THE HUMORAL RESPONSE OF SOWS AND YOUNG PIGS TO FOOT AND MOUTH DISEASE VACCINATION

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1985

This thesis was submitted in fulfilment of the degree of Doctor of Philosophy.

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SUMMARY

Groups of pregnant sows were inoculated with type O_1 foot and mouth disease (FMD) oil emulsion vaccine at various times before farrowing and samples of the sow's serum, colostrum and milk, and piglets' serum were collected for analysis.

Pregnant sows responded well to vaccination regardless of their state of gestation. Single vaccination produced protective levels of antibody in three out of four sows while double vaccination produced protective levels in all six sows tested. Although there was no evidence of a fall in the neutralizing antibody titres over one year post vaccination the IgG antibody population did show signs of a change in its heterogeneity and avidity.

No FMD neutralizing antibodies were detectable in the piglets' serum at birth but they were present 1.5hr after suckling and peak titres were reached one to three days later. Samples of colostrum/milk collected from different teats three days after farrowing showed significant ($P<0.005$) fore to hind variation. A significant correlation was also observed between the sow's serum titres and colostrum titres at farrowing ($r=0.90$), and between sows colostrum titres at farrowing and their three day old piglets serum titres ($r=0.99$).

When sows were vaccinated 12 to 13 days before farrowing (dbf) the predominating neutralizing antibody at parturition was IgM and the observed half-lives of the maternally derived antibodies in the piglets were short (four to eight days). However, when sows were last

vaccinated 30 to 32dbf, the maternally derived neutralizing antibodies in the piglets were predominantly IgG and the observed half-lives were seven to 21 days. If corrections were made for increase in blood volume the decay rates of IgM antibodies in piglets were seven to 18 days while the decay rate for IgG was greater than 408 days.

The response of young pigs to FMD vaccination and the effect of maternally derived antibodies on this response was also studied. Piglets born to non-immunized sows were able to respond to vaccination when one week old, with no deleterious effect on their growth rate. However, total suppression of the vaccination response was observed in one, two and four week old piglets born to immunized sows and a partial suppression occurred in eight week old piglets. This maternal antibody suppressive effect could be mimicked by the passive transfer of neutralizing IgG antibodies into older piglets.

ACKNOWLEDGEMENTS

I should like to express sincere thanks to my supervisors, Dr M Butler and Dr L Black, for their continued support and guidance throughout this study. I should also like to thank Mr B Dyson and Mr B Taylor for looking after the animals, Mr P Hingley for his advice on logistic analysis, and Prof. F J Bourne and his staff for supplying the anti-porcine immunoglobulin conjugates. I am especially grateful to Mrs B Matthew for her assistance in typing and preparing this final manuscript. Finally, I should like to thank my family and colleagues for their continued encouragement.

Financial support and facilities which enabled this work to be undertaken were provided by the Wellcome Foundation Ltd.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

While oil-emulsion vaccines appear to provide the best means of prophylaxis against FMD in pigs there are still many questions relating to the response of sows and young piglets that need to be answered.

To date, little attention has been directed towards the sows' vaccination regimen and the class of neutralizing antibody class present in their sera and colostra at time of farrowing. This is important because the anti-FMD antibody class predominating at this time may affect both the uptake and persistence (half-life) of maternally derived antibodies in the young piglets. Furthermore, there is a need to determine the earliest age at which piglets are competent to respond to oil vaccination and whether passively acquired antibodies have any suppressive effect on this response, since reports to date are unclear on this matter.

This study has therefore been designed to investigate:

1. The effect of the sow's vaccination regimen on her serological response, and the relationship between the sow's immune status at farrowing and that of her litter for one week after birth.

2. The half-lives of neutralizing antibodies in the piglets and the factors affecting the rate of decay, such as the antibody class and the piglet's growth rate.

3. The response of young piglets, born to vaccinated and unvaccinated sows, to oil vaccination.

4. The effect of injected antibody on the pig's response to oil vaccination.

The results obtained will provide useful information on the relationship between passive protection and active immunization against FMD in this species and, thus, should enable vaccine manufacturers to recommend vaccination regimens for breeding stock on a more rational basis.

1.2 PORCINE IMMUNOLOGY

a) The immune system of sows and their young

Three main immunoglobulins, analogous to human IgG, IgA and IgM, have been identified in pig serum and colostrum (Kim, Bradley and Watson, 1966(b); Karlsson, 1966) and of these IgG was the first to be isolated (Metzyer and Fougereau, 1967). These workers described two distinct subclasses, IgG_1 and IgG_2, with identical sedimentation coefficients of 6.7-7.0S but which could be separated by their electrophoretic mobilities. More recently three, possibly four, subclasses of IgG have been identified by Kaltreider and Johnson (1972). In 1969, Porter identified an IgM rich fraction from serum which was contaminated with a similar molecular weight protein, α_2 Macroglobulin. Porcine IgM has since been shown to occur as a pentameric form in serum with a sedimentation coefficient of 17.8-19.0S (Bourne, 1971; Porter and Allen, 1972). In the same year, Bourne (1969) successfully isolated porcine IgA from milk and colostrum, and this has also been isolated from serum, saliva, urine, respiratory tract secretion and vaginal mucous (Porter and Allen, 1972). Porcine IgA in external secretions occurs mainly as dimeric (9.3-9.5S or 10.8-11.6S) molecules but in serum it occurs as monomeric (6.4-7.0S) and dimeric (9.3-9.5S) molecules in equal proportions. The 10.8-11.6S secretory IgA also has a secretory component with a sedimentation value of 4.3S and a molecular weight of 80-90,000 daltons. Two structural forms of IgA have been identified in pigs, similar to IgA_1 and IgA_2 in man (Bourne, 1971).

Immunoglobulin G is quantitatively the major antibody class in porcine serum and colostrum, constituting 80% of the total. Curtis and Bourne (1971) found that the concentration of IgG in colostrum was approximately 3-fold higher than in sow serum (Table 1.1), the mean value being between 50 and 60mg/ml. However, the actual concentration of IgG in colostrum may vary between 11.74 and 101.39mg/ml (Inoue, Kitano and Inoue, 1980). A five-fold drop occurs in this concentration in the first 24 hours of lactation followed by a thirty-fold drop in the first week. Thus IgG accounts for only 20-30% of milk immunoglobulins. The IgA concentration is four times higher in colostrum than in serum, although in absolute terms, it is a minor component of both since the mean concentration of IgA in colostrum is 12.26mg with a range of 5.63-28.14mg/ml (Inoue, 1981(a)). A three-fold drop in IgA concentration occurs in the first 24 hours of lactation but it still accounts for 50-60% of total milk antibody. Immunoglobulin M is present in low concentration in serum (10-13%), colostrum (4%) and milk (18%) (Bourne, 1971) with the mean concentration in colostrum reported as 4.16mg/ml with a range of 1.50-7.74mg/ml (Inoue, 1981(b)).

Virtually all colostral IgG and a high proportion of IgM are derived from serum, as is 40% of IgA. Thus, colostrum should be regarded as a serum transudate and not a true secretion, since 90% of its immunoglobulin content is of serum origin (Bourne and Curtis, 1973). Furthermore, there is evidence to suggest that IgG_1 and IgG_2 are preferentially transferred from the serum to the colostrum at different times post partum (Franek et al, 1975). The source of milk immunoglobulin is different, about one-third to one-half of IgG and IgM are derived from serum but only a minor amount of IgA, (2-17%). Thus, the bulk of milk immunoglobulins, in particular IgA, are formed locally

Table 1.1 : Immunoglobulin Levels in Serum, Colostrum and Milk

	Mean Immunoglobulin Concentration (Mg per ml) (± S.E.)				Total Serum and Whey Protein (Mg per ml)
	IgG	IgG_2	IgA	IgM	
Serum (Pork Pigs)	18.31 (±0.67)	12.41 (±0.48)	1.44 (±0.12)	3.15 (±0.19)	70
Serum (Sows)	24.33 (±0.94)	14.08 (±0.49)	2.37 (±0.20)	2.92 (±0.18)	70
Colostrum (Ohr)	61.80 (±2.44)	40.29 (±1.66)	9.66 (±0.59)	3.19 (±0.21)	196
Milk (24hr)	11.83 (±4.82)	8.04 (±3.21)	3.76 (±0.99)	1.79 (±0.31)	41
Milk (2 days)	8.16 (±3.17)	5.02 (±1.80)	2.72 (±0.67)	1.81 (±0.41)	35
Milk (3-7 days)	1.91 (±0.64)	1.31 (±0.32)	3.41 (±1.01)	1.17 (±0.23)	33
Milk (8-35 days)	1.37 (±0.62)	0.99 (±0.45)	3.04 (±0.74)	0.89 (±0.25)	33

(Curtis and Bourne 1971, Bourne 1971)

in mammary lymphoid tissue. Colostrum is therefore suited to its immunological role of providing the newborn piglet with circulating immunoglobulins, whereas milk is adapted to a role of providing intestinal tract antibody during the post-natal and pre-weaning period (Bourne, 1976).

It has been reported that in piglets, IgA production does not contribute significantly to serum levels during the first 7-12 days of life, nor does IgG production during the first 14 days. However, IgM is produced in the first week of life (Curtis and Bourne, 1973), reaching a peak at 14 days after birth in colostrum-deprived piglets (Klobasa, Werhahn and Butler, 1981). A study of cells in the intestinal tract has shown that there is a slight predominance of IgM secreting cells over IgA secreting cells during the first 3 weeks of life, after which time IgA secreting cells predominate. Immunoglobulin G secreting cells are present in considerable but smaller numbers at all stages. At one month old the plasma cell population is similar to that seen in the adults (Brown and Bourne, 1976) and an interesting observation is that the spleen and mesenteric lymph nodes of either adult or young pigs contain relatively few mature plasma cells when compared to the laminar propria of the gut (Bourne, 1976). Thus it appears that,in the pig, the gut is an important site for the production of all antibody classes.

Immunological competence of the pig foetus has been demonstrated after the 55th day of gestation (Bourne et al, 1974) and the main immunoglobulin involved was 19S IgM, with smaller amounts of 4S IgG, 7S IgG, 19S IgG and 7S IgA. The lack of 9S and 11S IgA at this time

suggested that the piglets' gut immune system had not developed fully. A study of young piglets has shown that by 16 weeks of age they possess a mature serum immunoglobulin profile (Curtis and Bourne, 1971).

