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Virulence of *Streptococcus suis* Type 2 Strains in Newborn Germfree Pigs Depends on Phenotype

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To determine whether the virulence of *Streptococcus suis* type 2 is associated with the phenotype of the strain, we infected newborn germfree pigs with 10 strains of *S. suis* type 2 categorized by three phenotypes. In an earlier study, the phenotypes were distinguished by the presence or absence of the muramidase-released protein (MRP) and an extracellular factor (EF) and were designated MRP$^+$ EF$^+$, MRP$^+$ EF$^-$ and MRP$^-$ EF$^-$. Pigs were first inoculated with *Bordetella bronchiseptica* to predispose them to infection and were then intranasally inoculated with the streptococci. Strains of the MRP$^+$ EF$^+$ phenotype induced fever and increased the number of polymorphonuclear leukocytes in blood. Specific clinical signs of disease such as nervous disorders and lameness were also observed. At necropsy bacteriologic and pathologic examination disclosed meningoencephalitis, polyserositis, and polyarthritis. Strains of the MRP$^+$ EF$^-$ phenotype induced only nonspecific clinical signs of disease such as recumbency, lack of appetite, and fever; only slight pathologic changes were detected in the serosae. The four strains of the MRP$^-$ EF$^-$ phenotype induced no signs of disease. These findings indicate that the 110-kDa EF and, to a lesser degree, the 136-kDa MRP may be associated with the virulence of the bacterium. The results demonstrated that *S. suis* type 2 strains producing both MRP and EF are pathogenic for pigs.

Streptococcus suis type 2 is frequently isolated from pigs with meningitis, arthritis, septicemia, and bronchopneumonia (2, 5, 7, 8, 13, 18). The organism can also be isolated from the tonsils of healthy carrier pigs (3, 23). Occasionally *S. suis* causes meningitis with various complications in humans (1, 9). Two studies (4, 17) have indicated that strains of the species *S. suis* type 2 may differ in virulence for pigs. To differentiate various strains by virulence, virulence factors or markers must be identified. In an earlier study we identified two proteins that were presumably associated with virulence for pigs: the first was a 136-kDa cell wall-associated protein called muramidase-released protein (MRP), and the second was a 110-kDa extracellular protein provisionally designated extracellular factor (EF) (20). On the basis of the presence of MRP and EF, three phenotypes of *S. suis* type 2 were distinguished. Most isolates from various organs of diseased pigs belonged to the MRP$^+$ EF$^+$ phenotype, whereas most isolates from the tonsils of healthy pigs belonged to the MRP$^-$ EF$^-$ phenotype. Most isolates from human patients, however, belonged to the MRP$^+$ EF$^-$ phenotype. In the present study, we tested whether the virulence of *S. suis* type 2 strains is correlated with phenotype, as defined by the presence or absence of MRP and EF. The experimental model developed in an earlier study, in which pigs were predisposed to infection by inoculation with *Bordetella bronchiseptica* (17), was used in this study as well.

MATERIALS AND METHODS

Pigs. Fifty-two germfree pigs, cross-breeds of Great Yorkshire and Dutch Landrace, were obtained from four sows by caesarian sections performed in sterile flexible-film surgical isolators for two experiments. Sows in both experiments were full sisters. Pigs were allotted to 12 groups, each consisting of four or five pigs. Each group was housed in a sterile stainless steel incubator. Housing conditions and feeding regimens have been described earlier (17).

Inocula. Ten *S. suis* type 2 strains belonging to phenotype MRP$^+$ EF$^+$, MRP$^+$ EF$^-$, or MRP$^-$ EF$^-$ were obtained from three sources: from a pig with meningitis, from healthy pigs at slaughter, and from human patients (Table 1). The strains were biochemically and serologically typed as described earlier (18). Strains were stored as stock suspensions on glass beads in nutrient broth with 15% glycerol at −70°C. A 1-day-old colony of each strain, grown on Columbia blood agar base (code CM 331; Oxoid, Columbia, Md.) containing 6% horse blood, was incubated overnight at 37°C in Todd-Hewitt broth (code CM 189; Oxoid). To obtain early-stationary-growth-phase cultures, we diluted the overnight cultures in Todd-Hewitt broth (1:10) and incubated them at 37°C. Incubation was stopped after approximately 4 h, when a spectrophotometer measured the optical density at 600 nm as being 0.5. Cultures containing approximately 1×10^9 to 3×10^9 CFU/ml were then centrifuged at 4,000 × *g* for 15 min. The supernatant was analyzed for MRP and EF. The pellets were then washed and suspended at an A_{600} of 1 in a solution of phosphate-buffered saline (PBS) (136.89 mM NaCl, 2.68 mM KCl, 8.1 mM Na_2HPO_4, 2.79 mM KH_2PO_4, pH 7.2) and then used as an inoculum.

