Appendix 31

Cedivac-FMD; Duration of Immunity in cattle, sheep and pigs.

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Abstract:

In this study, the FMD antibody profiles in cattle, sheep and pigs were investigated for a period of 6 months following the single administration of a vaccine dose corresponding with a potency of 6 PD_{50}.

Monovalent double oil emulsion Cedivac-FMD vaccines containing either O_1 Manisa, A Turkey 14/98 or Asia1 Shamir were used. For each serotype, two or three batches of vaccine were formulated. Each vaccine batch was administered to 5 cattle, 5 pigs and 5 sheep. Blood samples were collected at regular intervals for up to 6 months following vaccination. Antibody titers were determined using the virus neutralization test. Statistical analyses were done using an ANOVA for repeated measurements.

For all FMD strains, the antibody titers increased rapidly in the first month. In the following months the antibody titers remained stable or still increased but at a much lower rate. For all FMD strains and for all species, antibody levels at 6 months were higher than at 4 weeks post vaccination.
Although there were significant differences in the antibody profiles between the species for all FMD strains tested, the clinical relevance of this finding is probably limited. For each of the FMD strains tested, there were no significant differences between the antibody profiles raised by the various batches of Cedivac-FMD.

The studies demonstrate that Cedivac-FMD DOE vaccines confer a duration of immunity of at least 6 months in cattle, sheep, and pigs. The data suggest that even beyond this period high levels of antibodies are maintained for O_1 Manisa, A Turkey, and Asia1 Shamir.

Introduction:

The duration of immunity (DOI) is an important characteristic of the efficacy of a vaccine and should be supported by data of laboratory and field trials (1). In the literature, surprisingly little data can be found on the DOI of regular or emergency FMD vaccinations.
In FMD-endemic countries it is desirable to use FMD vaccines that provide as long a DOI as possible in order to avoid frequent re-vaccination. In the European Union, the DOI of FMD vaccines has become important since the vaccinate-to-live strategy was adopted after the massive FMD outbreaks round the turn of the century. Before that time, ring vaccinations were only used to prevent spreading of the disease from the source of contamination. Within weeks following the conformation of an outbreak and the subsequent emergency vaccinations, all animals, both infected and vaccinated, were killed. A DOI longer than those few weeks was not necessary. The new EU regulations permit vaccinated animals to be kept alive. After an outbreak, a region is only declared free of FMD after a period of 6 months without new FMD outbreaks. It is therefore important to know whether or not during that period re-vaccination of animals is necessary.
In this study, antibody titers in cattle, pigs, and sheep, were measured during a six-month period following the single administration of monovalent Cedivac-FMD double oil emulsion vaccines containing 6 PD_{50} of the FMD strains O1 Manisa, A Turkey 14/98, or Asia1 Shamir.

Materials and Methods:

1.1. Vaccines
All vaccines administered were manufactured by the Animal Sciences Group, Lelystad, The Netherlands and contained inactivated, purified FMD antigens using a mineral oil as adjuvant in a double oil emulsion formulation. Batches of monovalent vaccines were formulated using antigens derived from FMD strains O1 Manisa (n = 3), A Turkey 14/98 (n = 3), and Asia1 Shamir (n = 2). In previously conducted PD_{50} experiments, the potency (PD_{50}/ml) for each of the used formulations

had been assessed. Based on these data, and for each of the FMD serotypes, the volume corresponding with exactly 6 PD_{50} was calculated and administered to each animal.

1.2. Animals and husbandry
All animals used in the experiments were conventionally bred and obtained from established commercial suppliers (Dumeco BV., Boxtel, The Netherlands and Topigs BV., Helvoirt, The Netherlands). Cattle were approximately 3 months of age and sheep and pigs were approximately 2 months of age at the time of vaccination. All animals were housed in appropriate facilities of the Animal Sciences Group, Lelystad and were fed conventionally.

1.3. Experiment design
All animals received a volume of vaccine corresponding with exactly 6 PD_{50}. Representative strains of the FMD serotypes O1, A and Asia1 were chosen and for each FMD strain, two or three batches of vaccine were formulated. Six PD_{50} doses of each batch of vaccine were administered to 5 cattle, 5 pigs and 5 sheep. Blood samples were collected at regular intervals for up to 6 months following vaccination and the resulting serum samples were analyzed in the virus neutralization test (VNT).
In analogy with European Pharmacopoeia requirements (3), the serological levels at 4 weeks post vaccination were considered to indicate acceptable levels of immunity. The differences between VNT titers of the species, batches, and time points were analysed using an ANOVA for Repeated Measurements and, if appropriate, followed by a Tukey-Kramer multiple comparison.