It is generally accepted that foetal pigs develop within an impermeable epitheliochorial placenta and there is therefore normally no placental transmission of maternal immunoglobulins (Nelson, 1932; Young and Underdahl, 1949; 1950; Brambell, 1958; Sterzl, Rejnek and Travicek, 1966). So the newborn piglets are virtually devoid of immunoglobulins at birth (Kim et al, 1966(a); Porter, 1969; Bourne et al, 1974). However, some workers have observed that in concentrated globulin fractions of sera from colostrum-deprived piglets antibodies may be detected (Myers and Segre, 1963). Porter (1969) identified a component present at a concentration of <50µg/ml which showed antigenic determinants in common with IgG and this appears to be an actively synthesised immunoglobulin which is made up of typical IgG heavy and light chains but which has a sedimentation coefficient of only 4S (Prokesova et al, 1969; Jonsson, 1973). Others have also found varying amounts of IgG, from trace levels up to 225µg/ml, in the serum of piglets prior to their first colostrum intake (Yabiki, Kashimazaki and Nomioka, 1974; Vior et al, 1975; Senft, Klobasa and Habe, 1976; Movsesijan et al, 1977; Chaniago et al, 1978). Concentrations of IgG as high as 1.3 to 3.8mg/ml have been reported (Payne and Marsh, 1962) although these may have been due to placental damage at birth. While the function of these precolostral immunoglobulins remains largely obscure, it has been suggested that they may play a role in competence of the young pig to respond to an antigenic stimulus (Jonsson, 1973). However, since they appear to be antigenically distinct from adult IgG and are present in such low concentrations it is reasonable to assume

that they do not provide the young pig with any significant protection against infection and thus the transfer of maternal antibodies from the colostrum by intestinal absorption is of major importance to the survival of the neonatal pig.

Immediately after birth the young pigs' intestine has the capacity to absorb antibodies and other macromolecules present in the sows' colostrum (McArthur, 1919; Nelson, 1932; 1934; Lecce, 1966). These antibodies are subsequently concentrated in the piglets' blood and lymphatic systems (Earle, 1935; Foster et al, 1951; Jakobsen and Moustgaard, 1950; Nordbring and Olsson, 1957; Rutquist, 1958) and thus provide passive protection against infection (McArthur, 1919; Nelson, 1934). The transmission of maternal antibodies to the piglets has been extensively reviewed by Brambell (1970) who has proposed a possible mechanism for the absorption of immunoglobulins in the gut (Brambell, 1966). The endoderm cells of the neonatal intestine would absorb proteins in solution by pinocytosis. The immunoglobulins transmitted would then become attached to specific receptors in the walls of the vacuoles which would protect them from degradation, following the junction of the pinocytotic phagosomes with lysosomes to form phagolysosomes, before they are passed out into the circulation. This hypothesis has been supported by subsequent experimental data (Lecce, 1966; Perry and Watson, 1967(a); Burton and Smith, 1977; Szeky et al, 1979; Smith, Burton and Munn, 1979; Werhahn, Klobasa and Butler, 1981). Although it appears that this absorption mechanism is selective for immunoglobulins from different species (Witty, Brown and Smith, 1969; Burton and Smith, 1977) it has been shown that the major immunoglobulins (IgG, IgM and IgA) from the same species are all non-selectively absorbed (Brown, 1976). The pigs' capacity to absorb immunoglobulin from colostrum

rapidly declines, following the first order reaction rate law, during the first 24hr after birth (Speer et al, 1959) and generally ceases altogether after 24 to 48hr (Speer et al, 1959; Miller et al, 1962; Yabiki, Kashiuazaki and Namioka, 1974; Vior et al, 1975; Murata and Namioka, 1977; Werhahn, Klobasa and Butler, 1981). However, the duration of immunoglobulin absorption is related to the nutritional state of the piglets (Lecce and Matrone, 1960). Thus piglets that were allowed to nurse immediately after birth, or that were fed cows' milk, ceased to absorb immunoglobulins at 12 hours after birth, while piglets that received only water or were starved retained the ability to absorb for at least 106 hours (Payne and Marsh, 1962). Similar observations have been made using polyvinylpyrrolidone (PVP). In this example nursing pigs lost their capacity to absorb PVP 24-36 hours after birth, whereas starved pigs were still able to absorb PVP when 86 hours old (Lecce and Morgan, 1962). One explanation for this phenomenon is that once the epithelial cells of the intestinal mucosa have been saturated with gammaglobulins, or other soluble proteins, further absorption ceases. The protection of maternal antibodies from proteolytic enzymes during protein absorbtion can be satisfactorily explained by the presence of a trypsin inhibitor in the sow's colostrum (Laskowski, Kassell and Hagerty, 1957) which has been shown to have a positive influence on IgG and IgA absorption (Jensen and Pedersen, 1982). Indeed it has been suggested that the early cessation of protein absorption is due not so much to gut closure as to the start of protein digestion as the level of inhibitor falls (Baintner, 1973). Variations observed in colostral immunoglobulin levels may be partly due to differences in the output by the sow which in turn may be influenced by the district, number of parturitions, kind of feed, type of farming and the number of sows raised on the farm (Inoue, Kitano and Inoue, 1980). Furthermore,

variation in colostral IgG concentration has been shown to increase rapidly up to 12 hours post partum, thereafter decreasing rapidly, (Frenyo et al, 1980/81) and it is interesting to note that antibody titres may also vary in colostrum samples collected from different teats of the same sow (Perry and Watson, 1967(b); Wu, Wang and Chang, 1980).

Bourne (1971) showed that 24 hours after birth the peak concentration of maternally derived IgG in piglets was 18.7 to 39mg/ml and this fell to a minimum value of 6.3mg/ml within the following 36-40 days. The IgA and IgM peak concentrations were 2.1 to 8.6mg/ml and 1.1 to 2.0mg/ml respectively at 24 hours and these both fell very rapidly to reach minimum values of 0.15mg/ml and 0.6mg/ml within 17-22 and 8-14 days respectively. It has been calculated that the half-lives of passively acquired maternal IgA, IgM and IgG were 2-3.5, 1.3-4.5 and 6.4-22.5 days respectively (Porter and Hill, 1970; Curtis and Bourne, 1971; Curtis and Bourne, 1973; Prochazka, Franek and Krejci, 1979; Frenyo et al, 1980/81; Klobasa, Werhahn and Butler, 1981). The marked differences in the reported decay rates may be partly due to differences in growth rates between individual pigs (Curtis and Bourne, 1971; Curtis and Bourne, 1973) and values may be exaggerated by the young piglets' rapidly expanding blood volume (Frenyo et al, 1980/81). However, few authors have corrected their data for body weight or blood volume increase. One report on porcine parvovirus (PPV) has shown that a half-life of 18.5 days, calculated in 16-24 week old piglets, becomes 29 days when corrected for weight gain. Furthermore, the PPV antibody half-lives were found to be shorter (14 days) in 0 to 6 week old piglets than in 8 to 14 week old piglets (21.7 days) (Paul, Mengeling and Pirtle, 1982), suggesting that the rapid growth rate in the younger

piglets has a marked effect on the observed half-life of passive antibody. It is interesting to speculate that the short half-life of colostral IgA in piglet serum may be the result of its redistribution to mucosal sites since it has been demonstrated that serum-derived IgA is selectively transported from serum into respiratory tract secretions (Bradley, Bourne and Brown, 1976). This would confer additional surface immunity to that provided by milk IgA which coats the intestinal epithelium of the young pig (Porter and Allen, 1972).

b) The response of sows and young pigs following exposure to antigens, other than FMD virus

The immune response of sows and piglets to both non-environmental antigens (for example, heterospecific red blood cells, ferritin, bovine serum and egg white) and environmental antigens (for example, nematodes, bacteria and viruses) has been examined.

(i) Non-environmental antigens

Parenterally administered sheep red blood cells (SRBC), bovine serum or egg white do not produce a measurable serological response in piglets under 8 weeks old which have been deprived of colostrum. However, if the animals receive 'normal' colostrum they respond at 3 weeks old which suggests that colostrum intake is important for the development of active immunity and it has been suggested that maternally derived immunoglobulins may be required for the recognition of foreign antigens (Hoerlein, 1957). The first antibodies formed in germ-free piglets after immunization with SRBC are of macroglobulinemic (IgM) nature with a sedimentation coefficient of 19S. They are followed by the formation of more slowly sedimenting 7S antibodies of the IgG type (Proskesova et al, 1969). However, using keyhole limpet haemocyanin as an antigen, Kim, Bradley and Watson (1968) showed that in the true primary response of germ-free piglets the first antibody synthesised was 19S IgG which was antigenically identical to late immune 7S IgG and distinct from 19S IgM. In addition there was a sequential synthesis of 7S IgG before the appearance of IgM or IgA. Furthermore this response was not dependent on the piglets having pre-existing 'natural' antibodies or maternally derived antibodies (Kim, Bradley and Watson, 1967).

The passive transfer of SRBC specific antibodies via colostrum can interfere with active antibody production during the first 3 to 6 weeks of life and can also sensitise the piglets to anaphylactic shock when they are challenged with the antigen intravenously (Hoerlein, 1957) which suggests that IgE-like antibodies may also be passively passed from sows to piglets.

More recent studies using a hapten-carrier conjugate of trinitrophenylated sheep erythrocytes (TNP-SRBC) have shed light on the regulation by maternal antibodies of the immune response in neonatal piglets. It was found in this system that passively acquired maternal antibody would completely inhibit the *in vitro* primary response to SRBC while leaving the response to TNP intact. It was suggested that the maternal antibody was regulating the *in vitro* response at the B-cell level since T-cell helper function to SRBC could not have been inhibited in order for an anti-TNP response to have remained intact. In these experiments, the regulation of immune responses in the piglets by maternal antibody disappeared before they were 3 months of age (Muscoplat, Setcavage and Kim, 1977).

