Prediction of protection by FMD vaccines on the basis of LPBE results

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Abstract

Liquid-phase-blocking ELISA (LPBE) titres of cattle vaccinated against serotypes A or Asia were correlated with vaccine potency values (PD50) determined by cattle challenge tests according to the European Pharmacopoeia. The LPBE was found to be well suited for the prediction of protection by vaccine batches and will allow to replace the challenge test for many routine batch tests.

Introduction

The assessment of potency of foot-and-mouth disease (FMD) vaccines according to the European Pharmacopoeia (EP) requires that vaccine batches are tested in groups of cattle inoculated with different doses of the vaccines so that the quality of a batch can be expressed in terms of fifty percent protective doses (PD50). This test method can be replaced by other methods provided that a correlation to the PD50 method is established. Animal welfare concerns, the limited capacity of high security stables and the risk that virus excreted by infected animals may escape to the environment prompted us to look for an alternative potency test. Previous studies with neutralization assays (Hecke et al., 1960; Stellmann et al., 1968, Pay and Parker, 1977, Pay and Hingley, 1986) had already shown a correlation between SNT titre and protection. However, neutralization tests are usually less reproducible than ELISAs. In the 1980th, R. Ahl established a method for the assessment of vaccine potency in cattle by measuring the antibody response in vaccinated cattle in a plaque reduction test based on a cell suspension plaque assay with BHK21 CT cells (Ahl et al., 1990). While the plaque reduction test solved the problem of reproducibility, it was very laborious and difficult to transfer to another lab. Several groups looked for an alternative potency test based on the liquid-phase blocking sandwich ELISA (LPBE) for the detection of antibodies against foot-and-mouth disease virus, which had been published by Hamblin et al in 1986. In 1989, Hamblin and coworkers published that LPBE antibody levels in cattle sera following vaccination were highly related to protection. They considered the ELISA to be more reliable than the VN and recommended its use for the evaluation of immunological responses following infection as well as following vaccination. Furthermore, the LPBE does not require infectious virus, but works also with inactivated virus (Ferris et al., 1990) allows to reduce disease security risks. Van Maanen and Terpstra, 1989, compared the LPBE and VNT for the assessment of vaccine potency and concluded that, because the SNT and the LPBE are highly correlated and the LBE is more reproducible, the LPBE should predict protection more reliably than the SNT. Amadori et al., (1990) stated that the plaque reduction test and LPBE gave precise estimates within certain limits of potency and recommended a combination of these tests. The LPBE was also established at the BFAV, now Friedrich-Loeffler-Institut (FLI). Preliminary results on the correlation of LPBE titres of cattle vaccinated against serotypes A or Asia with vaccine potency values (PD50) determined by cattle challenge tests will be reported below. In South-America, the LPBE, in a variation employing monoclonal antibodies, was extensively and successfully used for in-vitro testing of FMD vaccines. In 1993, Periolo et al. published a paper on the large-scale use of the LPBE for the evaluation of protective immunity against aphthovirus in cattle vaccinated with oil-adjuvanted vaccines in Argentina. It was found that, with few exceptions, animals with titres of at least 2.1 log 10 were protected against challenge with serotypes C85, A87, 01 Caseros and A79. Further data were published by Robiolo et al. (1995). The lowest expected protection (LEP) at a 95% confidence of 245 FMD commercial vaccines was calculated from the LPBE titres of cattle sera obtained from 3920 animals at 60 days postvaccination (d.p.v.) and challenged with live virus at 90 d.p.v. It was found that LEP evaluation was highly specific (able to predict the failure in 100% of the cases) although its sensitivity (ability to predict the approval) was only about 65%. It was possible, nevertheless, to improve the evaluation by using an alternative coefficient (Ro), exclusively dependent on the number of animals exhibiting the highest and lowest LPBE titres in a particular vaccine trial. This coefficient was capable of predicting the approval of 90% of the vaccines, yet maintaining acceptable levels of safety. Based on these results and further studies (Smitsaart et al., 1998) challenge was finally discontinued in Argentina. Unfortunately, data from Argentina can not be pooled with data from Europe, mainly because of the different challenge regimes. In Argentina, lots of 16 animals were vaccinated and challenged at 90 days d.p.v. with one of four virus strains. If 13 out of the 16 animals were protected, the vaccine batch passed. If at least 11 animals were protected, the manufactures could apply for a retrial. Serum samples from vaccinated and control cattle were collected 60 and 90 d.p.v. and the level of FMDV specific antibodies were determined. In Europe, however, 3 groups of five cattle are vaccinated with receive different volumes of vaccine. 21 d.p.v., the animals are challenged and eight days later the test is read and the PD50 content is calculated, usually by the Kärber method. A vaccine has passed if it contains at least 3 PD50, but the customer may ask for higher values, e.g. 6 PD50 for emergency vaccines. The LPBE was also used to examine the degree of
relation between viruses and predict heterologous protection (Kitching, 1989). Lunt et al. (1994) published an improved method, measuring the LPBE titres at equal OD values for the test virus and the reference virus, which attempted to increase the accuracy of the test.

