Humoral response of pregnant sows to foot and mouth disease vaccination

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SUMMARY

Four groups of sows were inoculated, either once or twice, with O_1BFS 1860 foot and mouth disease oil-emulsion vaccine during pregnancy and samples of serum, for analysis, were collected at intervals for > 300 days.

The pregnant sows responded well to vaccination regardless of their state of gestation. Single vaccination produced protective levels of antibody ($> 1.53 \log_{10}SN_{50}$) in 3 out of 4 sows while double vaccination produced protective levels in all 6 sows tested. Anti-FMD IgM antibodies could be detected for 40–60 days after vaccination or revaccination. Anti-FMD IgG antibodies appeared within 10 days of vaccination and persisted, in each sow, for the duration of the study. The anti-FMD IgA response observed was less easy to characterize due to significant animal to animal variation. Although there was no evidence of a fall in the neutralizing antibody titres over one year post vaccination the anti-FMD IgG antibody population did show signs of a change in its heterogenity and avidity.

INTRODUCTION

The effectiveness of foot and mouth disease (FMD) oil emulsion vaccines has been widely studied in fattening pigs from 1 to 2 up to 10 months old (McKercher & Giordano, 1967a; Anderson, 1969; Basarab & Pay, 1982; Ouldridge, Francis & Black, 1982). However, little information is available on the immune response of pregnant sows to FMD vaccination. Such information is essential for the design of rational vaccination regimens for breeding stock. This will also ensure that adequate levels of passive antibody of the correct immunoglobulin class are passed onto new-born piglets (Francis & Black, 1984a, b).

This study was designed to provide information on the level and persistence of FMD virus (FMDV) neutralizing antibody in the pregnant sow, the antibody classes involved and whether the nature of the antibodies changes with time after vaccination.

MATERIALS AND METHODS

Vaccination schedule

Ten pregnant Large White sows, never previously vaccinated against nor exposed to FMD, were divided into 3 groups of 2 and 1 group of 4 and vaccinated

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intramuscularly with a 2 ml dose of FMD oil emulsion vaccine (containing either 5·2 μg, groups A–C, or 13·4 μg, group D, of intact 146S antigen) according to the following schedule:

Group A: Sows A1 and A2 vaccinated once at 12–13 days before farrowing (dbf).
Group B: Sows B1 and B2 vaccinated once at 30–32 dbf.
Group C: Sows C1 and C2 vaccinated twice at 51–52 and 31–32 dbf.
Group D: Sows D1, D2, D3, and D4 vaccinated twice at 87–89 and 30–32 dbf.

Serum sampling

Serum samples were collected from each sow at the time of vaccination or revaccination and at frequent intervals (generally weekly) thereafter for more than 300 days post initial vaccination (dpiv) and tested for neutralizing activity. Sera collected up to 80 dpiv from sows in groups A, B, and C and up to 90 dpiv from group D were also tested by ELISA, at a single dilution of 1 in 50, for immunoglobulin class activity using IgG, IgM or IgA specific conjugates.

Samples collected from one singly vaccinated sow (A1) and one doubly vaccinated sow (C1) were further analysed by ELISA for IgG antibodies using (*a*) a titration method and (*b*) a logistic analysis.

All serum samples were stored at −20 °C and inactivated at 56 °C for 30 min prior to testing.

Neutralizing antibody assessment

The neutralizing activity of the sow serum samples against 100TCID$_{50}$ of FMDV was examined using a microneutralization test (Francis & Black, 1983). Each test was performed in triplicate and the results were recorded as the mean log$_{10}$ reciprocal of the serum dilution which gave confluent cell sheets in 50% of the microplate wells.

Correlation of neutralization test results with protection

In order to evaluate fully the FMDV neutralizing antibody titres reported it was important to determine their correlation with protection from infection. Sera collected from 156 pigs prior to O$_1$BFS 1860 FMDV needle challenge (Burrows, 1966) were tested on three separate occasions for *in vitro* FMDV neutralizing activity and the data produced was used in a probit analysis.

