     |
| 3  | 0 (0)  | 0 (0)  | 3 (25)   | 9 (75)                           | 12    |
| 4  | 0 (0)  | 0 (0)  | 2 (40)   | 3 (60)                           | 5     |
| 5  | 0 (0)  | 0 (0)  | 1 (25)   | 3 (75)                           | 4     |
| 7  | 0 (0)  | 0 (0)  | 0 (0)  | 7 (100)                          | 7     |
| 8  | 0 (0)  | 0 (0)  | 1 (25)   | 5 (75)                           | 4     |
| 9  | 0 (0)  | 0 (0)  | 8 (89)   | 1 (11)                           | 9     |
| 10   | 0 (0)  | 0 (0)  | 1 (100)  | 0 (0)                            | 1     |
| 16   | 0 (0)  | 0 (0)  | 1 (100)  | 0 (0)                            | 1     |
| 25   | 0 (0)  | 0 (0)  | 0 (0)  | 3 (100)                          | 3     |
| NT   | 0 (0)  | 0 (0)  | 2 (20)   | 6 (80)                           | 10    |

MRP<sup>+</sup> = 136 kDa MRP; MRP<sup>s</sup> = lower molecular weight variant of MRP; MRP<sup>\*</sup> = higher molecular weight variant of MRP; EF<sup>+</sup> = 110 kDa EF; EF<sup>\*</sup> = higher molecular weight variant of EF

NT: Not typable isolates

### *3.5 Serotypes, phenotypes and sites of isolation*

*S. suis* serotype 2 strains which belonged to an EF-positive phenotype (either MRP<sup>+</sup>EF<sup>+</sup>, MRP<sup>s</sup>EF<sup>+</sup> or MRP<sup>-</sup>EF<sup>+</sup>) were frequently isolated from tissues typical for a *S. suis* infection (Table 6). The same results were obtained for serotype 1, 1/2 and 14 strains, isolated from tissues typically affected by *S. suis*. Most of these strains had a MRP<sup>+</sup>EF<sup>+</sup>, MRP<sup>s</sup>EF<sup>+</sup> or MRP<sup>-</sup>EF<sup>+</sup> phenotype, suggesting that also in these serotypes expression of EF seemed to be associated with virulence.

Different results were obtained for serotype 9 strains. Although serotype 9 strains were frequently isolated from tissues typically affected by *S. suis* none of these strains produced EF. More than 80% of the serotype 9 strains had a MRP<sup>\*</sup>EF<sup>-</sup> phenotype, irrespective their site of isolation.

None of the serotype 7 strains, neither isolated from tissues associated with *S. suis* infections nor isolated from lungs, produced EF. Therefore EF seemed not to be important in serotype 7 strains.

## **4. Discussion**

We previously showed that a high percentage of virulent *S. suis* serotype 2 strains produce MRP and EF (Vecht et al., 1991). In the present study we determined the serotypes and MRP/EF phenotypes of a considerable number of *S. suis* strains isolated from diseased pigs in seven European countries. It appeared that high percentages of *S. suis* serotype 1, 2, 1/2 and 14 strains isolated from typical *S. suis* tissues (brains, serosae, joints, heart) or parenchymatous organs (liver, kidney, spleen) of diseased pigs expressed EF, indicating that in these serotypes expression of EF (either with or without MRP) is possibly associated with virulence. In contrast, a high percentage of strains of serotypes 7 and 9, isolated from tissues typically affected by *S. suis* showed EF-negative phenotypes (either with or without MRP). This suggests that in serotype 7 and 9 strains expression of EF is not associated with virulence. Remarkably however, more than 80% of all *S. suis* serotype 9 strains produced a MRP<sup>\*</sup> protein (higher molecular weight variant of the 136-kDa MRP). This could suggest that in serotype 9 strains expression of MRP<sup>\*</sup> is associated with virulence. Whether MRP<sup>\*</sup>EF<sup>-</sup> and MRP<sup>-</sup>EF<sup>-</sup> strains of serotype 9 differ in virulence has to be determined in an experimental animal model. Among the 23 strains of serotype 7 investigated, only one produced an MRP<sup>\*</sup> protein. This suggests that expression of MRP in serotype 7 strains is not related to virulence.

