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Cedivac-FMD can be used according to a marker vaccine principle

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Abstract

In this study, we investigated whether Cedivac-FMD, an emergency vaccine against foot-and-mouth disease (FMD), is suitable for use conjointly with a screening program intended to confirm freedom from disease in vaccinated herds based on evidence of virus replication in vaccinates. Different sets of sera were tested using the Ceditest® FMDV-NS ELISA for the detection of antibodies against non-structural proteins (NSP) of FMD virus. During a vaccine safety study, serum samples were collected from 10 calves, 10 lambs and 10 piglets following administration of a double dose and a repeat dose of high payload trivalent Cedivac-FMD vaccine. All serum samples collected both two weeks following the administration of a double dose as well as those collected two weeks after the single dose booster (given 2 weeks after the double dose) were negative in the Ceditest® FMDV-NS ELISA. In a series of vaccine potency experiments, serum samples were collected from 70 vaccinated cattle prior to and following exposure to infectious, homologous FMD virus. When testing cattle sera collected 4 weeks after vaccination with a regular dose of monovalent >6 PD_{50} vaccines, 1 of 70 animals tested positive in the NSP antibody ELISA. After infection with FMD virus, antibodies to NSP were detected in 59 of 70 vaccinated cattle and 27 of 28 non-vaccinated control animals within 7 days. Cedivac-FMD vaccines do not induce NSP antibodies in cattle, pigs or sheep following administration of a double dose or a repeat dose. FMD-exposed animals can be detected in a vaccinated group within 7-14 days. Because Cedivac-FMD does not induce NSP antibodies, the principle of 'marker vaccine' applies.

Introduction

In the aftermath of the 2001 FMD outbreaks, European Council directive 2003/85/EC was drafted. This directive retrospectively concludes that when control strategies were implemented in 2001, too much importance was attached to trade aspects and insufficient consideration was given to the possibility offered by the use of emergency vaccination and subsequent tests to detect infected animals in a vaccinated population. Directive 2003/85/EC makes provision for emergency vaccination and reducing significantly subsequent killing of the vaccinated animals following appropriate testing to substantiate the absence of infection. It has been well documented that exposure of susceptible animals to infectious FMD virus elicits the production of antibodies directed against structural as well as non-structural proteins (NSP). Non-structural proteins, which are coded for in the FMD virus genome, are a group of enzymes and other

proteins required in the different steps of the virus replication process including the assembly of the virus capsid structure. Previous studies have identified a number of antigenic non-structural proteins of which 3ABC appears to be the most reliable marker of FMD virus replication (Mackay et al. 1998, Sørensen et al. 1998). ELISAs for the detection of antibodies against non-structural proteins will likely play an essential role in the serological survey of livestock herds in future post-outbreak situations. Commercially available ELISA kits, and particularly the Ceditest® FMDV-NS ELISA, have been shown to have a high diagnostic sensitivity and specificity (Brocchi et al. 2006). It should be obvious that the use of such an ELISA in FMD surveillance programs following vaccination is only useful if the vaccine itself does not elicit an antibody response to NSP. The down-stream processing applied in the antigen manufacturing process of Cedivac-FMD vaccines results in a concentrated, purified intermediate product. The purification steps incorporated in the downstream processing separate whole FMD virus particle from proteins that have a significantly different molecular mass. Non-structural proteins are significantly smaller than whole FMD virus particle. Vaccines manufactured using antigen purified in this manner are therefore not expected to contain the amount of NSP necessary to induce an NSP antibody response in vaccinates. The quality requirements of information presented to support this claim in an application for a marketing authorization in the EU are stated in the Position Paper on Requirements for Vaccines Against Foot-and-Mouth Disease (EMEA/CVMP/775/02-final) which was drafted by the European Medicines Agency (EMEA) and came into effect in late 2004 (Anonymous 2004). This guideline provides important information to both FMD vaccine manufacturers as well as competent authorities on the requirements that FMD vaccines should meet before a marketing authorisation can be granted in the EU. Guidance is also specifically provided on how to obtain information to support that a vaccine does not induce antibodies to NSP. This information is also important for policy makers in that it gives guidance on how to evaluate if a given vaccine might interfere with the identification of infected animals in combination with an appropriate diagnostic test in a post-outbreak surveillance program. The EMEA position paper recommends generating data using an immunisation schedule consisting of three administrations of a double dose of vaccine containing the maximum antigen payload at intervals of two to four weeks to at least 10 animals of one or more species. Annex XIV of Directive 2003/85/EC however, lays down an immunisation schedule consisting of one initial and one subsequent booster vaccination. However, it is important to realise that the measure of assurance