B. bronchiseptica 92932, isolated from the nose of a pig with atrophic rhinitis, was used to predispose pigs to *S. suis* infection (10, 17). The strain was kept on Dorset egg medium. The inoculum was prepared by culturing a 48-h-old colony from sheep blood agar in brain heart infusion broth. After 18 h of incubation at 37°C, this medium contained approximately 10^9 CFU/ml. The brain heart infusion broth was diluted (1:100) in PBS to prepare the inoculum.

Electrophoresis and Western blot. The MRP and EF phenotypes of the *S. suis* strains used as inocula and of the isolates recovered at the end of the experiments were determined. Sodium dodecyl sulfate-polyacrylamide gel

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TABLE 1. Experimental design

S. suis strain	*S. suis* phenotype	Source[a] of *S. suis* isolation	Dosage (CFU [10^6]) of *S. suis* inoculation	No. of pigs inoculated
3	MRP^+ EF^+	Meninges, pig	1.84	5
3	MRP^+ EF^+	Meninges, pig	1.96	4
10	MRP^+ EF^+	Tonsil, pig	1.52	5
22	MRP^+ EF^+	Human	2.93	4
17	MRP^+ EF^-	Tonsil, pig	1.26	4
24	MRP^+ EF^-	Human	1.22	4
28	MRP^+ EF^-	Human	1.23	4
12	MRP^- EF^-	Tonsil, pig	1.05	5
12	MRP^- EF^-	Tonsil, pig	0.98	4
16	MRP^- EF^-	Tonsil, pig	0.70	4
18	MRP^- EF^-	Tonsil, pig	1.10	4
25	MRP^- EF^-	Human	0.97	4

[a] Strain 3 was isolated from a pig with meningitis during routine diagnostic procedures. Strains 10, 12, 16, and 18 were isolated at slaughter from the tonsils of healthy pigs. Strains 22 (830544), 24 (740113), 25 (821021) and 28 (760366) were isolated from human patients with *S. suis* type 2 meningitis (Numbers in parentheses refer to those of Arends and Zanen [1]).

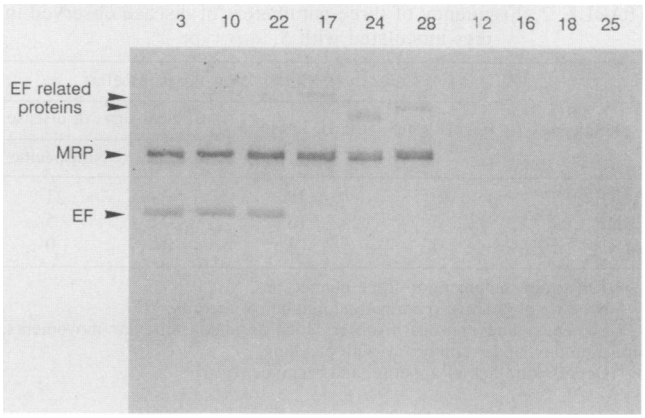

FIG. 1. Western blot of the cell culture supernatant of the 10 *S. suis* type 2 strains probed with a 1:1 mixture of mouse anti-MRP and anti-EF MAb. The blot shows three phenotypes of *S. suis* type 2: MRP^+ EF^+ (strains 3, 10, and 22), MRP^+ EF^- (strains 17, 24, and 28), and MRP^- EF^- (strains 12, 16, 18, and 25). Lane numbers correspond to strain numbers. The MAbs recognize the 136-kDa MRP, 110-kDa EF of strains 3, 10, and 22, and the high-molecular-mass proteins (>150 kDa) of strains 17, 24, and 28.

