Quantitation of the Antigenicity and Immunogenicity of Purified Foot-and-Mouth Disease Virus Vaccine for Swine and Steers

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The antigenicity and immunogenicity of a purified preparation of foot-and-mouth disease virus [type A₁₂, strain 119 (FMDV A-119)] inactivated with 6.0 mM N-acetylenimine at 37 C were compared in swine and steers. Three antigen doses were tested, 640, 160, and 40 ng. In accordance with findings for guinea pigs, as previously determined by dose-response curves, as little as fourfold changes in antigen in the region of the minimum effective dose produced marked differences in the serological and immune responses of swine. The minimum effective dose of antigen for antibody formation in swine and guinea pigs, as determined by mouse median protective dose (PD₅₀) values, was 160 ng. The minimum immunogenic dose for swine was also 160 ng. The vaccinated swine were challenged with either FMDV A-119 or with heterologous subtype A₂₄ strain Cruzeiro or type A strain A-CANEFA-1. Those immunized with 640 ng of antigen were about equally immune to the three challenge viruses; most swine having a mouse PD₅₀ value of 2.0 or greater were immune regardless of which strain was used for challenge. In steers, the smallest dose tested, 40 ng, was satisfactory in eliciting circulating antibodies and immunity. Physical and biological tests indicated that the antigen used in the vaccine is stable for at least 9 months at 4 C.

Foot-and-mouth disease virus (FMDV) vaccines prepared from virus grown in the BHK-21 line of baby hamster kidney cells have elicited undesirable reactions resembling delayed hypersensitivity after revaccination of cattle (12). Purification of FMDV before vaccine formulation under certain experimental conditions appears to reduce the incidence of such reactions (Bahnmann, personal communication). Moreover, the use of purified and concentrated virus in a vaccine allows potency and volume parameters to be accurately adjusted and eliminates or lessens the chances for immunological competition (6) caused by nonviral components of crude vaccines.

Because of the high costs of testing FMDV vaccines in cattle and swine, antigen dose versus neutralizing antibody data are usually developed only for what is considered to be the practical immunological range in lieu of establishing an entire dose-response curve. Such curves, which allow a more comprehensive and critical assessment of vaccine potency, have, however, been established in guinea pigs for purified and concentrated, acetylenimine (AEI)-inactivated high-passage FMDV, type A₁₂, strain 119 (A-119; 13, 15). It was the purpose of the present work to utilize this data in helping to establish the smallest dose of a similar vaccine which elicits measurable neutralizing antibody responses in both cattle and swine as well as the doses required to induce resistance to challenge in 50% of the animals.

MATERIALS AND METHODS

Virus inactivation and storage. FMDV A-119 was passaged once in suckling mice, 150 times in primary calf kidney cultures, and once in BHK cells (16) derived from line 21, clone 13 of MacPherson and Stoker (11). This virus, at 2.62 mg/ml, purified as previously described (2), was inactivated with 6.0 mM AEI at 37 ± 0.5 C for 48 hr in 0.2 M NaCl, 0.05 M sodium phosphate, pH 7.5 (14). Titrations in suckling mice and plaque assays in calf kidney cultures were used to determine the rate of virus inactivation. The inactivation was a first-order reaction which extrapolated to 10⁻⁴⁴ plaque-forming

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units (PFU)/ml at 48 hr. In addition, intradermal inoculation of 2 ml (0.65 mg/ml, 0.1 ml/site) after 48 hr of AEI treatment in each of six steers failed to demonstrate infective virus (9, 14). After inactivation, appropriate concentrations of antigen were obtained by dilution in the above buffer. No attempt was made to remove, assess, or maintain the presence of AEI after the 48-hr treatment. Absorbance-temperature profiles (1) and complex fixation activities (4) of the purified virus were essentially the same before and after inactivation as well as after 9 months of storage at 4 C.