that vaccination will not interfere with the subsequent identification of infected animals is directly related to the administration regime of the vaccine in question. Vaccines which require multiple or frequent injections to achieve and maintain immunity are justifiably subject to stringent data requirements to support that the vaccine does not induce antibodies to NSP. Since a single dose of Cedivac-FMD vaccine confers a duration of immunity of at least six months (Selman et al. 2006), repeat administration is not required to achieve immunity that will last probably until well after the post-outbreak surveillance has been completed. Therefore, if no NSP antibody response has been induced following one initial and one subsequent booster vaccination, it can be assumed that future use of the vaccine in an outbreak situation would not interfere with the subsequent identification of animals exposed to wild virus.

This report investigates the assumption that Cedivac-FMD vaccine does not induce an NSP antibody response by evaluating NS-ELISA results from sera collected in safety studies involving repeat vaccination and sera collected in vaccination-challenge experiments.

Materials and Methods

1.1. Vaccines

All vaccines administered were manufactured by Animal Sciences Group, Lelystad, The Netherlands, and contained inactivated, purified FMD virus antigens using a mineral oil as adjuvant in a double oil emulsion formulation. In the safety study, a trivalent vaccine formulated to contain a high antigen payload was used. This payload corresponded with 25 times the antigen required for one 50% protective dose (PD_{50}) for each of the strains: A Turkey 14/98, Asia1 Shamir and O1 Manisa. In the potency tests, monovalent vaccines were used containing a broad spectrum of vaccine antigens as listed in Table 1. The vaccines used were found to have a potency of at least 6 PD_{50}.

1.2. Challenge virus

The FMD challenge viruses used were prepared by giving the source virus material obtained from the World Reference Laboratory in Pirbright, U.K., one additional cattle passage. In the PD_{50} experiments inoculations with homologous virus were performed using a 50% infective dose (ID_{50}) of approximately 10,000 as determined by titration on cattle tongue.

1.3. Animals and husbandry

All animals used in the experiments were conventionally bred and obtained from established commercial suppliers (Dumeco BV., Boxtel, The Netherlands, Topigs BV., Helvoirt, The Netherlands). Calves, lambs and piglets used in the safety study were six weeks of age at the time of the first vaccination. Cattle used in the potency experiments were at least 6 months of age. Animals were housed in appropriate facilities and were fed rationed portions appropriate for their age. All experiments were approved by the Animal Ethics Committee of the Animal Sciences Group.

1.4. Sera sets

The safety study was performed following European Pharmacopoeia chapter 5.2.6 *Evaluation of Safety of Veterinary Vaccines and Immunosera*. The vaccination scheme involved the administration of a double dose (4 ml) of high payload trivalent Cedivac-FMD vaccine to 10 calves, 10 lambs and 10 piglets followed by the administration of a single dose 2 weeks later. Animals were monitored for 14 days following each vaccination. This resulted in a sera set from 30 animals collected at 3 time points: prior to the first vaccination, 2 weeks post vaccination (of a double dose) and 2 weeks post repeat-vaccination (of a single dose).

Potency experiments, carried out in order to determine the number of PD_{50} in a vaccine dose, were performed according to European Pharmacopoeia monograph 04/2005:0063 with the exception that cattle were challenged at 4 weeks post vaccination. Only results from sera collected from cattle that received a full dose of vaccine (2 ml) and sera from the unvaccinated controls are reported in this study. PD_{50} experiments were performed using FMD virus strains: A Turkey 14/98, A Iran 87, Asia1 Shamir, A22 Iraq, A24 Cruzeiro, O1 BFS, and O1 Manisa. Sera from 14 PD_{50} experiments were used resulting in a serum set from 70 vaccinated cattle and 28 unvaccinated controls sampled at 2 time points: 4 weeks post vaccination (coinciding with the day of challenge) and 7 days post infection. Using the European Pharmacopoeia criteria, 65 of the 70 cattle (≥ 90%) were protected from clinical FMD following challenge.

