

## Antibody response in pig nasal fluid and serum following foot-and-mouth disease infection or vaccination

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### SUMMARY

Nasal fluid and serum collected from pigs after exposure to live foot and mouth disease (FMD) virus or injection of single oil emulsion (w/o) or double oil emulsion (w/o/w) vaccines were examined for FMD neutralizing activity. After virus exposure the response profiles of serum and nasal mucus were similar to one another. In both, neutralizing activity rose to a peak at one to two weeks after exposure and then subsided slowly. After vaccination with either the w/o or w/o/w preparations a neutralizing response was demonstrable in the serum three to seven days after the first injection, and this was boosted by revaccinations 56 and 117 days later. The neutralizing activity was also detectable in nasal fluid seven days after the first vaccination, but subsequent revaccinations 56 and 117 days later provoked neutralizing titres which were no greater than those observed after the initial vaccination.

### INTRODUCTION

The respiratory tract is thought to be the primary site of foot and mouth disease (FMD) infection in pigs (Donaldson & Ferris, 1980) and consequently neutralizing antibodies in the secretions may have a role to play in providing protection. Previous reports have failed to demonstrate neutralizing activity in secretions of pigs after vaccination against FMD (Wittmann, Bauer & Mussgay, 1970; Wittmann, 1972) although such activity has been demonstrated in the secretions of cattle following either infection or vaccination (Hyslop, 1965; Figueroa, Ohlbaum & Contreras, 1973; Garland, 1974; McVicar & Suttmoller, 1974; Matsumoto, McKercher & Nusbaum, 1978; Pinto & Garland, 1979; Francis, Ouldrige & Black, 1983). The present work was undertaken to provide further information on the presence of neutralizing activity in the nasal secretions of pigs after either FMD virus infection or vaccination. For comparative purposes the profile of neutralizing activity in the serum was also followed.

### MATERIALS AND METHODS

#### *Animals*

Fifteen FMD-susceptible Large White pigs, 8-10 weeks old, were housed in quarantine conditions for the duration of the experiments.

### *Vaccines*

The vaccines were produced from A<sub>24</sub> Cruzeiro virus strain grown on baby hamster kidney 21 clone 13 suspension cells (Capstick *et al.* 1967). The virus harvest was inactivated with acetylethylencimine as described by Pay *et al.* (1971) and made up as either single (w/o) or double (w/o/w) oil emulsion vaccines (Basarab, 1978). These vaccines were formulated to contain identical payloads of 2 µg 146S antigen per 2 ml dose. This relatively low antigen payload was used to ensure that the effects of revaccination would be clearly demonstrable.

### *Virus exposure and vaccination schedules*

In the first part of the experiment nine pigs were exposed to FMD infection by injecting 100 ID<sub>50</sub> of pig-adapted O<sub>1</sub>BFS 1860 virus, in 0.1 ml 0.04 M phosphate buffer, into each heel bulb of one forefoot of each pig.

In the second part, six pigs were injected intramuscularly with 2 ml of A<sub>24</sub> Cruzeiro vaccine at 0, 56 and 117 days. Of these, three pigs received w/o vaccine and three received w/o/w vaccine.

### *Sampling*

Blood samples were collected daily for the first three days from the pigs exposed to live FMD virus and at weekly intervals thereafter for seven weeks. Nasal fluid was collected on the first day after virus exposure and then weekly for seven weeks. Similar samples were also collected from the vaccinated pigs at the time of vaccination, three days later and then weekly for eight to nine weeks.

Nasal fluid (ca. 0.5–1 ml) was collected using a method described by Baskerville & Lloyd (1977). Briefly, a PVC tube (1 mm internal diameter) attached to a plastic mucus extractor (Henley's Medical Supplies, London) was inserted into the ventral meatus of the pig's nostril and the outer tube of the extractor was connected to a vacuum pump. The extracted nasal fluid was diluted 1 in 2 in complete phosphate-buffered saline (pH 7.3) containing antibiotics, heat-inactivated at 56 °C for 30 min and stored at –20 °C until tested. Serum samples were also inactivated by heating to 56 °C for 30 min, and stored at –20 °C until used. If there was any evidence of bleeding in the pig's snout during sampling or blood tinging in the collection bottle the nasal fluid samples were discarded.