**Vaccination and bleeding.** Vaccines containing 640, 160, and 40 ng of inactivated virus were each tested in swine, steers, and guinea pigs. Vaccines were composed of 1 ml of an appropriate dilution of the AEI-inactivated virus preparation emulsified with 1 ml of an oil adjuvant consisting of one part emulsifier (Arlacel A) and nine parts light mineral oil (Marcol 52, Esso Research and Engineering Co., Linden, N.J.; reference 5). Nine Hereford steers were vaccinated subcutaneously, midway between the base of the ear and the point of the shoulder, with vaccines prepared with antigen stored for 5 months. Thirty-six swine were injected in the dorsal surface of the ear with vaccines which had been prepared from antigen stored for 9 months at 4 C. Two groups (15 per group, 5 per dose) of female Duncan Hartley strain guinea pigs were also vaccinated subcutaneously. Group 1 was vaccinated at the same time as the steers, and group 2 was vaccinated at the same time as the swine. Blood samples were collected from the cattle and swine at 0, 7, and 28 days postvaccination (DPV) and from the guinea pigs at 7 and 28 days postvaccination (DPV). Individual sera were prepared and stored at −10 C.

**Serum/virus neutralization determinations.** Low-passage tissue culture-produced FMDV [6,600 mouse median lethal dose (LD50)/ml] of the desired subtype was mixed with equal amounts of various dilutions of serum and incubated at 37 C for 1 hr. Each mixture was then injected (0.03 ml/dose) into 5- and 9-day-old unweaned Rockefeller H strain mice to detect unneutralized virus. Neutralizing capacities of sera were computed as mouse median protective dose (PD50) values (7).

**Challenge of immunity of vaccinated swine.** The immunity of each of the 36 vaccinated swine was challenged 28 DPV by injection with 1 ml of guinea pig FMDV-infected vesicular fluid diluted with Hanks balanced-salt solution to contain 40,000 mouse LD50 of the desired strain of FMDV. This virus is highly virulent for both swine and cattle (Tables 2 and 3). The injection was given intradermally in the ventral area of the panniculus of the left foreleg (3). The swine were in three groups, one of 18 animals and two of 9 animals. The group of 18 consisted of 6 swine vaccinated with each of the antigen doses, 640, 160, and 40 ng; similarly, each group of 9 contained 3 swine from each of the three antigen doses. These animal groupings were made without regard to antibody levels of the individual swine. The 18 swine were challenged with FMDV A-119, whereas one group of 9 was challenged with type A (subtype not designated) strain A-CANEFA-1 (A-CANEFA-1) and the other was challenged with type A34, strain Cruzeiro, Brazil (A34). In addition, each challenged group contained two nonvaccinated control swine, one of which was challenged along with the vaccinated swine. The swine were examined for signs of foot-and-mouth disease (FMD) at 2-day intervals for 14 days postchallenge (5).

**Challenge of immunity of vaccinated steers.** The immunity of the vaccinated steers was challenged 28 DPV by exposure to experimentally infected steers (8). Two of five nonvaccinated control steers placed in contact with the immunized animals were inoculated intradermally with 10,000 mouse LD50 of FMDV A-119 in guinea pig vesicular fluid. The steers were examined for signs of FMD at 2-day intervals for 14 days after exposure.

**RESULTS**

**Swine.** Neutralizing antibody measured as mouse PD50 values in the sera of vaccinated swine is shown in Table 1. By 7 DPV, the FMDV A-119 mouse PD50 values of serum from swine vaccinated with either 640 or 160 ng of virus were 10-fold higher than original values. By 28 DPV, the PD50 values had increased still further and to a greater degree in swine vaccinated with 640 ng of virus than with 160 ng. The PD50 values of sera from swine vaccinated with 40 ng of virus remained essentially the same as those values obtained from the sera of the nonvaccinated control swine.