1.5. NSP ELISA

Sera were tested for the presence of antibodies directed against non-structural protein 3ABC of FMD virus using a commercially available ELISA (Ceditest®

FMDV-NS ELISA, Cedi Diagnostics BV., Lelystad, The Netherlands). The assay was performed according to manufacturer's instructions. Results of 50% inhibition and higher were considered positive.

1.6. Virus neutralisation test

The virus neutralisation test was performed as described previously (Dekker and Terpstra 1996).

Results

When testing sera from the safety study, all of the samples collected either two weeks following the administration of a double dose or collected two weeks following the administration of the single dose (and four weeks following the double dose) were found to be negative in the NS ELISA (Table 2). The frequency distribution of the individual percent inhibition values appears practically identical at all time points (Figure 1).

When testing sera from the PD_{50} experiments, 1 of 70 samples collected four weeks post vaccination and 1 of 28 samples from non-vaccinated control animals were positive in the NS ELISA. One week later at 7 days post infection, 59 of 70 samples (84 %) from the vaccinated cattle and 27 of 28 samples (96%) from non-vaccinated control animals were positive in the NS ELISA (Table 1). The frequency distributions of the individual percent inhibition values for both vaccinated and non-vaccinated animals are similar only at 0 days post infection (Figures 2 and 3).

As can be seen in Table 3, the 11 vaccinated-challenged animals that remained negative for NSP antibodies at 7 days post infection were concentrated among animals with the highest VNT titres at four weeks post vaccination. These 11 animals were classified as protected. The Fischer Exact test revealed that VNT titre and response in the Ceditest® FMDV-NS ELISA were statistically significantly associated ($p = 0.0453$).

Discussion

The objective of this study was to evaluate whether Cedivac-FMD is suitable for use in FMD surveillance programs intended to substantiate or confirm freedom from infection in herds based on evidence of virus replication in vaccinates. Different sets of sera were tested for the presence of antibodies against non-structural proteins of FMD virus using the Ceditest® FMD NS-ELISA. The ELISA results of the safety study sera set indicate that Cedivac-FMD vaccines do not

induce antibodies to non-structural proteins of FMD 28 days after administration of a double dose or 14 days after a second dose in either of the three target species tested. When considering these results, it is important to note that the vaccine used was specifically formulated to simulate a “worst-case scenario” to investigate adverse effects following administration of an overdose and a repeat dose. This trivalent vaccine was formulated to contain an antigen payload 25 times the amount corresponding with one PD_{50} per dose of 2 ml for each of three strains. The payload of any non-structural proteins would also be maximised in this vaccine and would elicit an antibody response if present, especially following administration of 4 ml (double dose). The fact that the frequency distribution of the percent inhibitions of the 30 animals remains practically identical even 14 days after receiving a repeat dose indicates that Cedivac-FMD vaccines do not contain the amount of NSP sufficient to induce an antibody response in vaccinates. The data obtained during the safety studies meet the requirements of Directive 2003/85/EC. Although the data provides the required measure of assurance that vaccination will not interfere with the identification of infected animals in a post-outbreak surveillance program, the requirements for data as recommended in the position paper would not be met due to the different immunisation schedule used. It would appear that the data requirements in the position paper are more relevant for FMD vaccines that require multiple or repeated injections to achieve and maintain immunity.

The NS ELISA results of the PD_{50} sera at 0 dpi agree largely with those of the safety study sera set. However, two samples tested positive at this time point: one from a vaccinated and one from a non-vaccinated animal. Upon closer inspection of the percent inhibition values, it was noted that these samples are marginally positive (57% and 51% inhibition respectively). Since a small proportion of false positive and false negative results are practically inevitable in biological diagnostic assays, and have been described in comparative evaluation study of different NS ELISAs (Brocchi et al. 2006), the results observed are not considered unusual. In the PD_{50} experiments, 59 of 70 (84%) vaccinated animals and 27 of 28 non-vaccinated animals tested positive for antibodies to NSP within seven days of challenge with infectious FMD virus. Similar results were obtained for all FMD strains tested. The proportion of vaccinated animals that tested positive for antibodies to NSP following experimental infection was higher than the 54% (48 of 89 vaccinated-infected cattle) reported for the Ceditest® in a recent comparative evaluation study of different NS ELISAs (Brocchi et al. 2006). Factors including, but not limited to, the different vaccines used, different

formulations, origins of challenge virus, route of inoculation, and the breed or health status of animals in question likely determine the proportion that develop antibodies to NSP following infection.