No differences in phenotypes of *S. suis* serotype 2 were observed between strains isolated from the seven European countries, suggesting that in Europe the production of MRP and EF is associated with virulent strains of *S. suis* serotype 2. These results are in agreement with previous findings in Australia, Europe and the United States (Vecht et al., 1991; Mwaniki et al., 1994; Salasia and Lämmle 1995; Galina et al., 1996; Luque et al., 1999). In Canada, however, a correlation between

the production of MRP, EF and virulence of *S. suis* serotype 2 strains was not found (Gottschalk et al., 1998). None of the Canadian strains isolated from diseased pigs had an MRP<sup>+</sup>EF<sup>+</sup> phenotype.

In this study, serotype 2 appeared to be the most prevalent serotype within the strains collected. Moreover, not all serotypes seemed equally important in the various countries. Serotype 2 was the most frequently isolated serotype in France, Italy and Spain. Earlier, serotype 2 was shown to be the most frequently isolated serotype in Belgium, the Netherlands and Germany (Vecht et al., 1985; Hommez et al., 1986; Estoepangestie et al., 1993). However, here we have shown that the prevalence of serotype 9 increased in these countries during the last few years. In the Netherlands, an increase in the number of serotype 9 strains has also been earlier reported (Jacobs et al., 1995). Prior to these studies, *S. suis* serotype 9 was only shown to be a problem in Australia, where it was recovered in high percentages from outbreaks of septicaemia and meningitis in weaned pigs (Gogolewski et al., 1990).

Beside *S. suis* serotype 2 strains, serotype 1 and 14 strains were frequently isolated in the UK. In fact, all *S. suis* serotype 14 strains were isolated from pigs in the UK. Because a one-way capsular cross-reaction exists between *S. suis* serotypes 1 and 14 strains (Gottschalk et al., 1989) we assume that these strains are closely related. A close relationship between serotype 1 and 14 strains could be supported by our observation that all serotype 14 strains and almost 70% of the serotype 1 strains isolated in the UK had the MRP<sup>s</sup>EF<sup>+</sup> phenotype. Interestingly, three out of the four *S. suis* serotype 2 strains, which were isolated in the UK, had this characteristic MRP<sup>s</sup>EF<sup>+</sup> phenotype. This is suggestive for a clonal relationship between the serotype 1, 2 and 14 strains with the MRP<sup>s</sup>EF<sup>+</sup> phenotype. Interestingly, the age distribution of pigs affected by *S. suis* serotype 1 and 14 strains differed considerably. Serotype 1 strains were usually seen in 3-week-old pigs while serotype 14 strains were mostly isolated from 6.5-week-old pigs. Molecular fingerprinting experiments have to be performed to study the genetic relation between the serotype 1, 2 and 14 strains with the MRP<sup>s</sup>EF<sup>+</sup> phenotype further.

In this study, *S. suis* strains were isolated from pigs until 21 weeks of age. As reported previously, *S. suis* serotype 1 is mostly isolated from pigs at the age of 3 weeks while the strains of the other serotypes were mostly isolated from 7- to 8-week-old pigs (Reams et al., 1994).

In summary, *S. suis* serotype 2 was overall the most isolated serotype in Europe although the frequency of *S. suis* serotype 9 strains was emerging. In Belgium, Germany and the Netherlands serotype 9 was the most frequently isolated serotype. The production of EF seemed to be important in *S. suis* serotype 1, 2, 1/2 and 14 strains but not in serotype 7 and 9 strains. A high molecular weight variant of MRP may be important for serotype 9 strains since more than 80% of these strains produced this protein. Whether MRP<sup>\*</sup>EF<sup>-</sup> and MRP<sup>-</sup>EF<sup>-</sup> strains of serotype 9 differ in virulence has to be determined in an experimental animal model.