Sera from swine vaccinated with 640 ng of FMDV A-119 had a mean mouse PD50 value of 1.6 at 7 DPV against FMDV A-119. The cross-

**TABLE 1. Serological responses to vaccination and to challenge of swine vaccinated with the indicated doses of purified FMDV A-119 vaccine inactivated with acetylated ethylamine and emulsified in oil adjuvant**

| Dose of vaccine (ng) | 0 DPV (A-119) | Time postvaccination | 7 DPV | 28 DPV | Postchallenge (A-119) |
|---------------------|--------------|----------------------|-------|--------|----------------------|
|                     | A-119        | A-119                | A-119 | A-119  | A-119                |
| 640                 | 0.5          | 1.60.40.5             | 2.82.82.4 | 4.1     |
| 160                 | 0.4          | 1.5                  | 2.2   | 3.9     |
| 40                  | 0.4          | 0.5                  | 0.8   | 3.7     |
| Controls            | 0.6          | 0.30.20.5             | 0.80.50.6 | 3.8     |

* Mean serological response expressed as mouse PD50 values in log10 units to the indicated FMDV at 0, 7, and 28 days postvaccination (DPV).

* Type A (subtype not designated) strain A-CANEFA-1.
neutralization potencies of the 7 DPV sera against FMDV A-CANEFA-1 and subtype A24 were 0.4 and 0.5 PD$_{50}$, respectively, or essentially the same as the sera of the nonvaccinated controls. By contrast, sera from these same swine at 28 DPV demonstrated similarly high, i.e., 2.8 to 2.4, mouse PD$_{50}$ values against all three of these subtypes of type A FMDV.

When the group of 18 swine, containing 6 animals vaccinated with each of the antigen doses, was challenged at 28 DPV with the homologous strain FMDV A-119, 5 of 6 vaccinated with the 640-ng dose and 3 of 6 vaccinated with the 160-ng dose were immune (Table 2). All six swine vaccinated with 40 ng, the smallest dose used, became infected when challenged with FMDV. When challenged with FMDV A-CANEFA-1, two of three swine vaccinated with the 640-ng dose were immune, whereas all six swine vaccinated with either 160 or 40 ng were susceptible and developed frank signs of FMD. Swine vaccinated with the 640-ng dose and challenged with FMDV type A24 were immune to challenge, whereas only one of three vaccinated with the 160-ng dose and none of those vaccinated with 40 ng were immune. All six of the nonvaccinated control swine were susceptible.

Steers. Serological response and immunity of steers after vaccination are shown in Table 3. At 7 DPV, mean mouse PD$_{50}$ values of 1.8 from the sera of steers vaccinated with 640 ng of antigen were appreciably higher than those (ca. 1.0 PD$_{50}$) of animals receiving the lower doses. By 28 DPV, the antibody levels (3.1 to 3.7 PD$_{50}$) resulting from the different doses of vaccine were markedly higher than any at 7 DPV and were of a similar magnitude, regardless of vaccine dose. All vaccinated steers together with three normal steers were challenged by contact exposure to two steers experimentally infected with FMDV A-119. The two inoculated control steers developed typical FMD lesions within 48 hr, whereas the three noninoculated controls did not develop lesions for an additional 48 hr. All nine of the vaccinated steers resisted this exposure.

Guinea pigs. Guinea pigs in group 1, receiving vaccine concurrently with the steers in which the 640 ng of antigen had been stored for 5 months at 4°C, developed PD$_{50}$ values of 1.8 log units at 7 DPV, which increased significantly by 28 DPV to 2.6. Guinea pigs vaccinated with 160 ng demonstrated no response at 7 DPV, but did develop a measurable response (1.4 PD$_{50}$) by 28 days. Those inoculated with the 40-ng dose gave no detectable response at either bleeding date. The guinea pigs in group 2, which were vaccinated along with swine with either 160 or 640 ng of antigen (stored 9 months at 4°C), developed measurable antibody responses (1.1 to 1.6 PD$_{50}$) at 7 DPV; however, their PD$_{50}$ values were considerably lower (0.7 log unit) at 28 DPV. As with group 1 guinea pigs, serum antibodies were not detectable in group 2 guinea pigs vaccinated with the smallest dose, 40 ng, at either 7 or 28 DPV.