Using a ferritin antigen the influence of the route of vaccination on the systemic and local immune response in the lactating sow was examined and it was found that intramammary vaccination was superior to intramuscular vaccination for the production of not only local but also systemic responses. Although all three major immunoglobulin classes (IgG, IgM and IgA) were involved there was a slightly higher percentage involvement of IgA following the intramammary stimulation than after stimulation by other routes. Furthermore, vaccination of one mammary gland gave rise to significant antibody

activity in other glands (Bourne, Newby and Chidlow, 1975). Thus the route of vaccination of the dam could be important in providing not only local intestinal tract immunity but also systemic protection to the young suckling pig.

(ii) Environmental antigens

Nematodes - Infection of pregnant sows with nematode parasites (Hyostrongylus rubidus) elicits a strong agglutinin response in the serum and colostrum of predominantly the IgG antibody class. Such antibodies are passed to the young pigs within 4 days of birth and their presence appears to aid the production of agglutinins in the piglets if they subsequently become infected, while also reducing their parasite burden (Smith and Herbert, 1976).

Bacterial antigens - Toxigenic strains of Escherichia coli when present in the pig gut can result in neonatal diarrhoea which is an important cause of death. Studies on the serum of weaned piglets and sows infected with E. coli have shown that the specific antibody is largely confined to the IgM class (Porter and Kenworthy, 1969; Porter, 1969) and when passively acquired by piglets has a half-life of between 1.3 days (Porter and Hill, 1970) and 4.3 days (Curtis and Bourne, 1971). Colostrum has also been shown to have a high proportion of IgA directed against E. coli although after passive aquisition it did not appear to contribute greatly to the piglets' serum antibody titres. On the basis of this observation it was concluded that these 11.6S secretory IgA antibodies could not be absorbed by the young pig (Porter, 1969). Subsequently, however, Bourne (1971) has shown that piglets can absorb 11.6S IgA and it was concluded that previous results (Porter, 1969) may be explained by the short half-life of this form of IgA compared with

9.5S serum IgA. Further work (Porter, Noakes and Allen, 1970) has confirmed the predominance of 11.6S IgA directed against E. coli in pig secretions. In addition a low molecular weight component active against E. coli, and antigenically similar to IgG, has been identified in pre-colostral piglet serum but its function remains unclear.

Colostrum-deprived piglets fail to produce antibody to diptheria toxoid inoculated intraperitoneally at 3 weeks of age and respond only slightly to tetanus toxoid administered in the same manner. However, this immunological deficiency can be overcome by the suckling of colostrum, or by adminstration of immune swine or horse serum (Segre and Kaeberle, 1962). Furthermore, colostrum-deprived piglets fed on artificial bovine milk substitute responded to E. coli antigen at an earlier age than immune colostrum fed littermates, suggesting that the immune response may be suppressed by the presence of passively acquired antibody (Porter and Hill, 1970). Passively acquired antibodies also suppress the number of immunoglobulin secreting cells in the intestine of 19 day old piglets which have been fed heat-killed E. coli (Watson, Bennell and Chianiago, 1979). The suppressive effect of passively acquired antibodies has been observed with other bacterial antigens, for example Bordetella bronchiseptica (Pedersen and Jensen, 1980), Salmonella typhimirium (Senft and Heckelmann, 1980) and Brucella abortus (Hoerlein, 1957).

As noted earlier the route of inoculation influences the class of antibody which is formed. For instance, oral immunisation provoked secretion of IgA antibodies in 10-day-old piglets (Porter et al, 1974), whereas intramammary and intramuscular inoculations in the lactating sow provoked predominantly IgG antibodies in both the serum and

colostrum. Other antibody classes that were provoked were IgM by intramuscular injection, and IgM and IgA, equally, by intramammary injection (Chidlow and Porter, 1977).

Viral antigens - Transmissible gastroenteritis (TGE) is an enteric viral disease which causes diahorrea and high mortality in pigs under 2 weeks old. In the sow infection with TGE elicits the production of IgA class antibodies in milk while intramuscular or intramammary injection of virus results in predominantly IgG antibodies in this secretion. It is important to note that immunity in piglets is best after ingestion of IgA immunoglobulin (Bohl et al, 1974; Bohl and Saif, 1975; Saif and Bohl, 1977; 1979). A reason for this is that there is selective binding of IgA to intestinal epithelial cells which may act as an immunological barrier to the attachment of the virus (Saif and Bohl, 1979).

Rotaviruses have frequently been associated with mild diarrhoea in piglets both at and before weaning but colostrum-deprived piglets experimentally infected develop severe diarrhoea and quickly die. However, piglets can be protected for 37 days by feeding bovine colostrum containing rotavirus-neutralizing activity and piglets treated in this way are still capable of developing their own natural immunity (Bridger and Brown, 1981). The anti-rotavirus antibodies in colostrum are predominantly IgG and IgM while those in milk and faeces are predominantly IgA (Corthier and Franz, 1981) and it is the IgA antibodies that provide high long-lasting antibody activity in lacteal secretions (Hess and Bachmann, 1981). Thus protection from rotavirus appears to involve a similar mechanism to immunity to TGE.

Aujeszky's disease, also known as pseudorabies, is an acute infectious disease that is characterized by signs and symptoms of central nervous system disturbance. Sows which have been infected with Aujeszky's disease virus (ADV) are virtually immune for life (Akkermans, 1970) and will secrete antibodies in the colostrum after every farrow (Kojnok and Surjan, 1963; Akkermans, 1970). The colostral antibody can have a titre 11 to 16-fold greater than the circulating antibody but this will decline rapidly within 24 hours (McFerran and Dow, 1973). Unlike TGE, natural exposure to ADV elicits a major IgG antibody response, with minor quantities of IgM, in colostrum (Saif and Bohl, 1977). The half-life of maternally derived ADV antibody in piglets over the first 4 weeks of life is 8.5 days (McFerran and Dow, 1973). Such antibodies can protect 1-week-old piglets against contact challenge with ADV and will not interfere with an active antibody response in 2-week old piglets vaccinated with a DEAE dextran vaccine (Wittmann and Jakubik, 1979). However, maternal antibodies transferred from sows vaccinated with an oil vaccine, while providing at least 3 weeks passive protection against ADV, will prevent the active formation of neutralizing antibodies in 1, 2 or 3 week old piglets when vaccinated with an oil vaccine (Wittmann, 1981). Assuming that these observations are accurate, it is possible that DEAE dextran adjuvant may be able to overcome the suppressive effect of passive ADV antibody.

Swine influenza is a viral respiratory disease which produces bouts of dyspnoea and coughing followed, generally, by a rapid recovery. Piglets develop high antibody titres in their serum within 30hr of suckling from immune sows (Young and Underdahl, 1950). Infection of piglets possessing such maternally derived antibodies may result in clinical disease but the frequency and ease of viral isolation is

related to the concentration of anti-influenza antibody present at the time of exposure (Renshaw, 1975; Mensik et al, 1976). The concentration of maternal antibody present at the time of infection also influences the active immune response of the host. Thus, if the concentration is high the passively protected host is not immunologically stimulated (primed) while if the concentration is low the host becomes infected and is immunologically primed, although active antibody production is not always seen. It has been suggested (Uhr and Moller, 1968) that high concentrations of passive antibody exert suppression through a peripheral effect, by masking antigenic determinants and preventing them from reacting with receptors on immunocompetent cells, whereas lower concentrations of passive antibody have a central effect, by combining with antigen at a cellular level and thus reducing the immunocompetence of the lymphoid system (Feldmann and Diener, 1970; Renshaw, 1975). Other studies on swine influenza have shown that piglets infected or immunized when one day old produce antibodies within 5 days and a similar response has been shown in both colostrum-fed and colostrum deprived piglets (Mensik et al, 1976).

Hog cholera virus (HCV), also known as classical swine fever virus, causes a highly infectious septicaemia characterized by generalized haemorrages, 95 to 100 percent morbidity, and almost as high mortality. As long ago as the early 1900s it was recognised that sows immunized against HCV transmit immunity to their offspring and that this immunity was associated with the piglets suckling (McArthur, 1919). Nursing piglets acquired similar levels of HCV antibodies to those in their mother's serum within 24 hours of suckling and these antibodies have a half-life of 13 days (Coggins, 1964). The immune status of the piglets depends on the interval between vaccination of the sow and

farrowing and can be attributed to the quality of the antibodies transmitted in the colostrum (Corthier and Charley, 1978; Precausta, Kato and Brun, 1983). When present at a high level, transferred maternal HCV antibody will suppress the immune response to hog cholera vaccine (Coggins, 1964; Corthier, 1976; Mierzejewska et al, 1977; Launais, Aynaud and Corthier, 1978; Corthier and Charley, 1978). However, this suppressive effect can be overcome by increasing the vaccine virus dose (Coggins, 1964), and animals which have suppressive levels of maternal antibody are still primed for a secondary vaccination or virus challenge (Corthier, 1976; Corthier and Charley, 1978). Vaccination against the CL Chinese strain can be carried out in 7-day old piglets born to non-immune sows (Precausta, Kato and Brun, 1983) and will be fully effective in piglets having passive immunity when they are 1 to 2 months old (Mierzejewska et al, 1977; Launais, Aynaud and Corthier, 1978; Precausta, Kato and Brun, 1983), at a time when piglets are still passively protected by maternal antibody (Launais, Aynaud and Corthier, 1978; Precausta, Kato and Brun, 1983).

Infection with porcine parvovirus (PPV) generally results in either subclinical or mild clinical signs, although natural exposure of susceptible swine during gestation often results in embryonic and foetal death (Cartwright and Huck, 1967). Passively acquired antibodies have been shown to interfere with the active immunity to live PPV (Paul, Mengeling and Brown, 1980). The biological half-lives of antibodies to PPV, unadjusted and adjusted for body weight gain, are approximately 19.7 and 29.0 days, respectively. However, these values will vary with the age of the piglet and period over which observations are made (Paul, Mengeling and Pirtle, 1982).

1.3 FOOT AND MOUTH DISEASE

Foot and mouth disease virus (FMDV) is a member of the family Picornaviridae (very small, RNA-containing viruses). It has recently been placed in its own genus Apthovirus (Cooper et al, 1978), aptha being Greek for 'vesicles in the mouth'. The Apthovirus genus is distinguished from the three other Picornaviridae genera (Enterovirus, Cardiovirus and Rhinovirus) by being unstable below pH 5.6; having a buoyant density in caesium chloride of 1.43-1.45; and its clinical manifestations (Cooper et al, 1978).