Enzyme linked immunosorbent assay (ELISA)

Immunoglobulin class activity. Sow serum samples were tested for anti-FMD immunoglobulin class activity using an indirect ELISA method (Francis, Ouldridge & Black, 1983). Briefly, microplates were coated overnight at room temperature with FMDV 146S antigen. The plates were washed and test samples diluted 1 in 50 were added. After 1 h incubation at 37 °C plates were washed and anti-pig IgM, IgA or IgG peroxidase conjugate (Department of Animal Husbandry, Bristol University, UK) was added. After a further 1 h at 37 °C, the plates were washed and an enzyme substrate was added. After 2–8 min, colour development was stopped with 12·5% sulphuric acid and the optical density (OD) at 492 nm was measured in a Multiskan (Flow Laboratories).

Table 1. *Probit analysis of the O_1BFS neutralizing antibody titration and protection results for 156 O_1BFS challenged pigs*

Percentage protection	Predicted $\log_{10}$ antibody titre (PA value)*			
	Estimate	Standard error	95 % fiducial confidence limits	
1	−0·38	0·29	−1·21	0·05
10	0·37	0·18	−0·11	0·64
20	0·70	0·13	0·33	0·90
30	0·92	0·10	0·65	1·09
40	1·11	0·09	0·91	1·27
50	1·30	0·08	1·13	1·46
60	1·48	0·08	1·32	1·67
70	1·67	0·10	1·51	1·92
80	1·90	0·12	1·71	2·23
90	2·22	0·17	1·96	2·68
99	2·97	0·28	2·55	3·76

* PA value for 62·5 % protection is 1·53.

Anti-FMD IgG titration. Selected sera from sows A 1 and C 1 were serially diluted in 0·5 $\log_{10}$ steps from 1·0 to 4·0 $\log_{10}$ and tested in an indirect ELISA (as described above but using an anti-pig IgG peroxidase conjugate supplied by Miles Laboratories, UK). The mean results of duplicate tests were plotted graphically as OD at 492 nm against $\log_{10}$ reciprocal antiserum dilution and used to calculate the anti-FMD IgG antibody titre ($\log_{10}$).

Logistic model anlysis. The indirect ELISA technique described above was also modified, using a fixed serum dilution of 1 in 10 and variable FMDV 146S antigen concentrations ranging from 0 to 8 μg/ml, in order to provide data for logistic model analysis (Hingley & Ouldridge, 1985). The logistic model used a computer programme to fit a sigmoid shaped curve to the OD values obtained. The parameterisation of the model used was:

$$OD_i = OD_{max} \left[\frac{(\overline{K}x_i)^a}{1 + (\overline{K}x_i)^a} \right] + \epsilon_i$$

Where

OD_i Optical density corrected for no antigen (blank) for the ith observation.
OD_{max} Plateau value of optical density.
$\overline{K}$ Average intrinsic association constant.
a Heterogeneity index.
x_i Concentration of antigen for ith observation.
ϵ_i Normally distributed error (mean O, variance σ^2) for ith observation.