1.4. Virus Neutralisation Test
The virus neutralisation test (VNT) was performed according to OIE guidelines, chapter 2.1.1. End-point titers were expressed as the reciprocal of the final serum dilution (log_{10}) that neutralized 100 $TCID_{50}$ of FMD virus in 50% of the wells.

Results:

- In all cases, the antibody titers increased rapidly in the first month. In the following months the antibody titers remained stable or still increased but at a much lower rate (Figures 1-3).
- For all FMD strains and for all species, antibody levels at 6 months were higher than at 4 weeks post vaccination.
- For all FMD strains, significant differences between antibody profiles of the various species were found (in all cases $P < 0.001$).
- No significant differences were found between the VNT titers raised by the various Cedivac-FMD vaccine batches for each of the FMD strains (O1 Manisa: $P = 0.27$; A Turkey14/98: $P = 0.71$; Asia1 Shamir: $P = 0.45$).

Discussion:

The main objective of this study was to evaluate the duration of immunity in cattle, pigs, and sheep, following the single administration of monovalent Cedivac-FMD vaccines containing either O1 Manisa, A Turkey 14/98, or Asia1 Shamir-derived antigens. All individual animals received a dose which corresponded with a potency of 6 PD_{50}, a dose which is generally accepted as the minimal accepted potency for FMD emergency vaccines.
The results of this study clearly demonstrate that at the end of the study period of six months, for all species and for all of the FMD strains tested, the antibody levels were higher than corresponding antibody levels at 4 weeks post vaccination. Since according to EU regulations (3), the antibody titers raised by a 6 PD_{50} FMD vaccine at 3-4 weeks post vaccination are considered to provide a sufficient immunity in vaccinated animals, it can be concluded that the DOI of Cedivac-FMD vaccines is at least 6 months. The high levels of antibodies observed at 6 months post vaccination suggest that the DOI could extend beyond this period.

The onset of immunity, both for the various FMD strains tested and the various species, were highly similar with the exception of titers in pigs vaccinated with Asia1 Shamir. Although the onset in pigs was slower, antibody titers reached levels comparable to those the other species in the following months. Also for the other FMD strains tested, significant differences were observed in the VNT titers between species. In the case of O1 Manisa and A Turkey 14/98, these differences most probably have little clinical relevance.

For all three FMD strains, no significant difference in the VNT titers between vaccine batches was measured. This indicates a low batch-to-batch variability of Cedivac-FMD vaccines.

Conclusion:
The Duration of Immunity for Cedivac-FMD vaccines is at least 6 months in cattle, pigs and sheep. In a separate study we have demonstrated that vaccination with Cedivac-FMD vaccines do not raise antibodies against the non-structural proteins of FMD viruses (4). The relatively long DOI and the marker vaccine capabilities make Cedivac-FMD vaccines excellent candidates for use in both emergency situations and FMD-endemic regions.

References:

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2. EMEA/CVMP/775/02-Final, Position Paper on Requirements for Vaccines Against Foot-and-Mouth Disease
3. *European Pharmacopoeia* 5.1, 2005:0063; Foot-and-mouth disease (ruminants) vaccine (inactivated).
4. **Chenard, G., Orsel, K., Selman, P. et al.:** Cedivac-FMD, a marker vaccine?; In: Session of the Research Group of the Standing Technical Committee of the European Commission for the control of Foot-and-Mouth Disease; Paphos, Cyprus, October 2006.

Tables and Figures

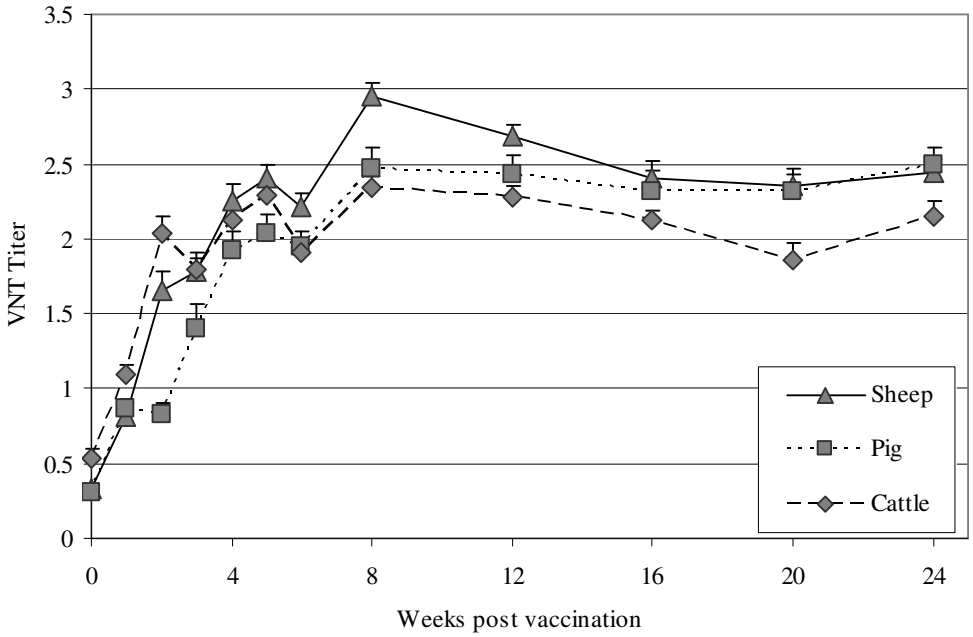

Figure 1. VNT (mean + sem) after vaccination with 6 PD_{50} of O1 Manisa.