The clinical signs of FMD infection were probably first described by Fractastorius in 1514 (see Bachrach, 1968). He noted that animals refused their feed, and the interiors of their mouths became permeated with a redness and small vesicles, which then descended to the feet. Evidence that FMD was a viral infection was produced by Loeffler and Frosch (1897) who showed that the infectious agent of the disease was filterable. This was the first animal virus to be described.

a) Antigenic characteristics

Not only was foot and mouth disease virus the first animal virus to be discovered but it was also the first virus in which antigenic differences between strains were recognised. The existence of more than one antigenic type was discovered by Vallee and Carre (1922), when they observed that recovered cattle in France became reinfected when brought into contact with sick animals from Germany. In a series of carefully controlled experiments they failed to obtain cross-immunity between two groups of strains and the viruses were named O (Oise) and A (Allemagne), after their areas of origin (Vallee and Carre, 1922; Waldmann and Trautwein, 1926). Four years later, a third type was described by Waldmann and Trautwein (1926) and called type C.

In the 1930's there was evidence that strains of virus isolated from outbreaks of typical foot and mouth disease in parts of Africa did not fit into the framework of the classification O, A and C and, in 1948, Galloway and his colleagues at Pirbright in England, confirmed the presence of three additional, immunologically distinct serotypes by cross-immunity test in cattle and pigs. These new strains of virus were isolated from regions of South* Africa where types O, A and C did not seem to occur and were called SAT (South* African territories) 1, 2 and 3 (Research Institute, Pirbright report 1937-1953). In 1954 a further new type was identified at the Animal Virus Research Institute, Pirbright, in material received from Pakistan and subsequently, in samples from India, Thailand and Hong Kong. This strain, the seventh immunologically distinct serotype of FMD was named ASIA 1 (Brooksby and Rogers, 1957). In addition to the seven major serotypes, immunological subtypes or variant strains are frequently encountered in natural outbreaks of the disease and give rise to difficulties in classification and immunization. Subtypes of FMDV (Waldmann and Trautwein, 1926; Bedson, Maitland and Burbury, 1927) were originally defined as strains within a type which, because of antigenic differences, did not cross-immunize completely. The criteria currently adopted, by the World Reference Laboratory at Pirbright, for subtype differentiation are based on serological relationships established by a variety of techniques, the most commonly used being complement fixation (Pereira, 1978).

b) Structure

The infectious virus particle is an icosahedron with a diameter of 23-25nm (Elford and Galloway, 1937; Bachrach and Breese, 1958; Bradish, Henderson and Kirkhom, 1960), a sedimentation coefficient of 140-146S (Breese, Trautman and Bachrach, 1960; Strohmaier, 1971) and an isodensity in caesium chloride of between 1.42 and 1.46g/ml (Trautman and Breese, 1962; Terry, Clark and Rweyemamu, 1982). It is composed of

*commonly misquoted as South but should be Southern

one molecule of single-stranded RNA (MW 2.6 x 10^6) (Bachrach, Trautman and Breese, 1964) and sixty copies of each of four structural proteins (VP1-3 MW 24 x 10^3 and VP4 MW 14 x 10^3) (Wild, Burroughs and Brown, 1969, Laporte, 1969). Virus harvests also contain non-infectious RNA-free empty capsids (Plantrose and Ryan, 1965; Graves, Cowan and Trautman, 1968) which have a sedimentation coefficient of 75S and an isodensity in caesium chloride of 1.31g/ml. These 75S particles are composed of 60 copies of VP1, VP3 and VP0 (a precursor of VP2 and VP4) (Vande Woude, Swaney and Bachrach, 1972; Rowlands, Sangar and Brown, 1975) and in a stable form are capable of evoking a strain-specific neutralizing antibody response (Rweyemamu, Terry and Pay, 1979). Heating the virus to 56°C for 30min or lowering pH to 6.5 disrupts the virus capsid to produce 12S particles (composed of VP1, 2 and 3), aggregated VP4 and free-RNA (Burroughs et al, 1971; Talbot et al, 1973). This particle will elicit complement-fixing antibodies but has a very low immunizing activity (Brown and Newman, 1963). A further antigen with a sedimentation coefficient of 4S and molecular weight of 56 x 10^3 was discovered in infected cell extracts by Cowan and Graves (1966). This was referred to as virus-infection-associated antigen (VIA antigen) and has subsequently been recognised as the RNA polymerase (Newman et al, 1979).

Of the four structural proteins VP1 appears to play a key role in the immunizing activity of the virion since cleavage of this protein by trypsin results in loss of immunizing activity (Wild, Burrows and Brown, 1969). Furthermore, VP1 is the only structural protein which, in its isolated form, will evoke neutralizing antibodies (Laporte et al, 1973; Bachrach et al, 1975). A more detailed study of enzymic and chemical cleavage fragments of O_1 Kaufbeuren VP1 has identified two areas (between amino acids 138-154 and 200-213) which are found on the surface of the virion and peptides containing these regions are able to induce

neutralizing antibodies against the homologous virus (Strohmaier, Franze and Adam, 1982). Application of recombinant DNA technology has allowed nucleotide sequences of the virus genome to be determined, from which the primary amino acid sequence of VP1 can be deduced. Independent groups of workers have used the published sequence of O_1 Kaufbeuren VP1 (Kurz et al, 1981) in order to chemically synthesize peptides corresponding to several regions of VP1 and have identified specific sites on the molecule (141-160 and 200-213; Bittle et al, 1982 and 144-159; Pfaff et al, 1982) which elicit neutralizing, and protective, antibodies to the whole virus. Although such peptides perform with an efficiency of 1-10% of that of the inactivated virus particle, on an equal weight basis, the titre of the antibodies produced is several orders of magnitude greater than the best results obtained with the whole VP1 protein (Arnon, Shapira and Jacob, 1983) and the antibodies will mimic the subtype specificity of the intact virion (Rowlands et al, 1983; Clarke et al, 1983). Such encouraging results have prompted active research into the possibility of totally synthetic vaccines for FMD.

c) Clinical signs and transmission

Cattle, swine, sheep and goats, in that order, are the species most frequently affected by FMD virus although all ruminants and cloven-footed animals are susceptible (Bachrach, 1968). The condition is characterized by the formation of vesicles on the mucous membranes of the mouth, on the nose, and on the skin between and adjacent to the claws of the feet (Shahan, 1962). In cattle, vesicles are most often seen on the tongue and feet; in nursing or milking cows they may also appear on the udder and teats, resulting in virtual cessation of milk yields. Rumen and heart lesions are frequently found at necropsy. There is usually a profuse drooling of ropey saliva, and the raised epithelial covering of the tongue vesicles invariably ruptures and

sloughs off, leaving large areas of raw tissue underneath. Mouth soreness prevents the animals eating, and foot lesions, which are usually invaded by bacteria, cause lameness (Bachrach, 1968). In pigs, vesicles form on the heels and may encircle the coronet, leading to 'thimbling' as the horn detaches and the old hoof is carried downwards by new growth. Lesions may also develop on the snout and tongue but these are more rare in the pig. Inflammation of the foot is often so severe that lameness occurs and this is significant in the diagnosis of the disease (Sard, 1978). Although mortality in adult stock is low, below 5%, it can approach 50% in young stock, with the animals dying without showing any signs of infection. Furthermore, in breeding stock abortion and a reduction in breeding ability are both common results of infection.

Transmission of FMD is primarily from the infected animal itself, especially during the early febrile stage when virus is present in the blood and all organs, tissues, secretions (saliva, lachryma, milk and semen), and excretions (sweat, urine and faeces) (Hyslop, 1970). The spread of FMD among animals in close proximity probably occurs chiefly through the dissemination of virus from saliva and foot lesions, which remain infectious for 9 to 11 days after infection. However, the mode of transmission has not been fully elucidated because infection often goes from one area to another or from one country to another without illness being evident in the intervening premises. Suspected means of transmission include wind-borne droplet infection (Sellers and Parker, 1969), migratory birds (Stockman, and Garnett, 1923) and carrier animals (van Bekkum et al, 1959). FMD is considered to be the most contagious disease known and morbidity in fully susceptible contact stock is often close to 100%.

d) Vaccines

The first effective vaccine against foot and mouth disease (FMD) was developed in the late 1930's (Waldmann, Kobe and Pyl, 1937). This vaccine, prepared from virus extracted from infected tongue epithelium and associated vesicular fluid, used a process of formaldehyde inactivation (Vallee, Carre and Rinjard, 1926) followed by aluminium hydroxide adsorption (Schmidt, 1936). It was referred to as the Vallee-Schmidt-Waldmann vaccine. However, as live animals were the source of virus the quantity of vaccine that could be produced was strictly limited. In 1947, Frenkel developed a technique in which the epithelium and superficial tissue of tongues from freshly slaughtered cattle was used to culture the virus on a large scale (Frenkel, 1950). The virus was inactivated with formalin and adsorbed onto aluminium hydroxide.

Frenkel and primary tissue culture vaccines, although still in use today, required a constant supply of tissue from recently slaughtered animals and suffered from the possibility of contamination with unwanted pathogens. In 1962, it was shown that cells derived from baby hamster kidneys (BHK 21 cells; MacPherson and Stoker, 1962) would provide a suitable continuous cell line for the production of FMD (Mowat and Chapman, 1962). These monolayer cells were subsequently adapted to suspension culture in 250ml vessels (Capstick et al, 1962; Capstick 1963). This advance enabled vaccine manufacturers to increase their production capacity dramatically and there are now commercial vaccine production plants operating culture vessels with capacities of 4000 litres (Mowat, Garland and Spier, 1978).

e) Vaccination of sows and young pigs

The systematic vaccination of cattle during the 1950's with aluminium hydroxide adjuvanted vaccines led to optimism about the possibility of rapid eradication of FMD from the European continent. However, the appearance of major pig epizootics in the 1960's dashed such optimism, particularly since the traditional aqueous cattle vaccines appeared to be far less effective at controlling the disease in pigs (reviewed by de Leeuw, 1980). This led to a period of intensive research into FMD vaccines for pigs which included several studies on maternal antibodies and their effect on protection and subsequent vaccination of young pigs.