Although it is difficult to carry out reliable investigations into the functional affinity (avidity) of FMDV/antibody interactions, due to the heterogeneity of the antigens and antibodies involved, the $\overline{K}$ value obtained by logistic analysis gives the association constant between FMD antigen fixed to the ELISA plates and the IgG antibodies in the serum of the sows and thus an increase in $\overline{K}$ may be taken as an increase in avidity of the antibody population being studied. The heterogeneity index (a) is a measure of the number of different molecular species of IgG which
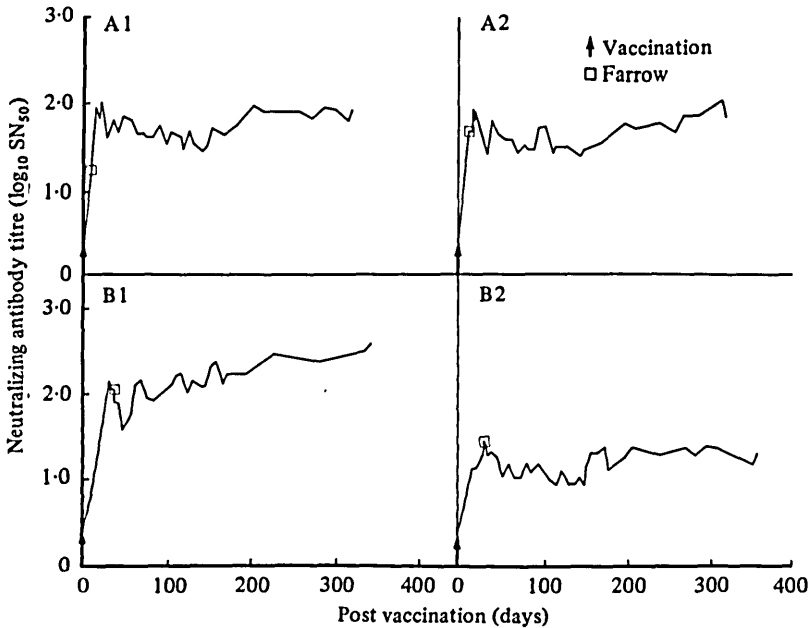
Fig. 1. Neutralizing antibody response of sows to single vaccination at 12–13 or 30–32 dbf.

can range from a value of 0–1; low values represent a large degree of heterogeneity, whereas monoclonal antibodies would have an index of 1.

RESULTS

Table 1 summarizes the predicted $\log_{10}SN_{50}$ titres (PA values) of the probit analysis carried out on the data obtained from 156 pigs at selective intervals between 1 and 99% protection, their standard errors and the 95% fiducial confidence limits. The PA value for 62·5% protection is of special interest since it corresponds to 5 out of 8 pigs protected which is the accepted passmark for oil emulsion vaccines in pig potency tests.

The mean results of the repeated virus neutralization tests are shown in Figs 1, 2 and 3. From Fig. 1 and 3 it can be seen that neutralizing activity reached peak levels 2–4 weeks after primary vaccination and that this activity began to subside within 4–8 weeks.

In those sows that did not receive a booster vaccination (Fig. 1) the neutralizing activity returned to its original peak level about 14–35 weeks after vaccination. The level of neutralizing activity was then sustained or increased steadily (sows B2 and A2) until the end of the experiment (10–12 months after the original vaccination).

In 3 out of the 4 singly vaccinated sows virus neutralizing antibody levels remained above the 50% protection value ($1·30 \log_{10}SN_{50}$) and close to the 62·5% protective level ($1·53 \log_{10}SN_{50}$) from 1 to 2 weeks after vaccination for more than 45 weeks. The exception was sow B2 the neutralizing antibody titres of which remained at or just below the 50% protective level. Figs. 2 and 3 show that
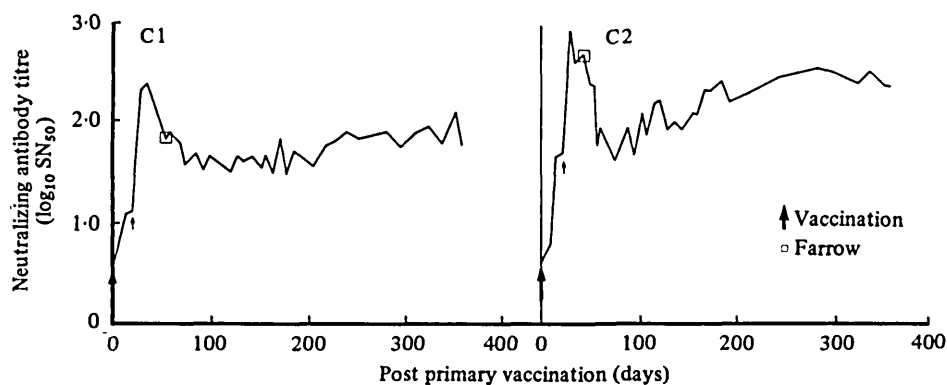
Fig. 2. Neutralizing antibody response of sows to double vaccination at 51–52 and 31–32 dbf.

