Elimination of strains of *Streptococcus suis* serotype 2 from the tonsils of carrier sows by combined medication and vaccination

B. SWILDENS, M. NIELEN, H. J. WISSELINK, J. H. M. VERHEIJDEN, J. A. STEGEMAN

The effect of vaccination with a killed whole-cell vaccine of extracellular factor-positive *Streptococcus suis* serotype 2 (*S suis* 2 EF$^+$) combined with medication with amoxicillin on the presence of virulent *S suis* 2 EF$^+$ strains on the tonsils of sows and their offspring was evaluated. In two herds, 14 pregnant sows that carried these virulent strains, as detected by PCR in three consecutive tonsillar brush samples, were selected and randomly assigned to be treated or left untreated as controls. The treated sows were vaccinated at six and three weeks before the expected farrowing date and medicated from one week before expected farrowing until the end of the experiment. Two weeks before parturition, the sows were housed in individual isolation farrowing rooms, and the sow and its litter were sampled by using tonsil brushes and tonsil swabs, respectively. Approximately 27 days postpartum, the sows and their piglets were euthanased and their tonsils were collected and analysed by PCR. No *S suis* 2 EF$^+$ could be detected in the tonsils of the seven treated sows, but the tonsils of the seven untreated sows remained positive. Only one of the litters of the untreated sows became infected, five days after birth, and none of the litters of the treated sows became infected.

Streptococcus suis has been implicated in the aetiology of meningitis, arthritis, pneumonia, septicaemia, endocarditis, polyserositis and sudden death in pigs. The infection is a zoonosis that has repeatedly been reported in pig-rearing and pork-consuming countries (Arends and Zanen 1988, World Health Organization [WHO] 2005). The virulent extracellular factor-positive strains of *S suis* serotype 2 (*S suis* 2 EF$^+$) have frequently been isolated from diseased pigs in many European countries, the USA and Australia (Mwaniki and others 1994, Salasia and Lammler 1995, Galina and others 1996, Wisselink and others 2000). Sows carrying *S suis* strains in their tonsils are considered the most important source of infection for their susceptible offspring (Clifton-Hadley 1984, Robertson and Blackmore 1989), which can become infected very early in their lives with different *S suis* serotypes, including virulent strains (Robertson and others 1991, Amass and others 1996a, Torremorell and others 1998, Cloutier and others 2003). Carrier sows can be detected by using a PCR for the detection of *S suis* 2 EF$^+$ strains in tonsillar swabs (Swildens and others 2005).

Several intervention studies have been carried out to try to reduce the clinical signs associated with *S suis* strains and the costs of control. For example, the vaccination of pregnant sows with a *S suis* serotype 14 bacterin resulted in central nervous signs in significantly fewer of their 13-day-old piglets after an experimental intravenous challenge with a homologous strain than in the control group (Amass and others 2000), and the vaccination of pregnant sows with a commercial serotype 2 vaccine significantly reduced the percentage of carriers of *S suis* serotype 2 in their one- to 15-day-old offspring (Torremorell and others 1998). However, the medication of early-weaned piglets was not successful in raising piglets free of *S suis* (Amass and others 1996b), and Clifton-Hadley and others (1984) reported failures to eliminate *S suis* from herds of slaughter pigs by different antibiotic regimens. However, to the best of the authors' knowledge, the effect of a combination of medication and vaccination of sows in order to protect their offspring from carrying *S suis* has not been investigated.

The aim of this experiment was to examine the effect of a combination of vaccination and medication of carrier sows on the *S suis* 2 EF$^+$ carrier status of the sows and their offspring.

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B. Swildens, DVM,
M. Nielen, DVM, PhD,
J. H. M. Verheijden, DVM, PhD,
J. A. Stegeman, DVM, PhD, Department of Farm Animal Health, Faculty of Veterinary Medicine, Utrecht University, PO Box 80151, 3508 TD Utrecht, The Netherlands
H. J. Wisselink, PhD, Division of Infectious Diseases, Animal Sciences Group, Wageningen University and Research Centre, PO Box 65, 8200 AB Lelystad, The Netherlands

MATERIALS AND METHODS

The experiment was carried out according to a protocol approved by the Animal Ethical Committee of Utrecht University, as required by Dutch law.