In the early 1960's studies on the immune response of sows and their young to the then current FMD aqueous cattle vaccines demonstrated that piglets from sows vaccinated repeatedly still had neutralizing antibodies at 3 to 4 months after birth (van Bekkum, Frenkel and Nathans, 1963; Nathans, 1965). However, at 2 months of age the antibody levels were only sufficient to protect 50% of the piglets from live virus challenge and attempts to boost the immune response of 6-10 week old piglets, already possessing maternally derived antibodies, by vaccination failed (van Bekkum, Frenkel and Nathans, 1963). Even at 16 weeks the maternal antibody titre was sufficient to inhibit a serological response to vaccination with both standard and experimental aqueous vaccines, the latter containing extra antigen and a saponin adjuvant. In this study it was apparent that the level of maternal antibody was critical thus where titres were above 1.35 $\log_{10}SN_{50}$ there was no serological response but below this a response to vaccination could occur (Nathans, 1965). However, the piglets which showed no response to primary vaccination appeared to have been primed because

they produced an intensified immune response to subsequent vaccinations. In 1966, van Bekkum showed that piglets from the same litter did not necessarily acquire the same level of maternal antibodies. Absorption of maternal antibody ended 2-3 days after birth and thereafter the levels declined with a half-life of 13-14days. Individual variations in the half-lives which increased with age may have been due to differences in growth rates of the piglets. The influence of other factors such as the frequency of the sows' vaccination and the interval between the final vaccination and parturition were also investigated and both appeared to be responsible for wide variations in passive antibody levels in the young. Revaccination of sows during pregnancy sometimes resulted in protracted persistence of maternal antibodies in piglets for up to 160 days but values between this maximum and zero were recorded.

In the early 1970's studies carried out using a commercial monovalent 'type C' aqueous vaccine, containing aluminium hydroxide and saponin as adjuvants, showed that sows and suckling piglets had similar serological responses. In both, antibody which was detected 7 days after vaccination persisted for approximately 90 days. The titres in the piglets being equivalent to (Dujin, 1971) or lower than (Dujin et al, 1975) those in the sows. However, in both reports the levels of antibody appeared to be unaffected by revaccination. The passively acquired antibodies in piglets born to sows revaccinated during pregnancy persisted for up to 35 days but titres were generally low and only likely to protect the piglets for 1-2 weeks (Dujin, 1971). Furthermore, the half-life of these maternal antibodies appeared to be 6.6 days, a lower value than that generally associated with IgG which has a mean half-life of 13.8-14.2 days (Curtis and Bourne, 1971; Curtis and Bourne, 1973). It seems possible that these low values reflected

the presence of IgM and IgA, both of which have shorter half-lives of 2.8-4.5 and 2.6-3.5 days respectively. This would be consistent with the generally accepted theory that the failure of aluminium hydroxide absorbed aqueous vaccines to elicit long term immunity in pigs is associated with the poor ability of such vaccines to provoke a switch from an IgM to an IgG response (Anderson, Masters and Mowat, 1971a;b; Ouldridge, Francis and Black, 1982).

Popovic et al (1975 and 1976) monitored the response of pigs to IFFA trivalent 'type O, A and C' and Wellcome monovalent 'type C' aqueous vaccines and observed that responses were generally low so that revaccination was often required to produce reasonable levels of antibody. Attempts to improve the response to the IFFA vaccine by using 2 to 100 times the normal recommended dose did not affect the antibody titres nor, incidentally, did it affect the health of the sows or their litters. However, the immune response to the higher doses persisted for 35-40 days longer than after the normal dose (Dujin, Popovic and Panjevic, 1975). Another interesting observation was that lower titres occurred in immunized piglets suffering from severe respiratory syndrome at the time of vaccination (Popovic et al, 1976) and this may account for some of the variations often observed in vaccination responses especially in view of the frequent incidence of swine influenza (Easterday, 1975).

Sidorov (1974) also studied the effect of pre-existing passive antibodies on subsequent immunization of piglets with aluminium hydroxide adsorbed saponin vaccines. He inoculated piglets with convalescent cattle serum, convalescent pig serum or immunolactone, and subsequent to vaccination detected antibody titres ranging from 0.5-2.8

$\log_{10}SN_{50}$ the highest levels being obtained from the animals initially injected with convalescent cattle serum. However, although these antibodies persisted at detectable levels for only 3-5 days, inhibition of both a post-vaccination and a post-infection immune response occurred for up to 10 days. A similar suppression of the immune response in calves, due to naturally acquired passive antibodies, has also been noted after use of aluminium hydroxide adsorbed FMD vaccines in the dams (Graves, 1963; van Bekkum, 1966; Srubar, 1966; Wisniewski and Jankowska, 1972; Mayr and Thein, 1972; Nicholls et al, 1984).

In 1961, Michelsen recognised that acceptable levels of immunity to FMD in pigs were unlikely to be attained by use of the aluminium hydroxide absorbed vaccines developed for cattle. He suggested that alternative adjuvants were necessary and carried out experiments using a water-in-oil emulsion first described by Armbruster et al (1960). These experiments provided the first evidence that oil-emulsion vaccines would protect a higher percentage of pigs than the existing aluminium hydroxide adsorbed aqueous vaccines. Further progress was not made until McKercher and Giordano (1967a) demonstrated that such single oil emulsion vaccines were capable of provoking levels of antibody which protected adult swine from FMD for at least 90 days, and their findings were subsequently confirmed by others (Graves et al, 1968; Giraud et al, 1969; Wittmann, Bauer and Mussgay, 1969; Anderson, 1969; Basarab, 1978).

Oil emulsion vaccines were also capable of inducing immunity in piglets at one month of age whether they were derived from vaccinated or unvaccinated mothers (Giraud et al, 1969). It was observed that the litter sizes appeared to be unaffected by vaccination of the sows as late as one month prior to farrowing and piglets born to such sows were

immune for up to one month. Piglet immunity was extended to 2 months when sows were given repeated vaccinations every 6 months, the last one being given in the first 3 months of pregnancy (Giraud et al, 1974). Under such a regimen, when sows were revaccinated five times at approximately 30 day intervals, the last being given 15 days before farrowing, they produced colostrum with high antibody titres and these antibodies were demonstrable for at least 6 weeks in their piglets (Ercegan, Panjevic and Ercegovac, 1976). In the same year, a study was carried out on piglets from sows which had a mean titre of 2.0 $\log_{10}SN_{50}$ at the time of farrowing. The antibody titre of the 3-day-old piglets was 2.72 $\log_{10}SN_{50}$ (that is 5 times higher than that circulating in the sows). This passive antibody declined to a mean titre of 0.8 $\log_{10}SN_{50}$ within 3 months, with a half-life of 12.71 days, and experimental exposure to live virus showed that 75% of the piglets were protected at 1 month, 73% at 2 months and 33.3% at 3 months. Vaccination of 10-week old piglets with no pre-existing antibody produced an antibody peak of 1.76 $\log_{10}SN_{50}$ at 42 days which declined to 1.16 $\log_{10}SN_{50}$ after a further 84 days at which time 80% were protected. A similar group of 10 week old piglets vaccinated and revaccinated after 21 days produced an antibody peak of 2.12 $\log_{10}SN_{50}$ at 42 days which declined to 1.52 $\log_{10}SN_{50}$ 84 days later at which time 100% of piglets were still protected. In these experiments, the vaccination of sows did not affect the size or quality of litters but Chappuis et al (1977) noted that the vaccination of 14-day-old piglets appeared to retard their growth and recommended regimens for use of oil-emulsion vaccines in pigs. For fattening pigs from unvaccinated sows vaccination at 8-10 weeks old was recommended, while vaccination of piglets from vaccinated sows should be delayed until the piglets were 10-12 weeks old. It was also recommended that breeder pigs should be vaccinated at 8-12 weeks and the vaccination

repeated at 6-8 month intervals. However, this was modified following the observations that vaccination of piglets at 1 and 2.5 months old did not retard their growth rate (Mougeot et al, 1980) and revaccination after 21 days extended the period of protection from 42 days to 126 days. The modified regimen was, therefore, that piglets from unvaccinated sows should be vaccinated at 5 weeks old and again 2-3 weeks later, while piglets from vaccinated sows should be vaccinated when 10 weeks old, which could be reduced to 5 weeks with revaccination 21 days later in high risk areas. Breeder pigs could be treated the same as the fattening pigs and then given a further vaccination at 6-8 months old followed by boosters every 6 months.

In 1978 Morgan and McKercher demonstrated that oil-emulsion vaccines elicited immunity in sows regardless of their gestation status. Furthermore, when the sows were vaccinated 21 to 72 days before farrowing, the immunity which was passed onto their piglets lasted for 1-4 weeks. The half-lives of these antibodies, as calculated by the present reviewer from the available data, were 6.9-9.6 days. However, if sows were revaccinated at 6 days prior to farrowing, the antibody passed to the piglets decayed more rapidly, with a half- life of 3.6 days and this was probably due to high levels of IgM antibodies which would have been present in the sow at the time of farrowing. If this were the explanation it is the first suggestion that the class of antibody at farrowing was important to the duration of the FMD neutralizing antibodies in their progeny.

The injection of oil-emulsion vaccines into piglets from immunized sows at four weeks after birth produced immunity which lasted for at least 4 months. It was claimed that such piglets also responded to oil

emulsion vaccination at 7, 14 and 21 days old regardless of the presence of maternal antibody (Morgan and McKercher, 1978).

In a later report Morgan and McKercher (1980) restated their 1978 conclusions cited above and added that the titres of antibodies in colostrum were directly related to the sow serum titre at the time of farrowing. They observed that piglets vaccinated with the oil vaccine produced 'a meaningful response' regardless of the FMD immune status of their mothers and that any failure in response of piglets would be due to 'something other than colostrally acquired antibodies'. It may be relevant that their observations on the piglet responses (Morgan and McKercher, 1978) were based on serological data of only 2 or 4 sample points and the results were not compared to matching groups of piglets from unvaccinated mothers. The only comparison between piglets from vaccinated and unvaccinated sows involved unmatched litters vaccinated at 7 or 28 days respectively and challenged 3-4 months later. Although almost all the piglets were protected the litter from the unvaccinated sow had a 10-fold higher neutralizing antibody titre at challenge.