electrophoresis (SDS-PAGE) as described by Laemmli (11) and Western immunoblotting were used to analyze cell culture supernatants of isolates recovered from nasopharynx of all pigs and from inflamed tissues such as meninges or joints of affected pigs. The separating gels contained 6% polyacrylamide. After electrophoresis, the proteins were stained with silver (12). For Western blot analysis, the proteins were electroblotted onto nitrocellulose by the Multiphor II Nova Blot system, according to the recommendations of the manufacturer (Pharmacia LKB, Uppsala, Sweden). Nitrocellulose filters were incubated either with a 1:1 mixture of mouse anti-MRP monoclonal antibodies (MAb) (11.3 mg/ml) and anti-EF MAb (8.4 mg/ml), each in a 1:200 dilution, or with a 1:500 dilution of polyclonal anti-MRP-EF rabbit serum (K191) (8.2 mg/ml) (19, 20). Filters were incubated with a 1:1,000 dilution of anti-mouse immunoglobulins conjugated with alkaline phosphatase or a 1:3,000 dilution of alkaline phosphatase-conjugated anti-rabbit immunoglobulin G(γ + κ) (Zymed Laboratories, Inc., San Francisco, Calif.). Bound antibodies were visualized by adding the substrate bromochloroindolyl phosphate (Sigma, St. Louis, Mo.)-Nitro Blue Tetrazolium (Merck, Darmstadt, Germany) in phosphatase buffer (100 mM NaCl, 5 mM $MgCl_2$, 100 mM diethanolamine [pH 9.5]).

Experimental design. The study consisted of two experiments with an interval of 5 months. Five-day-old germfree pigs were inoculated intranasally with a plastic disposable syringe filled with a suspension of *B. bronchiseptica* 92932 in brain heart infusion broth. The inocula contained 0.84×10^7 CFU in experiment 1 and 1.0×10^7 CFU in experiment 2. Two days postinoculation (p.i.) the pigs were similarly inoculated inside the sterile incubator with one of the 10 *S. suis* type 2 strains (Table 1). The mean (± standard deviation) inoculum size of these strains was 1.4 (± 0.60) $\times 10^6$ CFU. All inoculations consisted of a 0.5-ml bacterial suspension in each nostril during the inspiratory phase of breathing. In both experiments strain 3 (MRP^+ EF^+) was used as positive control and strain 12 (MRP^- EF^-) was used as negative control (see Results). Pigs were killed either when they became mortally ill or at the end of the experiment (3 to 4 weeks p.i.), and they were subsequently necropsied. Pigs were killed inside the incubators by intravenous administration of pentobarbital.

Disease monitoring. Pigs were monitored daily for clinical signs of disease, such as fever, dysfunction of the central nervous system (CNS) and lameness. Blood samples from each pig were collected three times weekly by venipuncture of the cranial vena cava. Leukocytes were counted with a conducting counter (Contraves A.G., Zurich, Switzerland) (6). The number of neutrophils was calculated after differential count of Giemsa-stained blood smears. To monitor infection with *S. suis* and *B. bronchiseptica* and to assess whether pigs were bacteriologically contaminated inside the incubators, we collected swab specimens of nasopharynx and feces daily and plated these directly onto Columbia agar containing 6% horse blood. The presence of *S. suis* type 2 and of *B. bronchiseptica* was confirmed by slide agglutination test in which a suspension of the monocultures was mixed with the appropriate hyperimmune rabbit serum (DLO-Central Veterinary Institute, Lelystad, The Netherlands). After the pigs were killed, they were examined for pathologic changes. Tissue specimens of the CNS, serosae, liver, spleen, and tonsils were bacteriologically and histologically examined as described before (17).

RESULTS

Electrophoresis and Western blot. When immunoblots were used to analyze culture supernatants of the *S. suis* strains before inoculation, three phenotypes were distinguished. Strains 3, 10, and 22 belonged to the MRP^+ EF^+ phenotype, strains 17, 24, and 28 belonged to the MRP^+ EF^- phenotype, and strains 12, 16, 18, and 25 belonged to the MRP^- EF^- phenotype (Fig. 1). The rabbit polyclonal antibodies recognized proteins that were greater than 150 kDa in the culture supernatants of the MRP^+ EF^- strains. These high-molecular-mass proteins were also detected by the anti-EF MAb, indicating that the 110-kDa EF and the >150-kDa proteins share epitopes (results not shown). In both the SDS-PAGE and Western blot, the phenotypes of the *S. suis* strains used as inocula were identical to the phenotypes of the isolates collected at the end of both experiments from tonsils and inflamed tissues of infected pigs.