revaccinating the sows at either 20 or 57 dpiv boosted antibody levels from approximately $1{\cdot}5 \log_{10}SN_{50}$ to more than $2{\cdot}2 \log_{10}SN_{50}$ within 7 days. The peak of this activity subsided during the next 7–10 weeks but, as in the case of the singly vaccinated sows, some of the sows titres gradually increased or else remained constant over the following months. Although the neutralizing activity never reached the revaccination peak levels it did persist at between 70 and 90 % protection levels for the duration of the experiment (312–359 dpiv). It is interesting to note that at the time of revaccination (20 dpiv) the serum antibody titre of sow C 1 ($1{\cdot}13 \log_{10}SN_{50}$) was almost identical with that of sow B 2 ($1{\cdot}14 \log_{10}SN_{50}$) and that revaccination boosted the antibody titres of C 1 to a 70–80 % protection level for more than 10 months while those of B 2 remained at only a 40–50 % protection level.

There was some indication of a limited fall in the SN_{50} titres immediately after farrowing but this was not severe and was only of short duration.

The mean optical density (OD) values from two immunoglobulin class-specific ELISA tests are presented as immunoglobulin class response curves for each animal in Figs. 4–6. It is not possible to make direct comparisons between the magnitude of each immunoglobulin class response as the activity of the specific conjugates used in the ELISA test could not be equated. However, it is possible to make comparisons between the profiles of response for the different antibody classes and to examine the effect of vaccination and revaccination on them. Following a single vaccination (Fig. 4) the IgM activity reached a peak within 1–2 weeks and then subsided to undetectable levels after 5–9 weeks. In contrast, the IgG response in the same sows did not reach a peak until 3–8 weeks after the primary vaccination and persisted for at least 11 weeks. The IgA response was generally more variable. For example, in sow A 2 the IgA response was especially pronounced and a peak of activity was observed 2–3 weeks after primary vaccination which persisted for more than 11 weeks. In sows A 1 and B 2 however, IgA activity was only present at very low levels. The remaining singly vaccinated sow (B 1) had a biphasic IgA response curve with peaks of activity at 27 and 73 dpiv. Revaccination of the sows (Figs. 5 and 6) resulted in further IgM activity similar to that observed in the
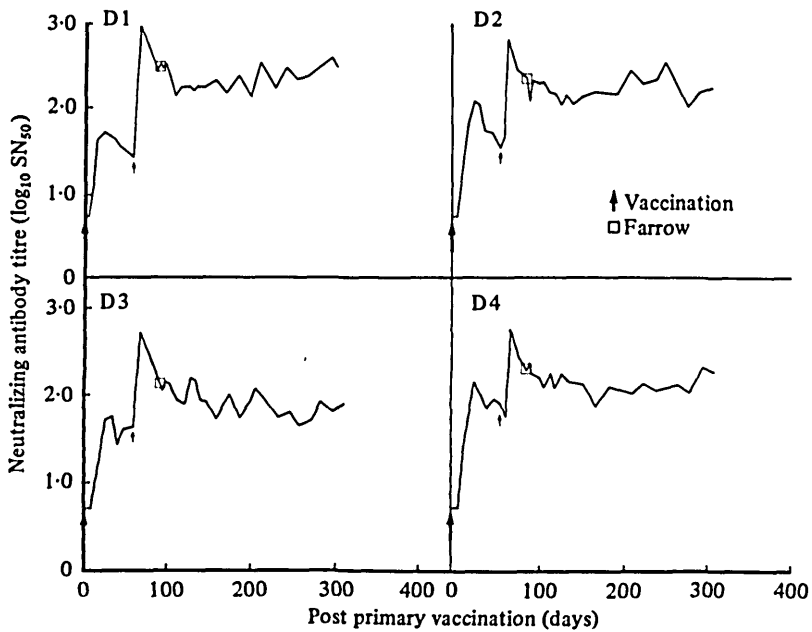
Fig. 3. Neutralizing antibody response of sows to double vaccination at 87–89 and 30–32 dbf.
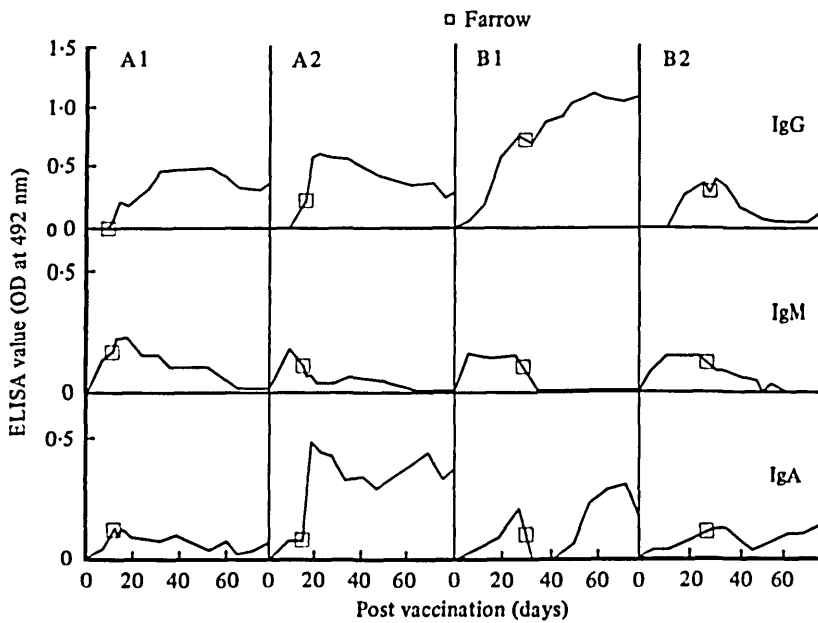

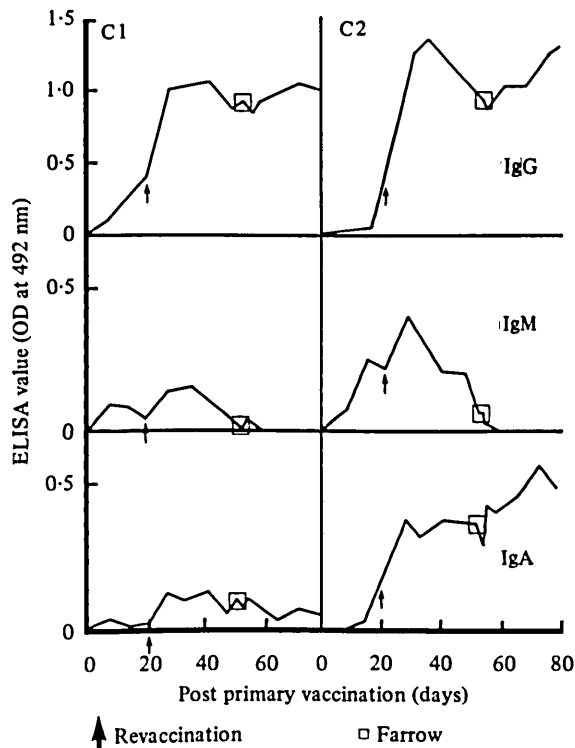Fig. 4. Immunoglobulin class response of sows to single vaccination at 12–13 or 30–32 dbf.