### *Neutralization tests*

The sera were examined using a modification of the method described by Golding *et al.* (1976). Briefly, 50 µl volumes of twofold dilutions of serum, prepared in Eagle's basal medium containing 2% steer serum, were mixed with 50 µl of a suspension containing 100 TCID<sub>50</sub> of homologous FMD virus, adapted to grow in IB-RS-2 cells (de Castro, 1964), in flat-bottomed microplates (Grade M29 ART, Sterilin Laboratories) and allowed to stand for 1 h at room temperature. Fifty microlitres of IB-RS-2 cells (1 × 10<sup>6</sup> cells/ml) was then added to each cup and the plates were sealed and incubated for 48 h at 37 °C. Finally, the plates were flooded with 10% citric acid in 0.85% saline to fix the cell sheets and inactivate remaining virus. The fixative was discarded after 30 min and the cells were stained by flooding the plates with 0.4% Naphthalene black in 0.85% saline. After a further 30 min

the plates were rinsed in sterile distilled water, shaken free of droplets and allowed to dry by evaporation. Each test was done in duplicate and the titration end-points were taken as the reciprocal of the serum dilution which gave confluent cell sheets in 50% of the cups. The neutralizing activity of the nasal fluid samples was determined by the same method except that the virus dose was reduced to 10 TCID<sub>50</sub>. The results of neutralization tests carried out on the nasal fluid of FMD-susceptible pigs prior to vaccination or virus exposure, using this reduced dose, were consistently lower than 0.9 log<sub>10</sub> (the lowest dilution tested), consequently titres of 0.9 log<sub>10</sub> and above were regarded as positive for neutralizing activity.

## RESULTS

Fig. 1 shows the mean FMD neutralizing antibody responses in both the serum and nasal fluid after exposure to live FMD virus. Both reached peaks at 8–15 days and remained at demonstrable levels for at least 50 days.

Figs 2*a* and 2*b* show the mean FMD neutralizing antibody responses in the serum and nasal fluid of pigs after w/o or w/o/w vaccination. In the serum the neutralizing activity was detectable at three to seven days and reached a peak between 21 and 28 days after the initial vaccination. Subsequent vaccinations at 56 and 117 days boosted the neutralizing antibody levels two- to four-fold, and new peak levels were reached 7–14 days after each revaccination.

Both groups of pigs also developed FMD neutralizing activity in their nasal fluid after a single w/o or w/o/w vaccination which could be demonstrated for approximately 50 days. Revaccination at 56 and 117 days again provoked neutralizing activity in the nasal fluid, the titres and duration of which were similar to those observed after primary vaccination.

Throughout these experiments the variation in response between individual pigs was measured. The mean coefficient of variation for the nasal fluid and serum titres after exposure to live virus was 17.2% and 10.1% respectively, and after vaccination 14.8% and 26.6% respectively.

## DISCUSSION

The neutralizing response profiles in the nasal fluid and serum after FMD virus exposure were similar to one another. Both reached their peak at one to two weeks after exposure and persisted for at least 50 days. The similarity of these response profiles may be contrasted with the results obtained in cattle, where the secretory neutralizing activity in the pharyngeal fluid reached a peak two to three weeks after that in the serum (Garland, 1974; McVicar & Suttmoller, 1974; Francis, Ouldrige & Black, 1983). The pig responses observed here suggest either that the nasal fluid neutralizing activity was serum derived or that pigs were able to mount a more rapid secretory antibody response to FMD antigen stimulation than cattle.

The serum antibody responses to primary w/o or w/o/w emulsion vaccines were similar to one another. Both provoked demonstrable responses within seven days which reached peak values at approximately 21 days, indicating that single and double emulsion vaccines were equally effective at immunizing pigs against FMD. Subsequent vaccinations provoked sharp increments in serum antibody titres

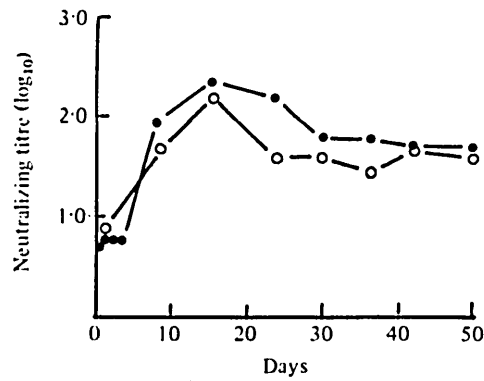


Fig. 1. The mean nasal fluid (○—○) and serum (●—●) neutralizing antibody responses following FMD virus exposure.