Clinical signs of disease. In both experiments, rectal tem-

TABLE 2. Frequency of three parameters of disease observed in pigs inoculated with *S. suis* type 2[a]

S. suis phenotype	Frequency[b] (%) of following parameter:			
	Fever (temp >40°C)	PML in blood (>10^{10}/liter)	Clinical signs of disease	
			Specific[c]	Nonspecific[d]
MRP$^+$ EF$^+$	40	78	57	21
MRP$^+$ EF$^-$	5	16	0	5
MRP$^-$ EF$^-$	0	3	0	0

[a] Ten strains belonging to three phenotypes.
[b] Number of positive records/total number of records.
[c] Lameness and nervous disorders such as ataxia, circular movements, opisthotonus, and recumbency with paddling.
[d] Depression, lack of appetite, and recumbency.

peratures of all pigs inoculated with strains of the MRP$^+$ EF$^+$ phenotype (strains 3, 10, and 22) increased from day 2 p.i. onwards, with temperatures peaking at 41.8°C between days 4 and 9. Rectal temperatures of 10 pigs that were inoculated with strains of the MRP$^+$ EF$^-$ phenotype (strains 17, 24, and 28) were higher than 40°C for short periods of 24 to 96 h between days 2 and 22. Frequency of fever (number of days when pigs showed fever/total number of days) was higher in the MRP$^+$ EF$^+$ group (40%) than in the MRP$^+$ EF$^-$ group (5%) (Table 2). Temperatures of pigs inoculated with strains of the MRP$^-$ EF$^-$ phenotype (strains 12, 16, 18, and 25) never exceeded 40°C.

The frequency of increased polymorphonuclear leukocytes (PML) in blood (number of blood samples with PML > 10×10^9 per liter/total number samples) was higher in the MRP$^+$ EF$^+$ group (78%) than in either the MRP$^+$ EF$^-$ group (16%) or the MRP$^-$ EF$^-$ group (3%) (Table 2). An analysis of variance was performed on the log of PML counts in blood samples of pigs inoculated with strains of the three phenotypes. Three days before inoculations no significant differences were found between the geometric mean PML counts of the three groups (Fig. 2). From day one p.i. onwards, the mean numbers of PML in blood samples of pigs inoculated with strains of the MRP$^+$ EF$^+$ phenotype were significantly higher ($P < 0.01$), than in either the MRP$^+$ EF$^-$ group or the MRP$^-$ EF$^-$ group. On day 20 p.i., the means in the MRP$^+$ EF$^+$ and MRP$^+$ EF$^-$ groups did not differ significantly from each other, but those means differed significantly ($P < 0.01$) from the means in the MRP$^-$ EF$^-$ group.

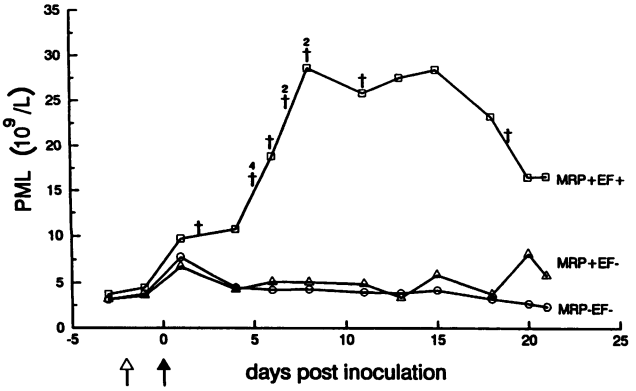

FIG. 2. Geometric mean PML counts (10^9/liter) after inverse transformation in three groups of pigs after inoculation with *S. suis* type 2. Groups consisted of pigs inoculated with one of the three phenotypes: curve MRP$^+$ EF$^+$ shows the mean counts of pigs inoculated with strains 3, 10, and 22; curve MRP$^+$ EF$^-$ shows the mean counts of pigs inoculated with strains 17, 24, and 28; curve MRP$^-$ EF$^-$ shows the mean counts of pigs inoculated with strains 12, 16, 18, and 25. †, death of a pig (if more than one, the appropriate number is given above this mark); ⇧, inoculation with *B. bronchiseptica*; ↑, inoculation with *S. suis* type 2.

Morbidity in pigs inoculated with strains of the MRP$^+$ EF$^+$ phenotype was 100%. From day 2 onwards, we observed nonspecific signs of systemic illness, such as depression, recumbency, lack of appetite, and fever. During the following days, pigs showed more specific signs of disease, such as ataxia, circular movements, opisthotonus, recumbency with paddling, and lameness. The frequency of specific signs of disease (number of days when pigs showed symptoms/total number of days) in the MRP$^+$ EF$^+$ group was 57% (Table 2). Nine pigs died in the course of the experiment, and three were killed in the terminal stages of disease. The mortality rate in groups inoculated with strains 3, 10, and 22 was thus 12 of 18 (67%). Nine pigs that were inoculated with strains of the MRP$^+$ EF$^-$ phenotype (strains 17, 24, and 28) developed fever or granulocytosis or showed other nonspecific signs of disease but did not show specific clinical signs, such as nervous disorders or lameness. The frequency of nonspecific signs in the MRP$^+$ EF$^-$ group was 5% (Table 2). Pigs in the MRP$^-$ EF$^-$ group did not develop clinical signs of disease.