Fig. 5. Immunoglobulin class response of sows to double vaccination at 51–52 and 31–32 dbf.

primary response. The IgG response, however, was significantly increased in magnitude to reach peak activity 1–2 weeks after revaccination. This effect was most apparent in sows revaccinated at 57 dpiv (D1–D4) as the primary response had either levelled off or was declining by then. Revaccination did appear to increase the IgA responses although the magnitude of these revaccination responses also varied from one animal to another.

Farrowing did not appear to have any marked effect except for a minor and short-lived drop in the immunoglobulin titres (see Fig. 5). However, it is worth noting that the relative distribution of class activity at the time of farrowing was greatly affected by the sow's vaccination regimens.

The calculated IgG titres for sows A1 and C1 are shown in Figs. 7a and b. For comparison, neutralizing antibody titres for each sample are also given, showing that the ELISA IgG response profiles for both sows were similar to the neutralizing antibody profiles, other than those values resulting from the 18 days post vaccination (dpv) sample for sow A1 and the 20 and 51 dpiv samples for sow C1. The ELISA test results in those cases may have been influenced by competition due to the presence of anti-FMD IgM activity in the sera.

Figures 7a and b also show that there were differences between the $\overline{K}$ and $\underline{a}$ curves of the two sows studied. Thus, in the serum samples from the singly vaccinated sow (A1) the $\overline{K}$ and $\underline{a}$ values increased steadily for at least 300 days indicating
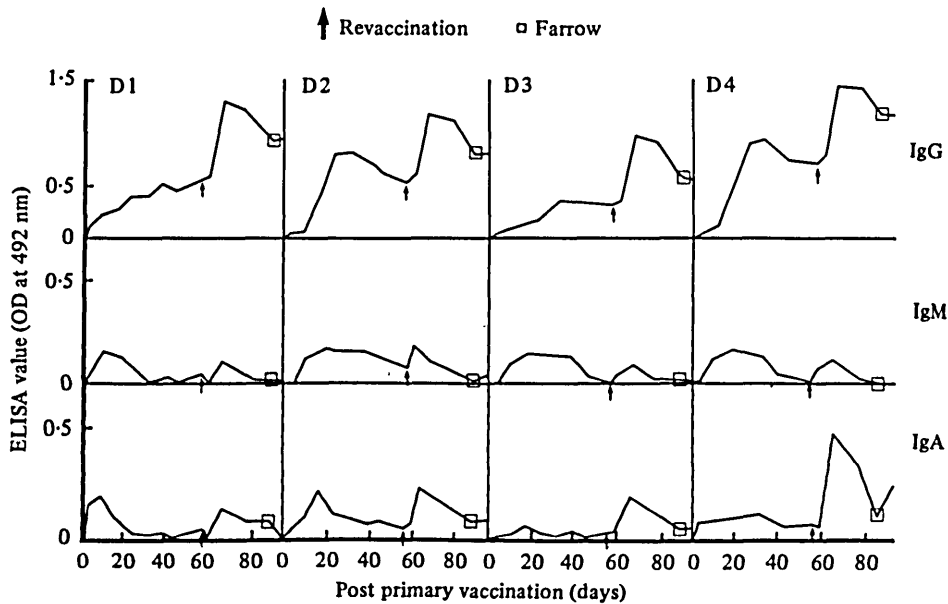
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Fig. 6. Immunoglobulin class response of sows to double vaccination at 87–89 and 30–32 dbf.

that the IgG antibody population became more avid and less heterogeneous with time after vaccination, while in the doubly vaccinated sow (C1) both $\overline{K}$ and $\underline{a}$ increased for 30–80 days after revaccination and then remained fairly constant for the next 200–250 days suggesting that from 80 days after revaccination the avidity and heterogeneity of the antibodies remained more or less unchanged.

