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PAPER 162

A REPRODUCIBLE *STREPTOCOCCUS SUIS* INFECTION MODEL USING ACETIC ACID AS PREDISPOSER

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Introduction and Objectives

Streptococcus suis serotype 2 is an important swine pathogen associated with cases of meningitis, arthritis, septicaemia and sudden death. The virulence of *S. suis* serotype 2 varies in between strains, and several potential virulence factors have been proposed by different researchers (3). Virulence testing of isolates of *S. suis* requires a reproducible challenge model. Challenge studies in mice and pigs have shown, that the virulence of *S. suis* is different in the two species (6). The virulence of *S. suis* is also dependent on the challenge route (1). In the present studies we worked with *S. suis* serotype 2 in an aerosol infection model. The non-infectious agent acetic acid was tested as a predisposer for the infection. Intranasal (IN) instillation of 1% acidic acid has previously been used to predipose for colonization of toxigenic *Pasteurella multocida* (5). The experiments were carried out in conventionally reared pigs from the Danish SPF-system.

Material and Methods

Groups of 4 pigs were inoculated with an aerosol of *S. suis*. One group had received 1% acetic acid IN as a predisposer, and the other group got no predisposer. *S. suis* serotype 2 strain P1/7 from Dr. Tom Alexander, Cambridge, UK was used. This strain is known to be virulent in an intravenous challenge model (4). In addition, a challenge experiment was made comparing strain P1/7 with strain 14958 from Danske Slagterier, since these two strains in our hands had been equally virulent in the IV challenge model.

The experimental animals were SPF-pigs from a commercial herd with no history of *S. suis* infection. The pigs were 8 w old at the time of challenge in experiment A, C and D and 6 w old when they were challenged in experiment B. The pigs were kept groupwise in pens.

Challenge was done by a culture grown at 37°C for 5 hours in Todd-Hewitt (TH) broth with 1% cystein. The culture was centrifuged and resuspended in cold broth to OD_{600} of 2.5 (experiment A+B) or 4-4.5 (experiments C+D). Before challenge the pigs were given 5 ml of 1% acetic acid in PBS (pH 3.4) IN while they were held in an upright position. The pigs were then left for 1 h before they were challenged with an aerosol of *S. suis*. The aerosol was made with a DeVilbiss nebulizer model Ultra-NEB 99. The nebulizer uses ultrasound to create an aerosol with a particle size of approximately 5 µm. Challenge was done over a period of 20 minutes in a tight box of 1x2x1m with controlled air in- and outlet. The nebulizer was connected to the inlet and sterile filter was mounted at the air outlet..

After challenge the pigs were attended at least twice a day. Clinical symptoms were recorded and seriously ill animals were euthanized. 14 days after challenge the experiment was stopped and remaining animals were euthanized. All animals were subjected to post mortem examination, where macroscopical lesions were recorded. At post mortem examination samples were taken for bacteriological examinations to reisolate *S. suis*. Samples were seeded on TH agar, and *S. suis* suspect colonies were reseeded before final identification.

Results and Discussion

In the pigs that died or were euthanized during the observation period the clinical and pathological findings corresponded to infection with *S. suis*, and the organism was reisolated from many seedings. The pigs that survived the challenge showed only minor clinical symptoms, and *S. suis* could not be reisolated from these pigs.

Table 1: Survey of design and results obtained after aerosol infection of 6-8 w old piglets with *S. suis* serotype 2.

Exp	OD_{600} of culture	*S. suis* strain	Acetic acid	No. of pigs	No. of pigs surviving
A	2.5	P 1/7	+	4	0
	2.5	P 1/7	-	4	0
B	2.5	P 1/7	+	4	0
	2.5	P 1/7	-	4	2
C	4.0	P 1/7	+	4	1
	4.0	P 1/7	-	4	3
D	4.5	P 1/7	+	4	0
	4.0	14958	+	4	3

Different infections such as *Bordetella bronchiseptica* (6) and PRRS (2) have been shown to predispose pigs to experimental *S. suis* infections. To studies with focus on *S. suis* a non-infectious predisposer is preferable to avoid confusion coming from findings from other diseases. The experiments presented above showed that it was possible to induce clinical disease with high mortality after aerosol exposure of pigs to *S. suis* serotype 2 even without using a predisposer. However, the morbidity was only 7 out of 12 (58%). IN instillation of acetic acid used as a predisposer improved the challenge model considerably, since the morbidity increased to 11 out of 12 (92%). Strain 14958 was less virulent than P1/7 in this model, even though they were equally virulent when IV challenge was used. This finding suggests that the aerosol model reveals differences in virulence not seen in the intravenous challenge model. Still, the number of animals in experiment D is too low to draw final conclusions regarding differences in virulence.

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