Pathologic findings. Severe and frequent inflammations of the CNS, serosa, and joints were only detected in pigs inoculated with strains of the MRP$^+$ EF$^+$ phenotype (Table 3). Signs of inflammation in the CNS were diffuse infiltration of mononuclear cells in the pia mater around the cerebrum, cerebellum, pons, mesencephalon, and medulla oblongata, sometimes with an effusion of fibrin and neutrophils in the subarachnoid space. Glia cell granulomas were observed in the cerebellar and cerebral cortex (Fig. 3). The choroid plexus contained mixed populations of mononuclear cells and neutrophils. Five pigs showed malacia in the white matter of the cerebellum, with leukocytic infiltration adjacent to the meninges (Fig. 4). Some areas also showed mononuclear cuffs around small blood vessels. Signs of inflammation of the serosae and joints were severe fibrinous pleuritis, peritonitis, serofibrinous pericarditis, and fibrinopurulent arthritis. We observed effusions of neutrophils and fibrin and proliferations of fibro(angio)blasts and fibrous tissue between the epicard and pericard; mononuclear cells were detected in the subepicardial areas. Two pigs inoculated with strain 10 showed signs of disseminated intravascular coagulation; one had thrombi in the coronary venules, and the other had hyalin membranes on the alveolar epithelium of the lungs.

Mild local chronic peritonitis, pleuritis, or pericarditis was observed in eight pigs inoculated with strains of the MRP$^+$ EF$^-$ phenotype (Table 3). Lesions of the serosae were minor compared with those of pigs inoculated with strains of the MRP$^+$ EF$^+$ phenotype. In the cerebellum of one pig inoculated with strain 24, we observed cuffs of mononuclear cells around small vessels and bleeding in the meninges, both signs of meningoencephalitis.

Pneumonia and bronchitis were observed in various forms (Table 3). Interstitial pneumonia with mononuclear infiltration around the septa and blood vessels and acute catarrhal bronchitis and peribronchi(oli)tis and bronchopneumonia with desquamated alveolar and bronchial epithelial cells were detected in the lungs of pigs inoculated with strains of