As mentioned earlier, 59 of 70 vaccinated animals tested positive for antibodies to NSP following experimental infection. This proportion is lower than the 27 of 28 non-vaccinated animals and is a logical consequence of the lower rate of virus replication as a result of vaccination. In total 11 vaccinated and challenged cattle tested negative in the NS ELISA indicating very low levels of virus replication in the animals which means a low risk of virus spread in a field situation. As one would expect, all these 11 animals were classified as protected.

Recent studies with vaccinated and non-vaccinated cattle, sheep and pigs have demonstrated that Cedivac-FMD is able to reduce and in most cases prevent virus transmission to contact animals under experimental conditions (Orsel et al. 2006). Given the available information, the authors believe that Cedivac-FMD meets important requirements for use in controlling an outbreak of FMD. Emergency vaccination with Cedivac-FMD and subsequent screening with the Ceditest® FMDV NS-ELISA would reduce the number of animals that would need to be culled in the aftermath of an outbreak of FMD. Using data from the 2001 FMD outbreak in the Netherlands (Pluimers et al. 2002) and a specificity of 99.0% for the Ceditest FMDV NS ELISA, a retrospective simulation of the number of false positive results was performed. Based on this analysis it was estimated that following an initial round of sampling and testing, approximately 50% of the premises where ring vaccination was applied would have been declared free from FMD and vaccinated herds spared of culling. A second round of confirmation testing combined with clinical examinations would raise the number of premises spared of culling even further (Dekker 2005).

The term marker vaccine has been most closely associated with vaccines containing specifically modified or tailored antigens that do not induce antibodies to a particular epitope (compared to wild virus) resulting in a “marker” to identify infected individuals. In the case of FMD, antibodies to NSP can be used as markers of infection only when there is a measure of assurance that previous vaccination has not induced antibodies to the same NSP. Because this measure of assurance has been demonstrated for Cedivac-FMD, it can be used according to a “marker vaccine” principle.

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References

Anonymous. 2004. The Position Paper on Requirements for Vaccines Against Foot-and-Mouth Disease EMEA/CVMP/775/02-final

Brocchi, E., Bergmann, I.E., Dekker, A., Paton, D.J., Sammin, D.J., Greiner, M., Grazioli, S., De Simone, F., Yadin, H., Haas, B., Bulut, N., Malirat, V., Neitzert, E., Goris, N., Parida, S., Sørensen, K. & De Clercq, K. 2006. Comparative performance of six ELISAs for antibodies to the non-structural proteins of foot-and-mouth disease. *Vaccine* 24 (47-48): pp. 6966-6979).

Dekker, A. and Terpstra, C. 1996. Prevalence of foot-and-mouth disease antibodies in dairy herds in the Netherlands, four years after vaccination. Research in Veterinary science 61; 89-91

Dekker, A. 2005. Monitoring after FMD emergency vaccination. Session of the research group of the European commission for the control of foot-and-mouth disease, Greifswald, Germany, 20 – 23 September 2005

Mackay, D. K. J., Forsyth, M. A., Davies, P. R., Berlinzani, A., Belsham, G. J., Flint, M. & Ryan, M. D. 1998. Differentiating infection from vaccination in foot-and-mouth disease using a panel of recombinant, non-structural proteins in ELISA. *Vaccine* 16 (5): pp. 446-459.

Orsel, K., Dekker, A., Bouma, A., Stegeman, J.A. & Jong, M.C. 2005. Vaccination against foot and mouth disease reduces virus transmission in groups of calves, *Vaccine* 23 (41): pp. 4887–4894.

Orsel, K., Dekker, A., Bouma, A., Stegeman, J.A. & Jong, M.C. 2007. The effect of vaccination on foot and mouth disease virus transmission among dairy cows. *Vaccine* 25 (2): pp. 327-335).