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## References

- Aarestrup, F.M., Jorsal, S.E., Jensen, N.E., 1998a. Serological characterization and antimicrobial susceptibility of *Streptococcus suis* isolates from diagnostic samples in Denmark during 1995 and 1996. *Vet. Microbiol.* 60, 59–66.
- Aarestrup, F.M., Rasmussen, S.R., Artursson, K., Jensen, N.E., 1998b. Trends in the resistance to antimicrobial agents of *Streptococcus suis* isolates from Denmark and Sweden. *Vet. Microbiol.* 63, 71–80.
- Awad-Masalmeh, M., Köfer, J., Schuh, M., Hinterdorfer, F., 1999. Serotypen, virulenzfaktoren und Empfindlichkeit gegenüber antibiotika von *Streptococcus suis*-stämmen isoliert aus klinisch gesunden und erkrankten schweinen in Österreich. *Wien. Tierärztl. Mschr.* 86, 262–269.
- Chengappa, M.M., Pace, L.W., Williams, J.A., Herren, C.H., Ascher, S.E., 1990. Efficacy of tiamulin against experimentally induced *Streptococcus suis* type 2 infection in swine. *J. Am. Vet. Med. Assoc.* 197, 1467–1470.
- Devriese, L.A., Ceysens, K., Hommez, J., Kilpper-Bälz, R., Schleifer, K.H., 1991. Characteristics of different *Streptococcus suis* ecovars and description of a simplified identification method. *Vet. Microbiol.* 26, 141–150.
- Estoeangestie, S., Lämmler, C.H., 1993. Distribution of capsular types 1 to 28 and further characteristics of *Streptococcus suis* isolates from various European countries. *Zbl. für Bakt.* 279, 394–403.
- Galina, L., Vecht, U., Wisselink, H.J., Pijoan, C., 1996. Prevalence of various phenotypes of *Streptococcus suis* isolated from swine in the U.S.A. based on the presence of muramidase-released protein and extracellular factor. *Can. J. Vet. Res.* 60, 72–74.
- Gogolewski, R.P., Cook, R.W., O'Connell, C.J., 1990. *Streptococcus suis* serotypes associated with disease in weaned pigs. *Aus. Vet. J.* 67, 202–204.
- Gottschalk, M., Higgins, R., Jacques, M., 1993. Production of capsular material by *Streptococcus suis* serotype 2 under different growth conditions. *Can. J. Vet. Res.* 57, 49–52.
- Gottschalk, M., Higgins, R., Jacques, M., Mittal, K.R., Henrichsen, J., 1989. Description of 14 new capsular types of *Streptococcus suis*. *J. of Clin. Microbiol.* 27, 2633–2636.
- Gottschalk, M., Higgins, R., Jacques, M., Beaudain, M., Henrichsen, J., 1991. Characterization of six new capsular types (23–28) of *Streptococcus suis*. *J. of Clin. Microbiol.* 29, 2590–2594.
- Gottschalk, M., Lebrun, A., Wisselink, H.J., Dubreuil, J.D., Smith, H.E., Vecht, U., 1998. Production of virulence-related proteins by Canadian strains of *Streptococcus suis* capsular type 2. *Can. J. Vet. Res.* 62, 75–79.
- Higgins, R., Gottschalk, M., Beaudoin, M., Rawluk, S.A., 1992. Distribution of *Streptococcus suis* capsular types in Quebec and western Canada. *Can. Vet. J.* 33, 27–30.
- Higgins, R., Gottschalk, M., Jacques, M., Beaudain, M., Henrichsen, J., 1995. Description of six new capsular types (29–34) of *Streptococcus suis*. *J. of Vet. Diagn. Invest.* 7, 405–406.
- Hoefling, D.C. 1998. Tracking the incidence of porcine respiratory diseases. *Vet. Med.*, 391–398.
- Hommez, J., Devriese, L.A., Henrichsen, J., Castryck, F., 1986. Identification of *Streptococcus suis*. *Vet. Microbiol.* 11, 349–355.