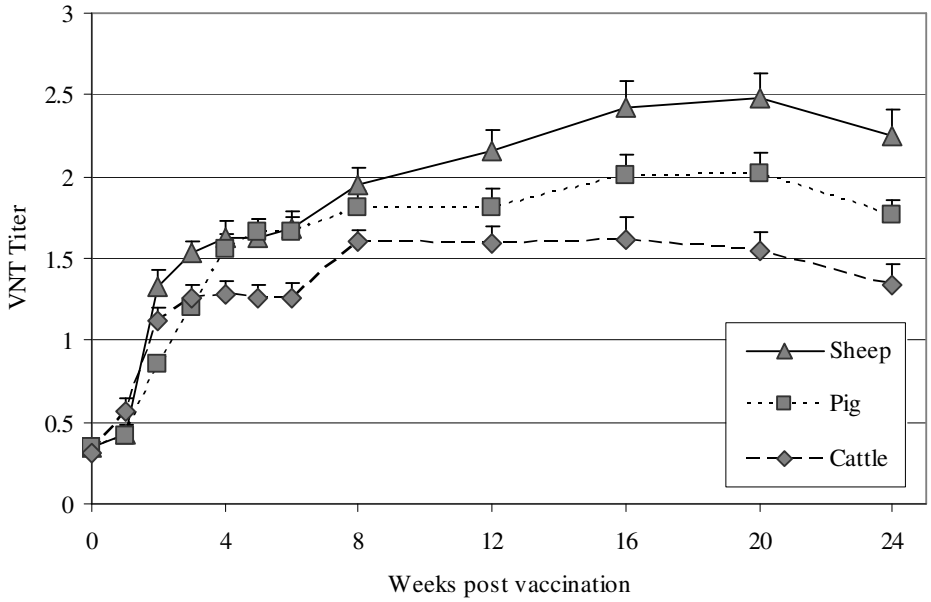

Figure 2. VNT (mean + sem) after vaccination with 6 PD_{50} of A Turkey 14/98.

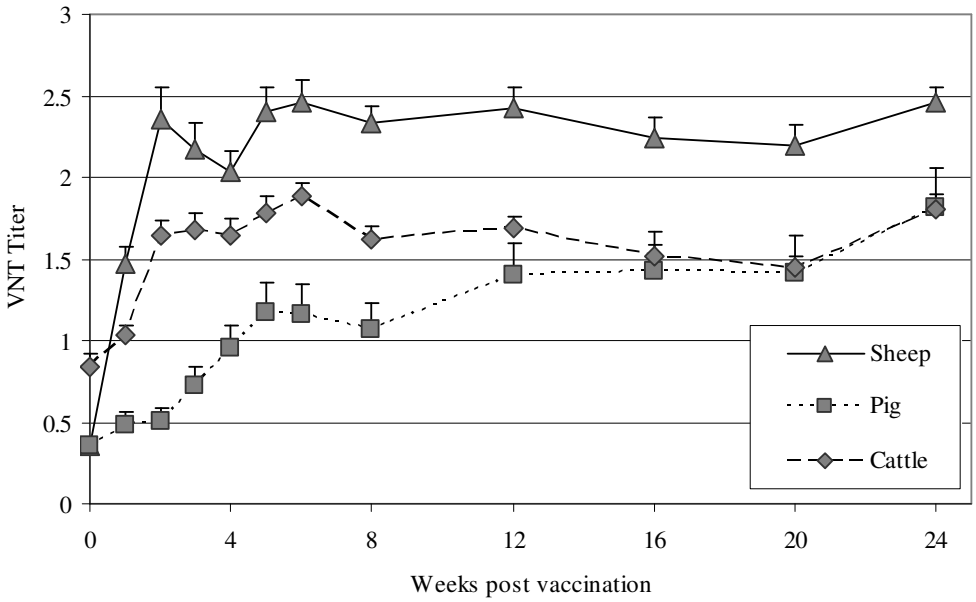

Figure 3. VNT (mean + sem) after vaccination with 6 PD_{50} of Asia1 Shamir.