In more recent studies it has been shown that piglets, from vaccinated or unvaccinated sows, vaccinated at 1, 3 or 5 weeks of age were protected from challenge at 2 months old while unvaccinated piglets were not (Black et al, 1982). However, when the serological responses of piglets from vaccinated and unvaccinated mothers were compared there was an indication that piglets, from vaccinated mothers, vaccinated at 1 or 3 weeks old (i.e. in the presence of maternally derived antibody) had lower titres in the early stages post vaccination (Black et al, 1982).

One problem associated with the use of oil-emulsion FMD vaccines in pigs has been the occurrence of local inflammatory reactions at the injection sites (Mussgay and Wittmann, 1968; Wittmann and Bauer, 1969) with subsequent damage being recognisable in the carcase. This effect has been minimised by using modified oil formulations (Giraud et al, 1969) reduced doses (McKercher and Morgan, 1969; Anderson, Masters and Mowat, 1971(a); McKercher and Bachrach, 1976) and by inoculating the pigs intramuscularly behind the ear (McKercher and Bachrach, 1976; Basarab, 1978; de Leeuw, 1980) but the search for better adjuvants continues. Most alternative adjuvants have been found to be less effective than the oil emulsion e.g. adjuvant 65 (Giraud et al, 1969; Wittmann, Bauer and Mussgay, 1969) and calcium phosphate (Turubatovic et al, 1972). Of these, DEAE-Dextran seems most promising since it appears to produce reasonable immunity in 11-15 week old piglets with minimal local reactions. Antibody titres were detected in these animals 4 days after vaccination and reached a peak at 14 days. It is interesting to note that the neutralizing antibodies detected in these experiments showed little correlation with results of challenge tests against live FMD virus (Wittmann, Bauer and Mussgay, 1970; 1972). Tests in younger, 6-8 week old, piglets produced slightly lower titres which nevertheless protected 75% of the animals 6-14 weeks later (Wittmann, Bauer and Mussgay, 1971). However, immunity to DEAE-Dextran vaccines has generally been short (1-3 months) despite the development of IgG antibodies (Anderson, Masters and Mowat, 1971(b)). There have also been some reports of tissue reactions caused by this adjuvant although these were not expected to be economically important (Wittmann, Bauer and Mussgay, 1970; 1972; Bauer et al, 1974). Thus on balance these adjuvants do not appear to provide an acceptable alternative to oil emulsion vaccines in pigs.

CHAPTER 2

MATERIALS AND METHODS

2.1 ANIMALS

Large White pigs which had not been previously vaccinated or exposed to FMD were housed in quarantine conditions at the Animal Virus Research Institue, Pirbright, England, for the duration of the experiments. The sows and weaned pigs were fed with Sow and Weaner Pellets (Billers Ltd, UK) twice a day and water was freely available at all times. Sows were housed 3 to a loose box until about 1 week before farrowing when they were placed in individual loose boxes. When farrowing was imminent they were placed in farrowing crates and kept there until the piglets were weaned at 8 weeks old. Newborn piglets were injected intramuscularly with 1ml Ferrofax10 Iron Supplement (C-Vet Ltd, UK) and then weekly until weaned to avoid any possibility of nutritional anaemia. The piglets' tails were docked at birth and male piglets were castrated when 4 weeks old.

Sows were identified using metal ear tags while piglets were tatooed in the ear at birth. Each piglet within any one litter was given a number prefixed by its mothers tag number e.g. piglets 96/01 to 96/10 were the ten piglets born to sow number 96.

2.2 VIRUSES

a) Virus strain

The FMD virus strain used throughout these experiments was O_1BFS 1860/67 which was isolated from the Wrexham area in 1967 during the last major FMD outbreak in Britain.

Virus for vaccine production, microneutralization and enzyme labelled immunosorbent assay (ELISA) was prepared from a master seed bank stock of O_1BFS 1860/67 stored at -70°C at the Wellcome Laboratories, Pirbright. This stock was originally obtained from the World Reference Laboratory in 1978, with a history of 10 passages in monolayer BHK 21 clone 13 cells (Stoker and McPherson, 1964) followed by one passage in BHK suspension (Susp) cells (Capstick et al, 1962), and was given a further two passages in BHK monolayer cells to produce the Wellcome stock.

Virus used for challenge of immunity was O_1BFS 1860 in the form of a 1 in 50 glycerinated filtrate of bovine tongue epithelium (Burrows, 1966), stored at -20°C.

b) Preparation of stock virus for microneutralization tests

Virus suspension from the master seed bank stock was thawed rapidly at 37°C and 1ml plus 4ml of virus maintenance medium (VMM, see Appendix 1) was used to inoculate a Roux flask containing a monolayer of Instituto Biologico - Renal Swine (IBRS) cells (de Castro, 1964). After adsorbtion for 30min at 37°C the culture was drained, 100ml of VMM containing 1% normal bovine serum was added and the flask was incubated at 37°C for 16hr. Virus was harvested once cytopathic effects (CPE) were advanced. To do this the medium was decanted into universal bottles and cell debris was sedimented by centrifugation (600 x g for 10min at room temperature). The supernatant was retained and used to infect a second flask in a similar manner to that described above. The virus suspension obtained from this flask was again centrifuged as described above to remove cell debris and dispensed in 2ml volumes, labelled O_1BFS 1860/67 BHK 10 Susp 1 BHK 2 IBRS 2 and frozen at -70°C.

c) Preparation of purified intact FMD virions

Two ml of the master seed bank stock virus was thawed rapidly at 37°C and diluted with 98ml of VMM. Ten confluent BHK monolayers, prepared in Thompson bottles, were each inoculated with 10ml of the diluted seed virus. The virus was allowed to adsorb for 30min at 37°C, cultures were drained and 200ml of serum-free VMM was added. The bottles were incubated at 37°C for 16hr and when CPE was advanced the medium was decanted into 1 litre centrifuge pots. The cell debris was removed by centrifugation (2,200 x g for 30min at room temperature) and the resulting supernatants were pooled.

The virus harvest (approximately 2 litres) was inactivated for 24hr at room temperature with 0.05% v/v acetylethyleneimine (AEI; Aeroject, USA). This has been shown to render the virus non-infectious without altering its immunogenicity (Brown and Crick, 1959). The residual AEI was inactivated by addition of 0.5% sodium thiosulphate (Brown et al, 1963).

Inactivated virus was concentrated by a method based on that described by Fayet (1969) and Wagner, Card and Cowan (1970). Polyethylene glycol (PEG; molecular weight 6,000 daltons) was added to the virus harvest to give a final concentration of 6% w/v. This mixture was stirred overnight at 4°C and the precipitate was sedimented (2,200 x g for 30min). After discarding the supernatant the sediment was resuspended in 0.2M Tris buffer (pH 7.6) containing 1.5M NaCl to give a final volume one tenth of the original harvest. Using this concentrate as starting material, the PEG precipitation procedure was repeated to produce 20ml of a 100x virus concentrate.

Purification was achieved by the method of Brown and Cartwright (1963). The concentrate was clarified by centrifugation at 10,000 x g for 30min and the virions in the supernatant were sedimented by ultracentrifugation (100,000 to 120,000 x g for 1hr). The pellet was resuspended in 2ml of 0.04M phosphate buffer and allowed to elute overnight at +4°C. Sodium dodecyl sulphate (SDS) was added to give a final concentration of 1% before layering the virus concentrate onto an 18ml linear sucrose gradient (15% to 45% w/w) containing 0.3M NaCl prepared in 23ml polycarbonate centrifuge tubes. The gradient was centrifuged at 95,000 x g for 2½hr and 20 x 1ml fractions were collected after a hole was pierced in the base of the polycarbonate centrifuge tube using a red-hot 18 gauge needle.

Fractions containing the intact purified FMD virions known as the 146S particles (Bachrach, 1952; Breese, Trautman and Bachrach, 1960) were identified by measuring the optical density at 259nm. The mass of virus in these fractions was calculated using the formula of Bachrach, Trautman and Breese (1964) which showed that an optical density of 1.0 at 259nm is equivalent to 131.5µg of virus per millilitre.

The isolated virions were further analysed for purity and integrity of viral proteins by a modification of the polyacrylamide gel electrophoresis (PAGE) method (Maizel, 1969; Laemmli, 1970). A gel plate (15cm x 17.5cm x 1mm) was prepared between two pieces of glass using a stock solution of 30% acrylamide and 0.8% N, N', -methylene-bis-acrylamide. A resolving gel, which contained 12.5% stock acrylamide made up in 0.375M Tris buffer (pH 8.8) containing 0.1% SDS, was poured into a well formed by the two pieces of glass to a height of 13.5cm. This was polymerised chemically by adding 4% v/v N, N, N', N'-tetra-methylethylenediamine (TEMED) and 5% w/v ammonium persulphate to the gel

immediately prior to pouring. Once the resolving gel had fully polymerised a stacking gel, which contained 4% acrylamide made up in 0.125M Tris buffer (pH 6.8) containing 0.1% SDS, was added to give a final gel height of 15cm. This gel was polymerised in a similar manner to the resolving gel. Before polymerisation of the stacking gel was complete a template, which formed twelve 15 x 11mm sample wells, was inserted into the gel. The purified virions were dialysed for 24hr against 0.0625M Tris buffer (pH 6.8) containing 2% SDS, 10% glycerol, 1% 2-mercaptoethanol and 0.001% bromophenol blue, heated to 100°C for 4min to dissociate the viral proteins and 100µL samples of this material were added to each sample channel on the gel. The space at the top of the gel and the electrophoresis apperatus reservoirs were filled with electrode buffer (0.025M Tris buffer containing 0.192M glycine and 0.1% SDS, pH 8.3) and 8 milliamps of constant current was applied to the gel using a power pack ('Vokam' Shandon Southern, UK). When the bromophenol blue marker reached the bottom of the gel (after approximately 16hr) the current was switched off and the gel was removed and stained with 0.1% coomassie brilliant blue in 50% methanol and 7% acetic acid for 2hr at 37°C. Finally the gel was destained by repeated washing in 7% acetic acid.

d) Preparation of challenge virus

Steers were inoculated intradermolingually with 2.0ml of a 1:10 dilution of O_1BFS 1860 collected from cattle and stored at -20°C as a glycerinated filtrate. Twenty sites were inoculated per tongue, approximately 0.1ml per site, under Rompun (Bayer UK Limited) sedation. Epithelium (approximately 20gm) was harvested from each steer at 28hr post inoculation and stored at -20°C in 50:50 glycerol:0.04M phosphate buffer with 0.01% phenol red. Virus was harvested from eight grams of tissue which was minced with scissors and ground in a mortar using a

pestle, sterile sand and 0.04M phosphate buffer to produce a 1:25 suspension. The suspension was clarified by centrifugation (1,800 x g at room temperature) and then filtered through a 0.22μ membrane. The filtrate was mixed with an equal volume of sterile glycerol to a final concentration equivalent to one part of the harvested infected bovine tongue epithelium in 50 parts glycerol/0.04M phosphate buffer, stored at -20°C and labelled C2.