Materials and Methods

Cattle were vaccinated with aluminium hydroxide vaccines and oil vaccines, type A22 (n=7) and A24 (n=3) as well as Asia 1 Shamir (n=10) of a European manufacturer. The vaccines included commercial as well as some experimental vaccine batches. Vaccine potency was determined according to the European Pharmacopoeia. Three groups of five animals received 1 dose, ¼ dose and 1/16 dose respectively. Sera were taken 21 d.p.v. and titres were determined in the LPBE following the protocol of the World Reference Laboratory for FMD with slight modifications. OD-readings were analysed by an Excel program, using interpolation to obtain precise titres. The group mean LPBE titre for each group of animals that had received a particular (full or reduced) dose of vaccine and the PD50 values determined for this batch of vaccine were used to calculate the regression between titre and protection. For groups that had received a reduced dose, the PD50 value was divided by 4 and 16 respectively.

Results

Data on individual animals showed a large "grey zone", where protection could not be predicted. For type A this "grey zone" ended at a titre of about 2.4, for type Asia at a titre of about 2.8 log10 units. Pooled data for 10 batches of A vaccines and 10 batches of Asia vaccines revealed a correlation between log10 LPBE titres and protection (log10 PD50) of R²=0.8365 and R²=0.8988, respectively.

Discussion

McCullough et al., 1992, found that neither the SNT nor ELISAs were able to predict the outcome of an infection of vaccinated cattle with certainty. They stated that these assays do not measure immunological protection but only antibody responses and described a "three zone" phenomenon: In the "white zone" antibody titres are high and donor animals likely to be protected; in the "black zone" antibody titres are low and donor animals are likely to be susceptible to infection and in the "grey zone" antibody titres are intermediary and no interpretation should be made with respect to protection. Looking at the results for individual animals, the findings of McCullough et al. could be confirmed, but using group mean titres resulted in correlation for types A and Asia in the range of 0.8 to 0.9. By this method, it could be confirmed that LPBE titres correlate well with protection in cattle, at least for serotypes A and Asia. Further statistical analysis, including probit analysis will be performed. It would be a great advantage to include sera from other manufacturers in the study to investigate whether correlations established for one type of vaccines are also valid for vaccines from other sources. For serotype O, we are not yet satisfied with the correlation found for 10 batches tested so far are. The reasons for this are being investigated in cooperation with the manufacturer. The phenomenon certainly is not due to technical problems with the LPBE, which is highly reproducible. WRL reagents gave similar results when used to test some of the sera. Currently, we are retesting all the sera by VNT in order to compare the results. We are also trying to establish a monolayer plaque reduction test that is less laborious than the plaque test established by Ahl (1990). We intend to use these methods also for heterologous tests in the frame of the FMD_ImproCon Project financed by the European Commission. This work will also contribute to the objectives of the "Position Paper on Requirements for Vaccines against Foot-and-Mouth Disease" issued by EMEA, in particular the reduction of challenge experiments.

References:


EMEA. 2004. Position Paper on Requirements for Vaccines against Foot-and-Mouth Disease, Committee for Veterinary Medicinal Products, EMEA/CVMP/775/02-Post-Consultation