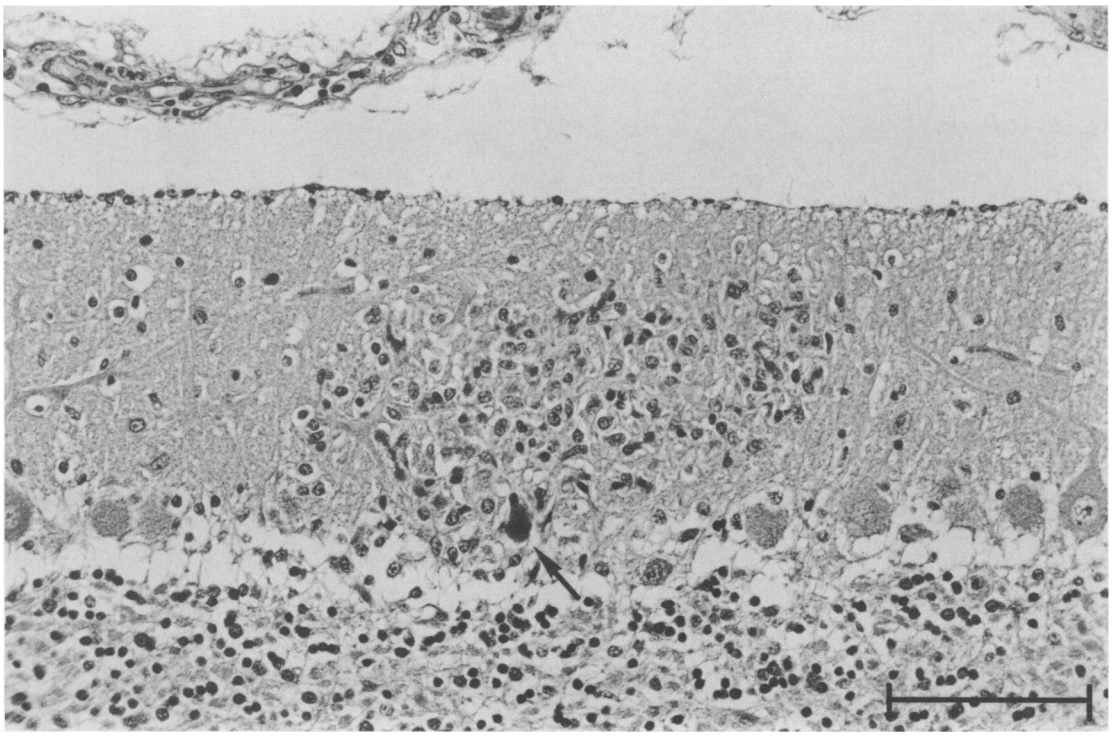

FIG. 3. Response of a pig 21 days after inoculation with *S. suis* type 2, strain 3 (MRP^+ EF^+). Glia cell granuloma with a pycnotic Purkinje cell (arrow) in the cerebellar cortex. Hematoxylin and eosin stain. Bar, 100 μm. Magnification, ×200.

all phenotypes. Acute fibrinous pneumonia was observed in the lungs of only three pigs, which had been inoculated with strains of the MRP^+ EF^+ phenotype.

Pigs inoculated with strains of the MRP^- EF^- phenotype had virtually no pathologic changes except pneumonic lesions (strains 12 and 16) (Table 3). One pig inoculated with strain 16 had chronic local epicarditis. Meningitis, polyserositis, and arthritis were not observed in these groups.

Follicle formation in B-cell areas and blast cell formation in T-cell areas of the white pulp of the spleen—signs of active immune response—were more frequently observed in pigs inoculated with strains of the MRP^+ EF^- phenotype (6 of 12 [50%]) than in pigs inoculated with strains of the MRP^- EF^- phenotype (5 of 22 [22%]) or strains of the MRP^+ EF^+ phenotype (2 of 18 [11%]) (Table 3). Some pigs inoculated with strains of the MRP^+ EF^+ phenotype showed lymphocytolysis in the germinal centers, while the marginal zone surrounding the white pulp was inflamed, signs of acute septicemia in young animals (16). Active follicles in tonsils were also more often seen in pigs inoculated with strains of the MRP^+ EF^- or MRP^- EF^- phenotype.

Bacteriologic findings. From day 1 p.i. till the end of the experiment, the streptococcal strains and *B. bronchiseptica* were isolated daily from nasopharyngeal and fecal swab specimens of all pigs. A *Bacillus* species was also isolated from day 6 p.i. onwards from swab specimens collected from pigs inoculated with strain 16 (experiment 1) and from day 19 p.i. onwards from pigs inoculated with strain 24 (experiment 2). Pigs in the other groups remained free from contaminating bacteria. The *Bacillus* species was not isolated from any of the inflamed tissues at necropsy. Apparently this bacterium did not interfere with the course of the streptococcal infections.

At necropsy, *S. suis* type 2 was mostly isolated from organs and tissues (CNS, serosae, and joints) that also showed pathologic changes. The streptococci were more often isolated from organs and tissues of pigs inoculated with strains of the MRP^+ EF^+ phenotype and less frequently from pigs inoculated with strains of the MRP^+ EF^- phenotype (Table 4). Twice *S. suis* was isolated from the joints of pigs inoculated with strains of the MRP^+ EF^- phenotype. *B. bronchiseptica* was only isolated from lungs and tonsils. Both *S. suis* and *B. bronchiseptica* were isolated from the tonsils of all pigs.

DISCUSSION

As only strains with the MRP^+ EF^+ phenotype induced specific clinical and pathological signs of disease and markedly increased PML numbers in blood, the present study demonstrated that pathogenic properties of *S. suis* type 2 for pigs are correlated with a MRP^+ EF^+ phenotype. Remarkably, strain 10, isolated from the tonsil of a healthy carrier pig, induced severe pathological signs of disease, whereas strain 25, isolated from a pig farmer who had meningitis, induced no pathological signs at all. Apparently the phenotype but not the source of isolation is related to the pathogenic properties of the isolate.