At two farrow-to-finish farms, a total of 16 *S suis* 2 EF$^+$ carrier sows (eight per farm) were selected for inclusion in the experiment. At both farms the weaned pigs suffered from clinical problems related to *S suis* 2 EF$^+$ strains. In a previous survey, the proportion of carrier sows had been estimated to be at least 0·55 (Swildens and others 2005). Four batches of four sows were studied. For each experimental batch, over 25 sows were sampled at the farms, 10, nine and eight weeks before the expected date of farrowing, and sows that tested positive in three consecutive weekly samplings were considered to be carriers. On each farm, the selected carrier sows were randomly allocated to the treatment or control group. Two to three weeks before the expected farrowing date, one from each group was randomly selected at each farm and transported to the experimental unit. These four sows were randomly assigned to individual isolation rooms, where they farrowed. In the treatment group a mean (sd) of 9·86 (0·38) piglets per sow were born, and in the control group 8·57 (2·30) piglets per sow were born. Samples were collected regularly from the sows and individual piglets. Twenty-two to 32 days after the piglets' birth, the sows and piglets were euthanased and their tonsils were removed with sterile scalpels and scissors, without making contact with other parts of the carcase.

Sampling of sows The sows were sampled by tonsillar brushing five times during the selection procedure, and in the week before they farrowed, within 24 hours after farrowing and weekly thereafter (Table 1). The samples were taken as described by Swildens and others (2005) with a slight modification; briefly, after the sow had been immobilised, a wedge was pushed between its teeth, and each tonsil was brushed for at least eight seconds using a sterile toothbrush attached to a 40 cm long metal rod.

Vaginal swabs were taken with a sterilised 40 cm plastic rod with a 5 cm cotton wool tip (Heinz-Herens) within 24 hours after parturition.

Sampling of piglets The piglets were sampled for the first time within 24 hours after birth, daily during the first week,

and then three times a week until they were euthanased (Table 2). To sample the piglets, a small wedge was gently inserted between their teeth, and a sterilised 15 cm plastic rod with a 1·5 cm cotton wool tip (Applimed 0538) was used to swab both tonsils for 10 seconds, while turning the swab between forefinger and thumb; on average, the whole procedure took less than 45 seconds.

Housing and hygiene protocol

The sows were housed in individual isolation rooms which, to prevent cross-contamination, were ventilated under negative pressure through high-efficiency particle arresting (HEPA) filters. A farrowing crate was placed in each isolation room, and food, water and medication were provided through closed systems, to minimise the need for technicians to enter the isolation rooms. Only technicians who had no other contacts with pigs sampled the animals, and before entering an isolation room they changed their clothes and put on disposable protective respiratory masks, gloves and hair caps.

Treatment

Vaccine Six and three weeks before expected farrowing, the sows in the treatment group were vaccinated (Table 1). A formalin-killed, whole-cell vaccine of the EF-positive *S suis* serotype 2 strain 4005 was prepared as described by Wisselink and others (2002). The vaccine contained approximately 10^9 colony forming units/dose; a water-in-oil emulsion (Stimune; Cedi-Diagnostics) was used as adjuvant. The sows received doses of 2 ml intramuscularly.

Medication The sows in the treatment group were medicated twice a day from one week before the expected farrowing date until they were euthanased. Each dose consisted of 40 mg/kg bodyweight of amoxicillin (Octacilline; Eurovet Animal Health, containing 80 per cent amoxicillin) in 2 litres of water.

Sample transport and storage

Directly after the samples were taken, the tonsillar and vaginal swabs and the tonsillar brushes were transported to the laboratory in a transport medium consisting of Todd-Hewitt broth (CM189; Oxoid) containing 0·25 per cent *Streptococcus* selective supplement (SR126; Oxoid) and 0·2 µg/ml crystal violet. At the laboratory the specimens were incubated in this medium for 18 hours at 37°C. Whole tonsils were transported in individual plastic bags on ice and processed within two hours of collection into homogenates, as described by Wisselink and others (1999); glycerol was added to a final concentration of 15 per cent. Individual samples and pooled samples from all the piglets in a litter were stored in 2 ml Greiner cryotubes at –70°C.

PCR test

The PCR assay for the detection of *S suis* 2 EF$^+$ strains and the preceding DNA preparations were performed as described by Wisselink and others (1999). The homogenates of the postmortem samples from the sows and piglets and the vaginal swabs from the sows were analysed by PCR. In addition, eight tonsillar brush samples taken during lactation from six treated sows and four taken from two untreated sows were analysed. All the pooled litter samples, and the individual samples taken within 24 hours after birth from the only litter of piglets that had positive individual homogenates, were also analysed by PCR.

Statistical analysis

To estimate the differences between the two treatment groups in the tonsillar carriage of *S suis* 2 EF$^+$, Fisher's exact test was performed in SPSS 9.0. Values of $P<0·05$ were considered significant.