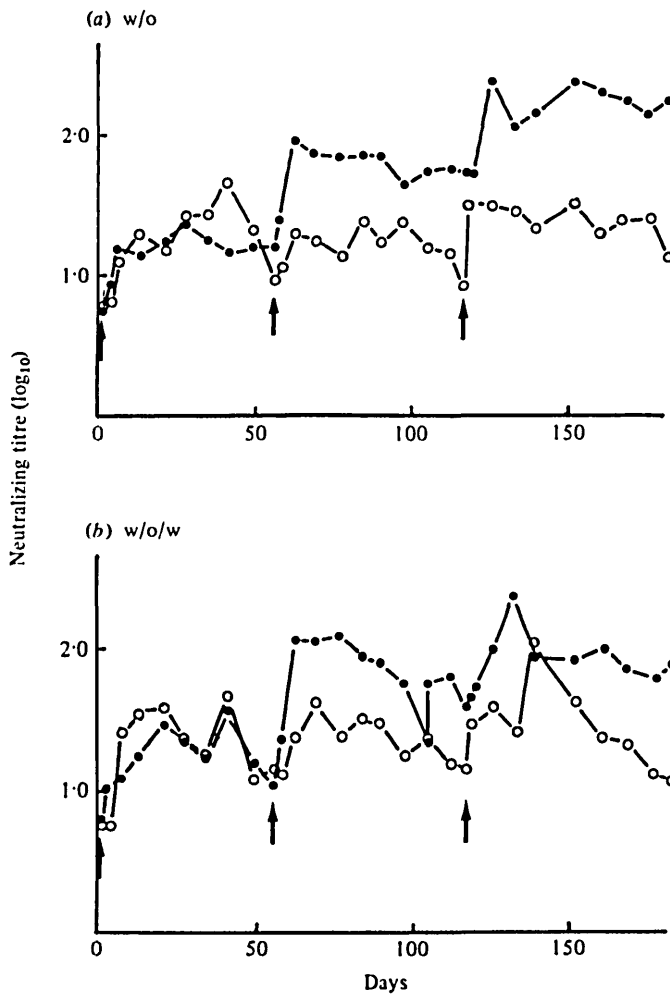


Fig. 2. The mean nasal fluid (○—○) and serum (●—●) neutralizing antibody responses following single (w/o) or double (w/o/w) oil emulsion FMD vaccination.

within 7 to 14 days, indicating that the animals had been primed by the first vaccination. These results support the findings of previous studies using Wellcome oil emulsion vaccines in pigs (Basarab, 1978; Ouldrige, Francis & Black, 1982).

The neutralizing activity observed in the nasal mucus after either w/o or w/o/w vaccination was generally lower than that observed in the serum, especially since the lower dose of virus used to examine neutralizing activity in nasal fluid had the effect of increasing the sensitivity of the tests. The differences between the nasal fluid and serum neutralization titres became especially marked after the second and third vaccinations, because revaccination appeared to provoke an anamnestic increase in the serum titres but not in the nasal fluid titres.

Previous studies have failed to detect neutralizing activity in pig secretions after FMD vaccination (Wittmann, Bauer & Mussgay, 1970; Witmann, 1972). However, these experiments involved either stimulation of secretion by injections of parasympathetic mimetic drugs or rinsing of the nasal cavity with large volumes of buffer, both of which may have reduced the concentration of any neutralizing antibodies to undetectable levels.

The fact that neutralizing activity was detectable in the nasal secretions of pigs after a single vaccination may be contrasted with the results obtained in cattle, where at least one revaccination was required before such activity could be demonstrated (Garland, 1974; Francis, Black & Rweyemamu, 1981). The early appearance of neutralizing activity in pig secretions suggests that mucosal protective mechanisms may be more important in pigs than in cattle. This may explain why poor correlations have sometimes been observed between the serum antibody titres and protection from infection by contact in this species (Wittman, Bauer & Mussgay, 1970, 1972).

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