Pigs inoculated with strains of the MRP^+ EF^- phenotype developed mild clinical signs of general disease but never developed specific signs, such as nervous signs or lameness. One pig showed some histopathologic signs of encephalitis, but tissue changes of the other pigs inoculated with MRP^+ EF^- strains were slight. Pigs inoculated with strains of the MRP^- EF^- phenotype showed no clinical or pathological signs of disease whatsoever (except for one pig with local

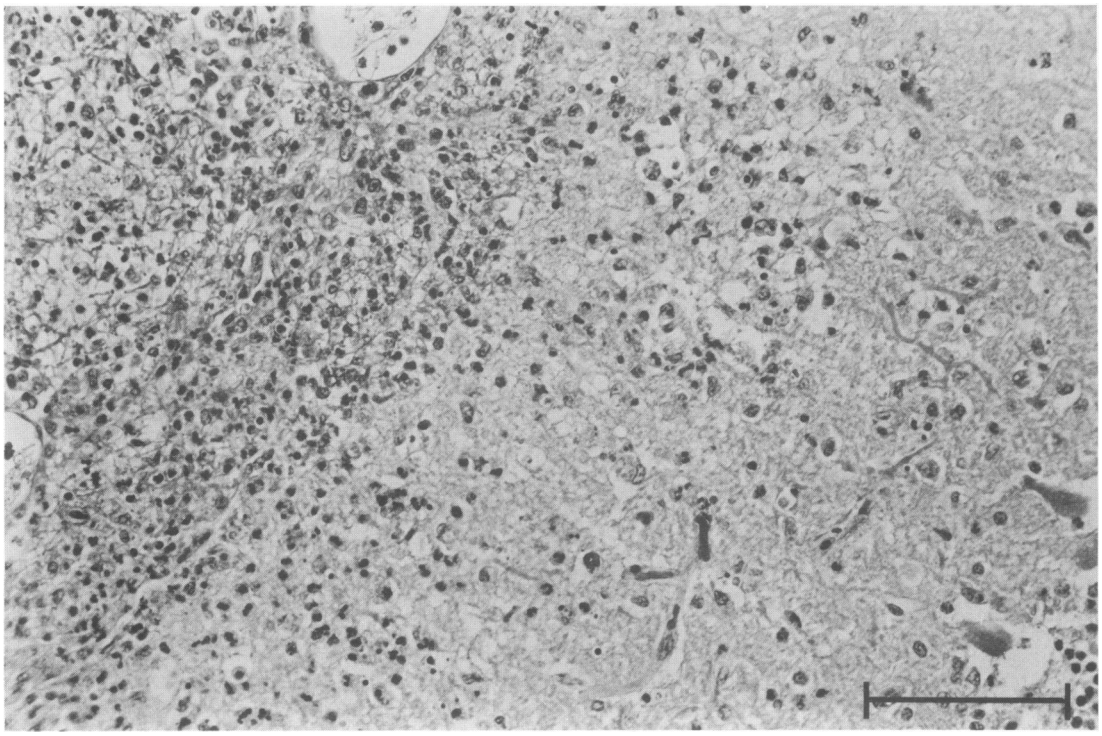

FIG. 4. Response of a pig 14 days after inoculation with *S. suis* type 2, strain 10 (MRP^+ EF^+). Exudative form of meningoencephalitis with neutrophil infiltration and malacia with karyorhexis and cavitation in the cerebellar cortex. Hematoxylin and eosin stain. Bar, 100 μm. Magnification, ×200.

epicarditis, possibly caused by needle injury during blood sampling).

Although a *Bacillus* species contaminated some pigs in the present study, no clinical or hematological signs were detected in these pigs, and the *Bacillus* species could not be isolated at necropsy from any of the tissues other than tonsil tissues. We therefore regard this contamination as insignificant.

Because MRP^+ EF^+ strains appeared more virulent for pigs than MRP^+ EF^- strains, EF is probably a more important virulence marker for pigs than MRP. The potential role of MRP as a virulence factor is mainly based on the finding that MRP^+ EF^- strains isolated from human patients were isolated at a high frequency (74%) (20). Strains of *S. suis* type 2 belonging to the MRP^- EF^+ phenotype have not been isolated. Therefore, the possibility cannot be excluded that both MRP and EF contribute to pathogenicity of *S. suis*. To prove that MRP and EF are virulence factors, isogenic strains that merely vary in the production of these proteins are needed. Until such strains are tested in vivo, MRP and EF should be regarded as markers of virulence only.