DISCUSSION

A single vaccination of pregnant sows with FMD oil emulsion vaccine resulted in neutralizing antibody levels that would be regarded as protective ($> 1 \cdot 53 \log_{10}SN_{50}$) in 3 out of the 4 sows tested. Furthermore, the antibody levels were sustained for nearly a year with no decline. This persistence was also observed in the sow with a low neutralizing antibody titre (B2) indicating that the duration of the response was not affected by the animals poor initial response. These observations compare favourably with anti-FMD responses seen previously in young fattening pigs (McKercher & Giordano 1967a; Graves, Cowan & Trautman 1968; Wittmann, Bauer & Mussgay, 1969; Anderson, 1969; Basarab, 1978; Ouldridge, Francis & Black, 1982).

Repeating the vaccination during pregnancy, with an interval of either 20 or 57 days between doses, provoked neutralizing antibodies which were maintained for nearly 1 year at levels that would be expected to afford 70–90 % protection from FMD in all six sows, including one that had shown a poor primary response. Thus it is possible that revaccination during pregnancy may overcome any problem of a poor primary response and would assure that the sow would have long lasting high titre antibodies against FMD virus. In these experiments farrowing had little
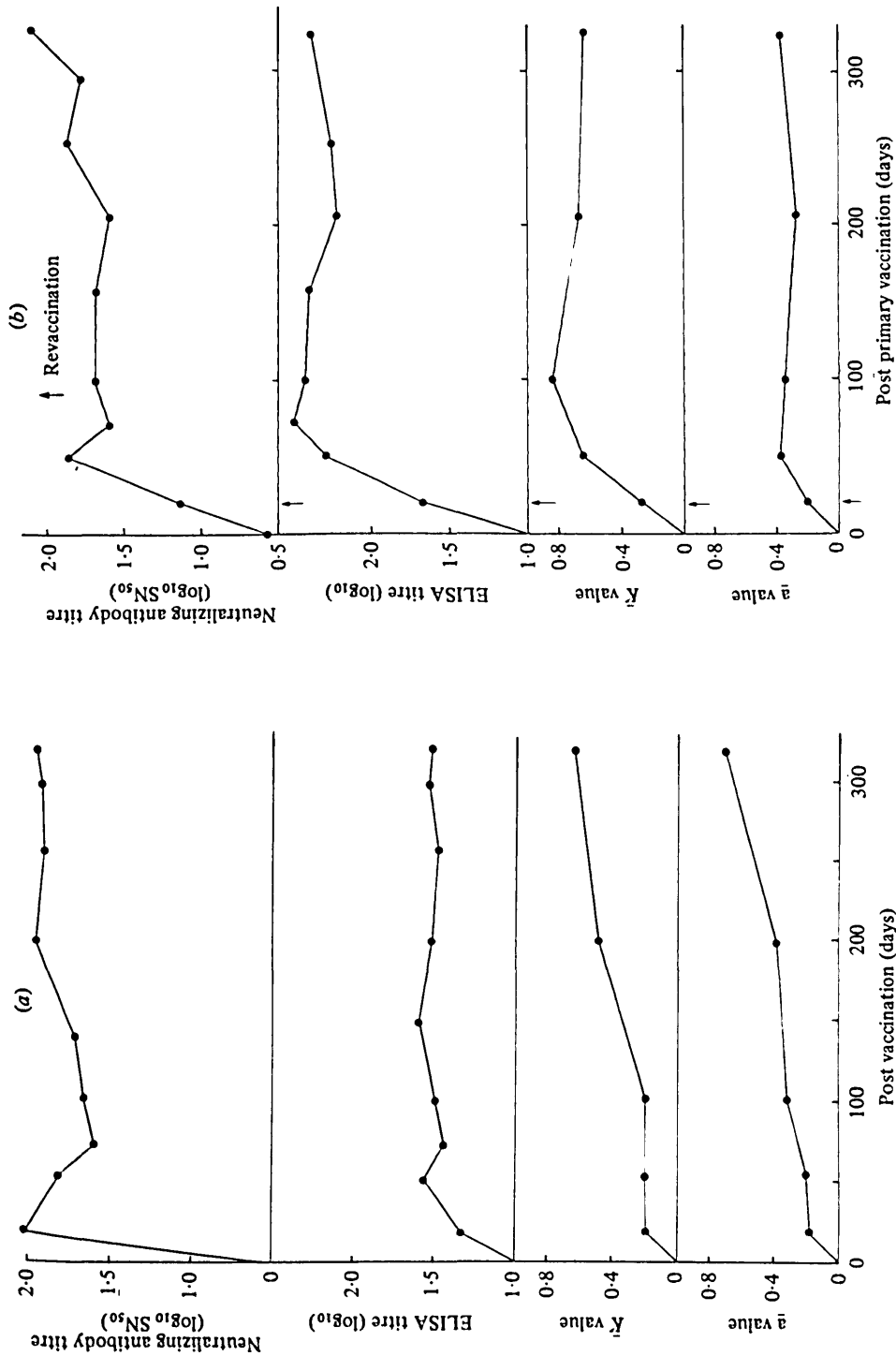
Fig. 7. Nature of the anti-FMD IgG response to (*a*) single vaccination (sow A1) and (*b*) double vaccination (sow C1).