**DISCUSSION**

The data provide quantitative information for the antigenicity and immunogenicity of the purified vaccine in steers and swine. A 160-ng amount of virus appeared to be the minimum

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**Table 2. Challenge of immunity at 28 days post-vaccination of swine vaccinated with the indicated doses of acetylateddimine-inactivated FMDV A-119 emulsified in oil adjuvant**

| Dose (ng) | Resistance to FMDV subtype |
|-----------|---------------------------|
|           | A-119 | A$^a$ | A$^{24}$ |
| 640       | 5/6$^b$ | 2/3 | 3/3 |
| 160       | 3/6   | 0/3 | 1/3 |
| 40        | 0/6   | 0/3 | 0/3 |
| Controls  | 0/2   | 0/2 | 0/2 |

$^a$ Type A (subtype not designated) strain A-CANEFA-1.

$^b$ Number of animals resistant to challenge over total number of animals tested.
effective antigenic dose in guinea pigs and swine and also to be close to the minimum immunogenic level for the latter. This dosage immunized 50% of the swine against the homologous virus type, whereas 40 ng was without effect and 640 ng protected five of six swine. By comparison, the smallest amount of antigen, i.e., 40 ng, used to vaccinate steers appeared to be in excess of the minimum effective dose. By 28 DPV, the three doses, 40, 160, and 640 ng, elicited similar serological responses in steers, all of which were immune to challenge. The higher inactivated vaccine dosage requirements for swine than for steers are in accord with the known requirements of immunizing swine during field outbreaks of FMD. The control of extensive outbreaks of FMD in swine requires a single vaccination of either monovalent Frenkel bovine tongue epithelium- or calf kidney cell-produced vaccine containing 4 to 10 times the antigen content required for cattle, or two inoculations of regular strength Frenkel vaccine spaced 2 weeks apart (17, 18). In limited experimental trials, vaccine containing incomplete Freund's adjuvant imparted protection to swine at a dosage similar to that used in cattle (10). However, the circulating antibody was considerably less than in cattle.

Vaccination of swine with purified-concentrated, inactivated FMDV A-119 antigen elicited serological and immunological responses to heterologous as well as to the homologous subtypes of the virus at 28 DPV with the 640-ng dose. Subtype cross-protection was not detected in serum taken at 7 DPV (Table 1), possibly indicating a higher degree of specificity for the 19S class of antibodies, the predominant species of antibody at the 7- DPV bleeding period.

Table 2 indicates relatively small differences in the immunity of the vaccinated swine to challenge with the three subtypes of FMDV. Examination of swine sera revealed that individual swine possessing an FMDV A-119 mouse PD50 value of 2.0 or greater were protected against all three challenge viruses, whereas those with PD50 values of less than 1.5 developed generalized FMD. Postchallenge FMDV A-119 mouse PD50 values in swine were essentially the same (3.7 to 4.1) regardless of previous vaccine experience or strain of challenge virus (Table 1).

In previous studies with this product, it was found that 10- to 16-fold differences in antigen were required to produce significantly different serological responses in guinea pigs in the region of the dose-response curve above the minimum effective dose (13). In the present work, vaccines with fourfold differences in antigen content in the region of the minimum effective dose produced distinctly different antibody responses in guinea pigs as well as different antibody and immunogenic responses in swine.

The purified concentrated antigen used for vaccine preparation in these experiments appeared to be stable for several months when stored at 4 °C. Because of space limitations, experiments in large animals cannot always be done at one time, and thus guinea pigs were vaccinated at the same time as the swine and steers to serve as a control of the vaccine. A change in the antigen during storage should be indicated by a difference in antibody response in the guinea pigs. The PD50 values obtained with the 28-DPV sera of the guinea pigs vaccinated at the same time as the swine could possibly indicate a deterioration of the antigen not detectable by either complement fixation or the absorbance-temperature tests, both of which indicated no significant changes in the antigen during storage. The latter two tests are in accord with the good immunogenicity of the stored antigen in swine and steers.

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