The infectivity titre was then estimated in pigs using a method described by Burrows (1966). Ten-fold dilutions of the C2 virus were prepared in 0.04M phosphate buffer and 0.1ml volumes were inoculated intradermally into the heel bulbs of four Large White cross-bred pigs (25-30Kg bodyweight). Each of four dilutions were tested using a separate foot, i.e. right fore 10^{-5}, left fore 10^{-4}, right hind 10^{-3}, left hind 10^{-2}. After 3 days the inoculation sites were examined for FMD lesions and sites showing signs of vesicle formation (blanching) were recorded as positive. The percentage of positive sites at each dilution on the four pigs was tabulated and the $\log_{10}$ ID_{50} titre was calculated using the following proportional distance formula:-

$$\text{log dilution next above 50\% positives} + \frac{\text{\% positive sites at dilution next above 50\%} - 50}{\text{\% positive sites at dilution next above 50\%} - \text{\% positive sites at dilution next below 50\%}}$$

$$= \log_{10} \text{ Pig foot } ID_{50} \text{ per 0.1ml.}$$

(1 $\log_{10}$ can be added to this value to give the pig foot ID_{50} per ml)

2.3 VACCINES

Standard batches of Wellcome foot and mouth disease oil emulsion vaccines, prepared by the production department at Wellcome Laboratory, Pirbright, were used. Baby hamster kidney 21 clone 13 suspension cells (Capstick et al, 1962; Capstick, 1963) were grown in stainless steel vessels equipped with automatic pH and temperature control (Telling and Stone, 1964; Telling and Elsworth, 1965). Cultures were infected with O_1BFS 1860/67 virus and the 24hr filtered virus harvest was inactivated with acetylethyleneimine as described by Pay et al (1971) and made up as a water-in-oil-emulsion with Marcol 52 (ESSO) and Arlacel A (ICI America) using Tween 80 (Difco) as emulsifier (Basarab, 1978). The batches used, coded O-115 and O-121, contained 5.23 and 13.4µg of virus antigen per 2ml dose respectively. Each batch was potency-tested 1-2 months prior to its use or had produced 100% protection in 8 to 10 week old pigs 21 days after vaccination with mean serum antibody titres for batches 115 and 121 of 2.7 and 2.8 $\log_{10}$ SN_{50} respectively.

2.4 VACCINATION AND CHALLENGE

Sows and piglets were inoculated with 2ml of the vaccine intramuscularly behind the ear using a an 18 gauge hypodermic needle.

Immunity was challenged by inoculation of 100 pig foot ID_{50} of O_1BFS 1860 C2 challenge virus into each of two heel bulbs on the left fore foot of each pig. Inspections for the development of local lesions were made 1, 3, 7 and 10 days later and pigs were classified as protected if lesions were found at the injection site and only one additional site on the body. Animals with more extensive lesions were regarded as unprotected. Two unvaccinated pigs in each test group were also challenged, as controls, to demonstrate the efficacy of the challenge virus.

2.5 SERUM, COLOSTRUM AND MILK SAMPLING

Blood was taken from the anterior vena cava of piglets (Carle and Dewhirst, 1942) using a 20 gauge 4cm hypodermic needle. New-born piglets were hand held during the procedure but larger animals, up to approximately 30Kg, were placed on their backs in a V-shaped trough with their heads extended downwards. One assistant held the forelimbs and head, while another held the hind limbs. Larger pigs, over 30Kg, were allowed to stand during bleeding and a 17 gauge 10cm hypodermic needle was used. A slip-noose over the upper jaw behind the canine teeth restrained the animal and pulled its head upwards to expose the required area. A finger was run down the side of trachea to a point just in front of the sternum to locate the hollow formed by the angle of the ribs with the trachea and at this site the needle was inserted into the anterior vena cava. Five ml of blood was collected from the piglets for the first month, 10ml during the second and third month, and thereafter 20ml volumes were obtained.

The blood was allowed to clot at room temperature and the clot was separated from the sides of the collection bottle with a glass rod. After leaving overnight at room temperature the serum was decanted and clarified by centrifugation at 600 x g for 10min. The clear supernatant was stored at -20°C. When required for testing the sera were thawed and inactivated at 56°C for 30min.

Samples of colostrum and milk were collected separately from two fore (one left and one right), two central and two hind teats of each sow at various times after farrowing. Collection was facilitated by injecting the sows with 1ml of Oxytocin (Leo Laboratories, UK) intravenously immediately before sampling. Some samples from individual teats were kept separately, while others were pooled, prior to centrifugation at 50,000 x g for 30min at +4°C. The whey was collected by carefully inserting a needle through the superficial fat layer, formed after centrifugation, and stored at -20°C. Before testing the thawed samples were inactivated at 56°C for 30min.

2.6 BODY WEIGHT AND BLOOD VOLUME DETERMINATION

Before blood samples were taken all the piglets were weighed. To do this piglets up to 1 month old were placed in a plastic bag and weighed using a spring balance (Salter, UK). Piglets 1 to 3 months old were placed in a specially constructed box and weighed on a bathroom scale (Salter, UK). Piglets older than 3 months were weighed on a commercial pig balance (Avery, UK). The body weight of each piglet was converted to blood volume using the formula of von Engelhardt (1966):

For piglets up to 25Kg body weight

$$BV = 9.5 \times W^{-0.068}$$

and for piglets above 25Kg body weight

$$BV = 17.9 \times W^{-0.27}$$

where

BV = blood volume in ml/100g of body weight, and

W = body weight (Kg).

2.7 CALCULATION OF OBSERVED ANTIBODY HALF-LIVES

The observed half-lives of maternally derived antibodies for each litter were calculated from the decrease in the mean $\log_{10}$ neutralizing antibody titres for that litter over time using a linear regression and the following two conditions. Firstly, only piglets with antibody titres >1.0 $\log_{10}$ at 3 days old were taken into account and secondly, in any litter where the antibody levels of individual piglets had fallen to their least detectable value (as indicated by subsequent bleedings) the mean half-life of the litter was determined up to that point.

2.8 CALCULATION OF ANTIBODY CATABOLISM AND/OR EXCRETION RATE

The antibody titres observed were corrected for increases in blood volume according to the following formula:

$$\frac{\text{Blood volume at sample point (a)}}{\text{Blood volume at start of the experiment (3 days after birth) (b)}} \times \text{FMD neutralizing antibody titre at sample point (c)} = \text{corrected titre}$$

In practice, the $\log_{10}$ values for the blood volumes were subtracted from each other ($\log_{10}a - \log_{10}b$) and the result was added to the $\log_{10}$ observed antibody titres ($\log_{10}c$) to give the corrected $\log_{10}$ titre. These corrected titres were used to calculate the half-lives of neutralizing antibodies due to the catabolism/excretion using a linear regression.

2.9 NEUTRALIZING ANTIBODY ASSAY

Test samples were assayed by a modification of the method described by Golding et al (1976). Fifty microlitre amounts of microtest medium (see Appendix 1) were dispensed into each well of 96 well flat-bottomed polystyrene microplates (M29ART; Sterilin laboratories, UK). An equal volume of test sample was then placed in the first well at the end of each pair of rows and doubling dilutions were prepared using a motorised hand diluter (Flow laboratories, UK). Fifty µl was discarded from the final dilution. Fifty microlitres of stock virus diluted to contain 100 tissue culture infective doses ($TCID_{50}$), was then added to each well and the plates were allowed to stand for 1hr at room temperature. Following this, 50µL of IBRS cell suspension (1×10^6 cells per ml) was added to each well, the plates were sealed with plastic plate sealers and incubated at 37°C for 48hr. After incubation the plate sealers were removed and the wells flooded with 10% citric acid in 0.85% saline, in order to fix the cells and inactivate infectious virus. Fixative was discarded after 30min and the cells were stained by flooding with 0.4% naphthalene black in 0.85% saline. Stain was washed off with tap water after 30min and the plates were blotted dry. Virus infection of the cells resulted in CPE which rendered them non-adherent to the plastic and they were easily washed away on staining. Therefore, blue-stained cell sheets were taken as an indication that either no virus was present or that infectious virus had been neutralized by the test sample, while clear unstained microplate wells indicated that infectious virus was present.

Control plates were included in each test as follows:

(a) Medium Control

This consisted of 4 rows of 8 wells containing 150µL of test medium.

(b) Cell Control

Fifty µL of IBRS cell suspension at the test concentration (1 x 10^6 cells per ml) were placed in 2 rows of 8 wells, 50µL of suspension at half that concentration (5 x 10^5 cells per ml) were placed in a further 2 rows of 8 wells and 50µL of cell suspension at double the test concentration (2 x 10^6 cells per ml) were placed in a third series of 2 x 8 wells. Each well also contained 100µL of test medium.

(c) Negative Serum Control

Two rows of a dilution series (1:2 to 1:256) of normal pig serum were prepared using the method described for test samples.

(d) Positive Serum Control

Convalescent serum from an O_1BFS 1860/67 infected steer was diluted from 1:2 to 1:4096 in 50µL of test medium using 6 wells per dilution. The microplate was then divided into pairs of rows and 50µL of three different virus doses (10,100 and 1000$TCID_{50}$) were added to each pair. After 1hr incubation at room temperature 50µL of cell suspension (1 x 10^6 cells/ml) were added to each well and the test was carried out as described.