TABLE 1: Sampling and treatment schedule for two groups of seven *Streptococcus suis* serotype 2–positive sows

Action	Material/method	Group	Timing
Tonsil sampling	Brush	T/C	10, 9, eight, six, three and one week before the expected farrowing date, within 24 hours after farrowing and every week thereafter
Vaginal sampling	Cotton wool swab	T/C	Within 24 hours after farrowing
Vaccination	*Streptococcus suis* serotype 2 strain 4005	T	Six and three weeks before the expected farrowing date
Medication	Amoxicillin	T	Twice a day from one week before the expected farrowing date until euthanasia

T Treated group, C Control group

RESULTS

A pair of sows from the same farm in the first batch were excluded from the experiment because the control sow was treated erroneously, leaving 14 sows in the experiment. No *S suis* 2 EF$^+$ strains could be detected postmortem in the tonsils of the seven treated sows, whereas they were detected by PCR in all the tonsils of the seven untreated sows ($P<0·001$). The same contrast was observed in the tonsil brush samples taken during the lactation period; the eight samples from the treated sows tested negative but the four samples from the untreated sows tested positive by PCR ($P=0·036$). No *S suis* 2 EF$^+$ could be detected in the vaginal swabs from the 14 sows.

None of the litters of the treated sows became infected with *S suis* 2 EF$^+$ strains, but one of the seven litters of the control sows became infected. In this litter, *S suis* 2 EF$^+$ strains were first detected in the pooled sample taken on day 5. The pooled sample taken on day 6 and five pooled samples taken between day 16 and day 25 were also positive, but the four pooled samples taken between day 7 and day 14 were negative.

DISCUSSION

No *S suis* 2 EF$^+$ strains could be detected by PCR in the whole tonsils of the treated sows postmortem. Earlier attempts by Amass and others (1996b) and Clifton-Hadley and others (1984) to eliminate *S suis* from the tonsils of weaned pigs and slaughter pigs by medication with therapeutic doses of antibiotics for five days and several months, respectively, failed. In contrast with those trials, the present experiment used sows instead of pigs and used a combination of antibacterial medication and vaccination rather than antibacterial treatment alone. It is not possible to attribute the successful results to either the medication or the vaccination of the sows, although the results of vaccination trials by Amass and others (2000) and Torremorell and others (1998) suggest that the vaccination may have made a significant contribution.

Owing to the imperfect test sensitivity of 0·88 (Swildens and others 2005) it is not possible to be certain that the treated sows were not still positive carriers at the end of the experiment, although the PCR on the whole tonsil was negative. However, in support of the negative postmortem find-

TABLE 2: Sampling schedule for the piglets from the seven treated sows and seven control sows

Action	Material/method	Timing
Tonsil sampling	Cotton wool swab	Day of birth and daily for the first seven or eight days; thereafter, three times a week

ings, all the tonsillar brush samples taken from the treated sows during lactation also tested negative. The specificity of the test was 0·96 (Swildens and others 2005), and it is therefore very unlikely that the sows were free of *S suis* 2 EF$^+$ strains at the start of the experiment and were included in the trial erroneously as carriers.

Only one of the seven litters of the untreated sows was infected with *S suis* 2 EF$^+$. This litter was probably not infected at birth, because *S suis* 2 EF$^+$ was first detected in the pooled sample taken on day 5. Moreover, the vaginal swabs taken directly after parturition were all negative, which is consistent with the findings of Clifton-Hadley (1984), who did not detect *S suis* in vaginal samples from 81 sows and gilts in two herds with a high carrier rate. The number of litters infected by the untreated sows was low compared with the results of Roberston and others (1991), who cultured *S suis* serotype 2 from all five litters in a trial. Torremorell and others (1998) cultured *S suis* serotype 2 from tonsillar swabs from 13 of 24 piglets from an unknown number of litters, five days after birth. However, both these studies were carried out in commercial farrowing units where infectious pigs could have infected susceptible pigs in neighbouring pens. In the present experiment a strict biosecurity protocol was used so that the role of the sow as the initiator of infections within its own litter could be evaluated.

By the use of individual isolation rooms for the sows and their litters and the use of a well characterised test, the results of this experiment show that it is possible to wean piglets free of *S suis* 2 EF$^+$ from positive sows, and that the incidence of vertical transmission in farrowing pens is low. By using the *S suis* 2 EF$^+$ -free weaners as replacement gilts, *S suis* 2 EF$^+$ could be eliminated from a farm provided that the gilts were not infected later on and that the incidence of transmission between gilts and sows and between sows was low. As a result, the costs of labour and the use of antibiotics to control disease associated with *S suis* could be reduced substantially. To determine the feasibility of this strategy, studies on the dynamics of animal contacts and the transmission of *S suis* 2 EF$^+$ within a farm are needed.

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