- Jacobs, A.A.C., Berg, A.J.G. van den, Baars, J.C., Nielsen, B., Johannsen, L.W., 1995. Production of suilysin, the thiol-activated haemolysin of *Streptococcus suis*, by field isolates from diseased pigs. *Vet. Rec.* 137, 295–296.
- Kataoka, Y., Sugimoto, C., Nakazawa, M., Morozumi, T., Kashiwazaki, M., 1993. The epidemiological studies of *Streptococcus suis* infections in Japan from 1987 to 1991. *J. of Vet. Med. Sc.* 55, 623–626.
- Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227, 680–685.
- Luque, I., Tarradas, C., Astorga, R., Perea, A., Wisselink, H.J., Vecht, U., 1999. The presence of muramidase-released protein and extracellular factor protein in various serotypes of *Streptococcus suis* isolated from diseased and healthy pigs in Spain. *Research in Veterinary Science* 1999, 66, 69–72.
- MacLennan, M., Foster, G., Dick, K., Smith, W.J., Nielsen, B., 1996. *Streptococcus suis* serotypes 7, 8 and 14 from diseased pigs in Scotland. *Vet. Rec.* 139, 423–424.
- Mwaniki, C.G., Robertson, I.D., Trott, D.J., Atyeo, R.F., Lee, B.J., Hampson, D.J., 1994. Clonal analysis and virulence of Australian isolated of *Streptococcus suis* type 2. *Epidemiol. Infect.* 113, 321–334.
- Perch, B., Pedersen, K.B., Henrichsen, J., 1983. Serology of capsulated streptococci pathogenic for pigs: six new serotypes of *Streptococcus suis*. *J. of Clin. Microbiol.* 17, 993–996.
- Reams, R.Y., Glickman, L.T., Harrington, D.D., Thacker, H.L., Bowersock, T.L., 1994. *Streptococcus suis* infection in swine: a retrospective study of 256 cases. Part II. Clinical signs, gross and microscopic lesions, and coexisting microorganisms. *J. Vet. Diagn. Invest.* 6, 326–334.
- Reams, R.Y., Harrington, D.D., Glickman, L.T., Thacker, H.L., Bowersock T.B., 1995. Fibrinohemorrhagic pneumonia in pigs naturally infected with *Streptococcus suis*. *J. Vet. Diagn. Invest.* 7, 406–408.
- Salasia, S.I.O., Lämmler, C., 1995. Distribution of serotype, virulence markers and further characteristics of *Streptococcus suis* isolates from pigs. *J. of Vet. Med. Series B* 42, 78–83.
- Sihovenen, L., Kurl, D.N., Henrichsen, J., 1988. *Streptococcus suis* isolated from pigs in Finland. *Acta Vet. Scand.* 29, 9–13.
- Smith, H.E., Vecht, U., Wisselink, H.J., Stockhofe-Zurwieden, N., Biermann, Y., Smits, M.A., 1996. Mutants of *Streptococcus suis* types 1 and 2 impaired in expression of muramidase-released protein and extracellular protein induce disease in newborn germfree pigs. *Infect. Immun.* 64, 4409–4412.
- Stockhofe-Zurwieden, N., Vecht, U., Wisselink, H.J., Lieshout, H. van, Smith, H.E., 1996. Comparative studies on the pathogenicity of different *Streptococcus suis* serotype 1 strains. *Proceedings of the 14th IPVS Congress. Bologna.* p. 299.
- Vecht, U., Leengod, L.A.M.G., Verheijen, E.R.M., 1985. *Streptococcus suis* infections in pigs in the Netherlands. *Vet. Q.* 7, 315–321.
- Vecht, U., Wisselink, H.J., Jellema, M.L., Smith, H.E., 1991. Identification of two proteins associated with virulence of *Streptococcus suis* type 2. *Infect. Immun.* 59, 3156–3162.
- Vecht, U., Wisselink, H.J., Dijk, J.E. van, Smith, H.E., 1992. Virulence of *Streptococcus suis* type 2 strains in newborn germfree pigs depends on phenotype. *Infect. Immun.* 60, 550–556.