297 Paton, D.J., De Clercq, K., Greiner, M., Dekker, A., Brocchi, E., Bergmann, I.,
298 Sammin, D.J., Gubbins, S. & Parida, S. 2006. Application of non-structural
299 protein antibody tests in substantiating freedom from foot-and-mouth disease
300 virus infection after emergency vaccination of cattle *Vaccine* 24(42-43): pp.
301 6503-6512.
302

303 Pluimers, F.H., Akkerman, A.M., Van der Wal, P., Dekker, A. and Bianchi, A.
304 2002.

305 Lessons from the foot and mouth disease outbreak in The Netherlands in 2001.
306 *Rev Sci Tech*. (3): pp. 711-721
307

308 Selman, P., Chénard, G. and Dekker, A. Cedivac-FMD: Duration of Immunity in
309 cattle, pigs and sheep. Presented at the 2006 Session of the Research Group
310 of the Standing Technical Committee of EUFMD Paphos, Cyprus - 15th - 21st
311 October 2006

312
313 Sørensen, K.J., Madsen, K.G., Madsen, E.S., Salt, J.S., Nqindi, J. & MacKay,
314 D.K.J. 1998. Differentiation of infection from vaccination in foot and mouth
315 disease by the detection of antibodies to the non-structural proteins 3D, 3AB and
316 3ABC in ELISA using antigens expressed in baculovirus. *Arch. Virol*. 143: pp.
317 1461-1476.
318

319 **Tables**

Table 1. NS ELISA results for sera collected during PD_{50} experiments

Number of cattle sera positive in NS ELISA out of total number sera tested at 4 weeks post vaccination (wpv) and 7 days post infection (dpi)		
Vaccine strain	4 wpv / 0 dpi	7 dpi
A Turkey 14/98	0/15	14/15
A Iran 2/87	0/10	10/10
Asia1 Shamir	0/15	12/15
A22 Iraq	0/5	5/5
A24 Cruzeiro	0/5	3/5
O1 BFS	0/5	3/5
O1 Manisa	1/15	12/15
Non-vaccinated control animals	1/28	27/28
Total	2/98	86/98

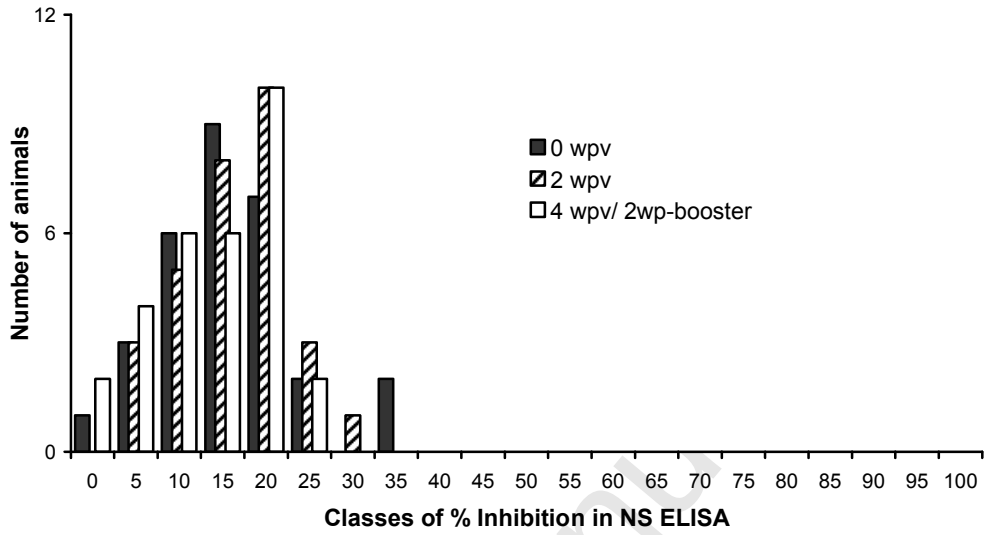
Table 2. NS ELISA results for sera collected during Safety studies