effect on the neutralizing antibody response which is in agreement with the findings of Morgan & McKercher (1978).

This study is the first in which anti-FMD immunoglobulin class activity in the pig has been studied using the ELISA technique. Previous investigations into the virus neutralizing activity of vaccinated pig sera have employed sucrose density gradient or gel filtration techniques (McKercher & Giordano, 1967b; Anderson, Masters & Mowat, 1971; Ouldridge, Francis & Black, 1982) in order to examine the IgM and IgG activity. Following vaccination with an aqueous vaccine, containing saponin and aluminium hydroxide as adjuvants, only IgM antibodies were detectable and it was necessary to use oil emulsion vaccines in order to evoke a switch to IgG production, which would induce long lasting immunity. The ELISA data obtained from pregnant sows supports these observations by demonstrating that IgM activity is transient, lasting for only 40–60 days after vaccination, and that it is the IgG antibodies that provide a sustained index of immunity. Although an IgA response was also present after both primary and secondary vaccinations no clearcut conclusions about the magnitude and duration of the response were possible because of marked animal to animal variation. However, if they were present in the local secretions, such antibodies would provide the animals with their first line of defence against airborn infection (Francis & Black, 1983).

The observation that the IgG antibody population becomes gradually less heterogeneous and more avid with time has been made previously with antibodies against polio (Svehag, 1965), bacteriophage (Finkelstein & Uhr, 1966) and influenza (Webster, 1968), in various animal species. This observation may account for the apparent gradual increase in the FMD virus neutralizing antibody titre since neutralization is a function not only of antibody concentration but also of affinity, valency and possibly molecular confiuration of the antibody (Blank, Leslie & Clem, 1972). In the present study revaccination of the sows also increased the avidity and decreased the heterogeneity of the antibody population. Furthermore, the change was more rapid and levelled off after 30–80 days suggesting that a population of memory cells, produced as part of the animals immune response to the primary vaccination, were being stimulated. However, a more extensive study using the logistic analysis method would be required before any firm statements could be made regarding the effect of FMD vaccination regimen on antibody avidity and heterogeneity.

In conclusion, the results have shown that pregnant sows respond well to FMD vaccination and that initial double vaccination, with the doses given 1 month apart, will provide adequate immunity for at least a year. Subsequent annual revaccination should then be sufficient to maintain neutralizing antibodies above the protective level. In areas where the risk of exposure to FMD is high a 6-month interval or regular revaccination at time of service would be advisable. Such a regimen would ensure that sows pass high titres of passive IgG antibody to their litters.

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