The ependyma is no real barrier to infection (15). Williams and Blakemore suggested that *S. suis* type 2 enters the CNS via the choroid plexus and speculated that the bacteria may be carried within mononuclear phagocytes to the cerebrospinal fluid compartment, joint spaces, and serosal cavities (21). Distribution of lesions within the compartment may be the result of passive accumulation of inflammatory cells caused by the flow of spinal fluid (22). In our study, mononuclear cells located beneath the surface were observed in several tissues of the CNS, serosae, and joint capsules of pigs inoculated with MRP^+ EF^+ strains, while an exudation of fibrin and granulocytes was detected at the surface of the tissues. These findings are more characteristic for an active hematogenous spread to the leptomeninges, serosae, and joints. The signs of disseminated intravascular coagulation seen in two pigs may be associated with septic shock, which has been described in human patients infected with *S. suis* (1, 9).

Our study indicates that for causing streptococci bronchopneumonia, are secondary to other microorganisms, such as *B. bronchiseptica*, since these were more often isolated from the lungs than were *S. suis* (Table 4). Virulent and nonvirulent *S. suis* strains induce pneumonic lesions alike, but probably only when other microorganisms are also present.

Activated white pulp in the spleen as a sign of immune response was most often (50%) detected in pigs inoculated with strains of the MRP^+ EF^- phenotype. Only 11% of the pigs inoculated with strains of the MRP^+ EF^+ phenotype had these signs; lymphocytolysis and early death apparently precluded the development of such a response.

In the culture supernatant of the MRP^+ EF^- strains, our anti-EF MAb recognized protein bands with lower electrophoretic mobility (molecular mass, >150 kDa) than EF (110 kDa) (20). These proteins were not recognized in culture supernatants of MRP^+ EF^+ and MRP^- EF^- strains. Preliminary studies show that DNA probes containing the gene that encodes EF hybridize with the DNA encoding this higher molecular mass protein (14). This finding confirms that both proteins are indeed related. In humans, the portals of entry of *S. suis* type 2 are small infected wounds (1); in pigs they are the mucosa of the nasopharynx and the palatine tonsils (3, 23). Therefore, the early pathogenesis of *S. suis* infection in pigs and humans may differ. The lower-molecular-mass (110-kDa) form of EF may be involved in the invasion of

TABLE 3. Pathologic lesions detected in various tissues of pigs inoculated with *S. suis* type 2[a]

Tissue and pathologic lesions	No. of pigs with pathologic lesions from following phenotype:		
	MRP$^+$ EF$^+$ (no. tested = 18)	MRP$^+$ EF$^-$ (no. tested = 12)	MRP$^-$ EF$^-$ (no. tested = 22)
CNS			
Meningitis[b]	12	0	0
Encephalitis[b]	10	1	0
Choroiditis	7	0	0
Malacia	5	0	0
Serosae or joints			
Peri- and epicarditis	11	1	1
Pleuritis	5	1	0
Peritonitis	14	6	0
Polyarthritis[c]	15	0	0
Lungs			
Catarrhal bronchopneumonia	1	1	1
Fibrinous pneumonia	3	0	0
Interstitial pneumonia	7	5	5
Bronchitis and peribronchiolitis	2	2	3
Liver, periportal and/or intralobular foci	11	8	3
Spleen			
Active white pulp	2	6	5
Active red pulp	4	0	2
Tonsil			
Active follicles	3	9	12
Exudation in crypts	1	5	6

[a] Ten strains belonging to three phenotypes.
[b] Affecting cerebrum, cerebellum, pons, mesencephalon, and medulla oblongata in various combinations.
[c] Affecting carpal, metacarpal, tarsal, metarsal, knee, elbow, shoulder, and hip joints in various combinations.

streptococci through the nasopharyngeal mucosa of pigs. This would explain why in our study MRP$^+$ EF$^-$ strains were only mildly virulent for pigs. It would also explain why such strains were isolated in a high rate from human patients (20). Further studies on the roles of MRP and EF in its lower and higher molecular forms in the pathogenesis of *S. suis* infections in pigs and humans are warranted.

TABLE 4. Isolation of streptococci from various tissues of pigs inoculated with *S. suis* type 2[a]

Tissue	No. of pigs from which *S. suis*[b] was isolated at necropsy from following phenotype:		
	MRP$^+$ EF$^+$ (no. tested = 18)	MRP$^+$ EF$^-$ (no. tested = 12)	MRP$^-$ EF$^-$ (no. tested = 22)
CNS	14	0	0
Serosae	9	2	0
Joints	13	2	0
Lungs	6 (9)	0 (2)	2 (8)

[a] Ten strains belonging to three phenotypes.
[b] Numbers in parentheses indicate number of pigs from which *B. bronchiseptica* was also isolated.

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