Number of sera positive in NS ELISA out of total number sera tested at weeks post vaccination (wpv) double dose and single dose		
Species	2 wpv	4 wpv (double) / 2 wpv (single)
Bovine	0/10	0/10
Ovine	0/10	0/10
Porcine	0/10	0/10
Total	0/30	0/30

Table 3. VNT titres at 4 wpv in relation to NS-ELISA result at 7 dpi for PD_{50} sera

	NS-ELISA positive	NS-ELISA negative
$\log_{10} \geq 1.95$	22	8
$\log_{10} \leq 1.80$	37	3

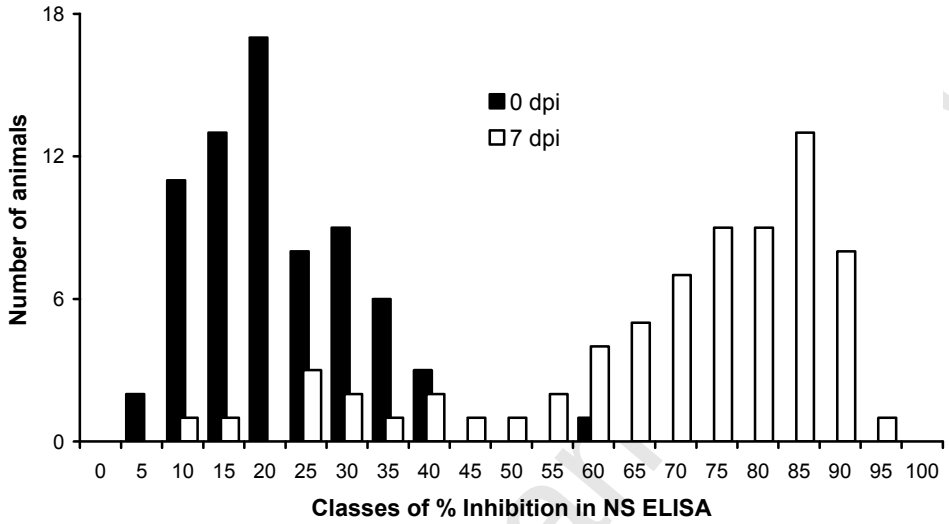
FIGURE 1



wpv = weeks post vaccination

Figure 1. Frequency distribution of safety study animals (n = 30)

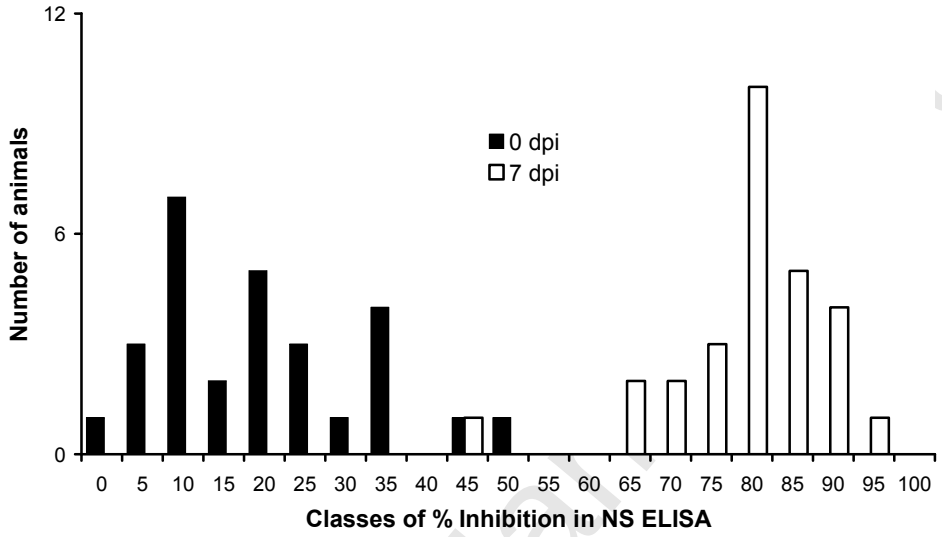
FIGURE 2



dpi = days post infection

Figure 2. Frequency distribution of vaccinated and challenged cattle in PD_{50} experiments (n = 70)

FIGURE 3



dpi = days post infection

Figure 3. Frequency distribution of non-vaccinated challenged cattle in PD_{50} experiments (n = 28)