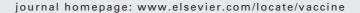


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# Efficacy of intradermally administrated E2 subunit vaccines in reducing horizontal transmission of classical swine fever virus

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#### **KEYWORDS**

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Horizontal transmission

To investigate if intradermal (ID) vaccination and intramuscular (IM) vaccination result in a comparable reduction of horizontal transmission of classical swine fever virus (CSFV), two registered E2 subunit marker vaccines were examined. Vaccine A was a water-in-oil emulsion containing the E2 glycoprotein originating from the Alfort/Tübingen strain and vaccine B was a water-oil-water emulsion containing the E2 glycoprotein originating from the Brescia strain. Eight groups, of ten pigs each, were vaccinated with either vaccine A or B, intramuscularly (IM) or intradermally (ID). Two different vaccination-challenge intervals were used for each vaccine. Furthermore, one group was vaccinated with a tenfold ID dose of vaccine A and one non-vaccinated group served as a control group. Five pigs from each group were challenged with the moderately virulent CSFV strain Paderborn, while the remaining five pigs served as contacts. Using vaccine A, full transmission to all contact pigs in both ID vaccinated groups occurred. No virus transmission was observed when IM vaccinated pigs were challenged 14 days post-vaccination (14 dpv) whereas only one out of five contact pig became infected when they were challenged 10 dpv. Using vaccine B no virus transmission was observed when pigs were ID or IM vaccinated and challenged 10 dpv. When challenged 3 dpv full transmission occurred in the ID vaccinated group, whereas four out of five contact pigs became infected in the IM vaccinated group. This result indicates that ID vaccination does not result in better protection against horizontal CSFV transmission compared to IM vaccination, for the vaccines studied. © 2007 Elsevier Ltd. All rights reserved.

### Introduction

The control strategy of the European Union for classical swine fever (CSF) outbreaks is based on zoo-sanitary measures, combined with stamping out infected and suspected herds. It may furthermore include massive pre-emptive

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1236 J.C.F.M. Dortmans et al.

culling to limit virus spread in the neighbourhood of infected herds [1]. This massive killing and destruction of mostly healthy animals is increasingly seen as wasteful and unethical. One alternative for pre-emptive culling might be emergency vaccination to control virus spread in the neighbourhood of infected herds. To make this alternative viable, the interval between vaccination and onset of immunity (reduced infectivity and susceptibility) must be short enough to ensure that virus replication and excretion reduces sufficiently quickly to diminish the virus infection load in the neighbourhood [2,3]. This requires evaluating available CSF-vaccines for their capacity to reduce virus transmission and the most effective way in which to administer them.

With respect to the application route, the intradermal route can be used as an alternative to the intramuscular route. Intradermal vaccination has been shown, in recent results in a number of animal species, to be a promising alternative for intramuscular vaccination of live attenuated, DNA or glycoprotein-based vaccines [4–7]; these studies show that intradermal vaccination may have greater immunogenicity than intramuscular vaccination. Furthermore, intradermal vaccination, generally requires less antigen to induce the same immune response [8,9] (Eble et al., unpublished), which may be relevant in emergency vaccination during an outbreak.

In this study, two registered subunit vaccines against CSFV were tested. Vaccine A was a water-in-oil emulsion containing the E2 glycoprotein originated from the Alfort/Tübingen strain, and vaccine B was a water—oil—water emulsion containing the E2 glycoprotein originated from the Brescia strain. Both vaccines allow discrimination between vaccinated and infected animals. Neither is as efficacious in clinical and virus transmission protection as modified live vaccines [3,10], and so we also sought to discover whether intradermal vaccination would improve their efficacy.

In this study the two CSF E2 subunit vaccines were used to investigate horizontal transmission of CSFV after intradermal versus intramuscular vaccination at different times post-vaccination.

#### Materials and methods

#### **Animals**

One hundred conventional pigs were used in the experiments and were randomly allotted into nine vaccine groups and one control group of 10 pigs each. All groups of pigs were housed separately under high containment conditions and were free of antibodies against pestiviruses. At the time of vaccination, the pigs were 8–10 weeks old.

#### Vaccine and challenge virus

The E2 subunit marker vaccines used in this experiment were

 Vaccine A: a water-in-oil emulsion containing 120 ELISA units (EU) of E2 as the active antigen component of the CSFV strain Alfort<sub>187</sub>/Tübingen per dose of 2 ml. Vaccine B: a water—oil—water emulsion (double oil emulsion; DOE) containing 32 μg of E2 as the active antigen component of the CSFV strain Brescia per dose of 2 ml.

Although the manufacturers of the marker vaccines recommend a double vaccination for optimal protection, a single vaccination dose was administered for both vaccines in this experiment to test their usefulness during an emergency vaccination. Vaccines were administered in the lateral neck region. Intradermal (ID) vaccinations were performed using the needle-less IDAL Vaccinator (Intervet International BV, Boxmeer, The Netherlands) and one dose contained 0.2 ml of vaccine. Intramuscular (IM) vaccinations were performed with a needle and one dose contained 2.0 ml of vaccine.

The pigs were challenged intranasally with the moderately virulent CSFV strain Paderborn,  $10^5$  TCID<sub>50</sub> of CSFV per pig (0.5 ml per nostril). This strain was used because isolates of recent outbreaks in the EU were moderately virulent strains [11,12].

# Experimental design

Four groups of pigs were vaccinated with vaccine A. Those vaccinated ID received 12 EU and were challenged 10 and 14 days post-vaccination (10 dpv), and those vaccinated IM received 120 EU and were also challenged 10 and 14 dpv. Four groups of pigs were vaccinated with vaccine B. Those vaccinated ID received 3.2 µg and were challenged 3 and 10 dpv, and those vaccinated IM received 32  $\mu g$  and were also challenged 3 and 10 dpv. The vaccination-challenge intervals for both vaccines were chosen to match the moment where recent experiments showed full protection against virus transmission after IM vaccination and at a moment where recent experiments did not yet show full protection against transmission [3,13,14] (Loeffen and de Bruin, unpublished). One additional group of pigs was vaccinated with a tenfold ID dose (120 EU) of vaccine A and challenged 14 dpv. The final group of pigs was not vaccinated and served as a transmission control group. Table 1 shows an overview of the experimental setup, listing type of vaccine, dpv and route of administration. Each of the 10 groups was temporarily divided in half, and five pigs from each group were challenged with the moderately virulent CSFV strain Paderborn. The remaining five pigs from each group were added to the challenged pigs 24h later, serving as contact animals. The experiment was terminated 28 days after challenge.

#### Clinical examination and sample collection

Body temperature was recorded daily, from 3 days before vaccination until the end of the experiment. Fever was defined as a body temperature  $\geq$ 40 °C during at least two consecutive days.

Serum blood samples and EDTA blood samples were collected from each pig at the day of vaccination, 1 week after vaccination, and three times a week after challenge, until death or the end of the experiment. Tonsil samples were collected, frozen in liquid nitrogen and stored at  $-70\,^{\circ}\text{C}$  from each pig that died during the experiment or was euthanized at the end of the experiment.

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