(e) Titration of Stock virus

Stock virus was diluted in half $\log_{10}$ steps, using microtest medium as diluent. All virus dilutions were expressed as $\log_{10}$ of the reciprocal of the dilution. The lowest dilution used was the expected $\log_{10}$ virus titre minus 2 $\log_{10}$, and the highest dilution was 3.5 $\log_{10}$ from this. The assay consisted of 24 wells each containing 50µL of test

medium, 50µL of one of the virus dilutions and 50µL of cell suspension (1×10^6 cells/ml).

All control plates were sealed, incubated and stained as described previously.

Tests were discarded if any of the control plates gave unsatisfactory results. Acceptable control parameters were:

(a) Medium Controls

Appeared clear red before the plates were stained indicating that the test medium was not contaminated.

(b) Cell Controls

At test dilution cell sheets were confluent, at half test concentration cell sheets were sparse and at double cell concentration the cell sheets were overgrown.

(c) Negative Serum Controls

Maximum CPE and the absence of cell sheets were taken to indicate that no non-specific neutralization was occurring.

(d) Positive Serum Controls

Titres (i.e. 50% end points) of the rows containing $100TCID_{50}$ fell within a predetermined range.

(e) Virus Dose Used Within the Test

The test virus titre ($TCID_{50}$ per 50µL was calculated from the number of wells on titration plates containing intact, blue-stained cell sheets using an adaptation of a formula described by Spearman (1908) and Karber (1931):

$$\left(\log_{10} \text{ highest dilution} + \frac{\log_{10} \text{ dilution step}}{2}\right) -$$

$$\left(\frac{\text{No. of wells with intact cell sheets}}{\text{No. of wells per dilution}} \times \log_{10} \text{ dilution step}\right)$$

The virus dose used for any particular test was an estimated $100TCID_{50}$ based on the mean of all titrations carried out previously on that particular virus (expected titre). However, as a virus titration was also included within each test, to give the observed titre, it was possible to calculate the exact virus dose used by applying the following formula:

$$\left(\begin{matrix}\text{Virus titre } (\log_{10}) \text{ observed} \\ \text{by titration}\end{matrix}\right) - \left(\begin{matrix}\text{Expected virus} \\ \text{titre } (\log_{10}) \\ \text{determined previously}\end{matrix} - 2 \log_{10}\right)$$

If the virus dose used was above 2.5 $\log_{10}$ or below 1.5 $\log_{10}$ the test was discarded.

The neutralization test results were expressed as the $\log_{10}$ reciprocal of the sample dilution, in the total volume of the reaction mixture (i.e. after the virus had been added), which demonstrated 50% CPE in the wells. This titre was subsequently standardised to a 2 $\log_{10}$ virus dose using a dose-correction factor calculated from the positive serum control and virus titration plate results. A worked example, with photographs of typical test and control plate results, is shown in Appendix 2.

2.10 ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA)

The indirect ELISA method first described for virus antigen and antibody detection by Voller, Bidwell and Bartlett (1976) and Voller and Bidwell (1976), and subsequently adapted for measuring FMD antigen and antibody by Abu Elzein and Crowther (1978), was used for this study. The optimum test conditions were established using methods described by Voller, Bidwell and Bartlett (1979).

Purified FMD virus (146S antigen) was diluted in 0.05M carbonate-bicarbonate buffer, pH 9.6, to give a final concentration of 3µg/ml, and 100µL was added to each well of flat bottomed flexible polyvinyl chloride microtitration plates (M29, Dynatech Laboratories, UK). The virus was allowed to adsorb onto the microplates overnight at room temperature. The plates were then washed three times with phosphate buffered saline, pH 7.3, (PBS) containing 0.05% Tween 20 (BDH Chemicals Ltd, UK). The test samples were diluted in PBS containing 0.05% Tween 20 and 1% ovalbumin (Grade III, Sigma London Chemical Company Ltd, UK) and added in 100µL volumes. After incubation at 37°C for 1hr in a humidified container, the plates were washed three times as before and 100µl of anti-pig immunoglobulin conjugated to horseradish peroxidase was added at a pre-determined dilution (1 in 2,000 for anti-pig IgG whole molecule, Miles Laboratories, UK; 1 in 1,000 for anti-pig IgG and IgM heavy chain specific, and 1 in 8,000 for anti-pig IgA heavy chain specific, Bristol University, UK) in PBS containing 0.05% Tween 20 and 1% ovalbumin. The plates were then incubated for a further 1hr at 37°C in a humidified container, washed as before and 100µL of substrate (0.04% O-phenylenediamine with 0.004% hydrogen peroxide in 0.01M phosphate/citrate buffer) was added to each well. After leaving the test plates in the dark at room temperature until

suitable colour development had occurred in +ve control samples, usually about 2 to 8min depending on conjugate used, the reactions were stopped by adding 100µL of 12.5% sulphuric acid to each well. The intensity of the colour which developed was recorded by reading the plates at 492nm in a 'Multiskan' spectrophotometer (Flow Laboratories, UK). The apparatus was zeroed against 8 blank wells at the end of each microplate, containing only substrate and 12.5% sulphuric acid. All samples were assayed twice and results were either expressed as a fixed dilution OD or as a titration end point (for details of methods and analyses, see Appendix 2).

The ELISA technique described above was also modified, by using a fixed serum dilution of 1:10 and variable antigen concentrations ranging from 0 to 8µg/ml, in order to provide data for logistic model analysis (Hingley and Ouldridge, 1985). This model provided estimates of changes in the avidity and heterogeneity of the total antibody population (for details of the methods of analysis see Appendix 2).

2.11 METHODS FOR THE ISOLATION AND CHARACTERISATION OF IMMUNOGLOBULINS

a) Gel filtration

Bio-Gel A-5M (Bio-Rad Laboratories, UK) with a fractionation range for proteins of 10,000 to 5,000,000 daltons was used (Burgin-Wolff, Hernandez and Just, 1971). To prepare the column 500ml of Bio-Gel A-5M slurry was mixed with 1L of column buffer (0.1M Tris buffer containing 0.3M NaCl and 0.005% Thiomersal, pH 7.2) and the gel was allowed to settle in a 2L glass measuring cylinder. The supernatant was aspirated and discarded, and the process was repeated to ensure that any fine particles were removed from the gel. The gel was transferred to a Buchner flask and degassed by evacuating the flask for 5min. A 26mm x

950mm glass column (LKB Instruments Ltd, UK) was mounted vertically, buffer was added to the bottom 10cm and an extension reservoir was fixed to the top. The top of the outlet tube was then positioned above the extension reservoir and the degassed gel slurry was gently poured down a glass rod into the column. Care was taken to avoid trapping air bubbles. The gel was allowed to settle for 30min before the tip of the outlet tube was lowered to give a hydrostatic pressure of 100cm H_2O in order to pack the gel. After packing the extension reservoir was removed and an upper flow adaptor and buffer reservoir were fitted. The gel was then washed with 1L of gel buffer at a hydrostatic pressure of 95cm H_2O.

A 2.5ml sample of serum was added to the top of the column via a 3 way valve system connected to a syringe and eluted fractions of 38 drops (approx. 2.5ml) were collected as they emerged from the bottom of the column using an LKB Ultrorac fraction collector. A total of 180 fractions were collected from each run. The protein separation was monitored by continuous scanning of the eluate at 280nm using a flow-through cell in an LKB Uvicord 'S' U.V. monitor and recording the OD profile on an LKB 2210 pen recorder.

b) Sucrose density fractionation

Following the method described by Ouldridge, Francis and Black (1982) pig serum samples were diluted to 50% in isotonic saline and layered in 1ml volumes onto a 20ml sucrose density gradients in 23ml polycarbonate ultracentrifuge tubes. The gradients, which were 5 to 25% sucrose in isotonic saline, were formed using an LKB gradient mixer. The tubes were spun at 95,000 x g for 17hr at 18°C in an MSE 'Prepspin' ultracentrifuge after which 1ml sample fractions were collected in

bijoux bottles from a hole punctured in the bottom of the tubes. Generally, 20 x 1ml fractions were collected from each gradient. Each fraction was examined for optical density at 280nm and for FMD neutralizing activity. Resultant peaks were also characterised using immunoelectrophoresis, immunodiffusion and sedimentation coefficient analysis.

c) Production of antiserum to normal pig serum

A method similar to that described by Dresser and Greaves (1973) was used. Each rabbit was injected intramuscularly (i/m) in one thigh with 1ml of a water-in-oil emulsion, containing 0.5ml Complete Freunds' adjuvant (Difco Laboratories, UK) and 0.5ml normal pig serum (NPS) diluted 1:5. One week later a similar i/m inoculation was given in the other thigh. After 4 weeks the rabbit was injected with 1ml of a water-in-oil emulsion, containing 0.5ml incomplete Freunds' adjuvant and 0.5ml NPS diluted 1:5, divided among several subcutaneous sites. After a further 14 days rabbits were test bled and the quality of the serum was assessed in immunoelectrophoresis and immunodiffusion tests. If the results were considered acceptable the rabbit was bled out and the serum was stored at -20°C.

d) Immunoelectrophoresis

The method of Graber and Williams (1953) was used. Microscope slides were covered with 2.5ml of molten 1% Noble agar (Difco Laboratories, UK) in 0.05M barbitone buffer; pH 8.6. After allowing the agar to set at +4°C wells were cut into the middle of each slide. The slides were then placed in an electrophoresis tank (Shandon Southern, UK) and 2µL of sample was added to each well using 'Microcap' pipettes (Drummond Scientific Co, UK). Some samples were mixed with 1%

bromophenol blue which acted as an electrophoresis marker. Paper wicks soaked in electrode buffer (0.1M barbitone buffer; pH 8.6) were connected across the ends of the slides to the buffer reservoirs and a constant current of 8ma/slide was applied to the gels using a 'Vokam' power pack (Shandon Southern, UK). When the bromophenol blue marker neared the ends of the slides (about 1 to 1.5hr later) the current was switched off and the slides were removed from the tank. Troughs were cut in the gel and filled with rabbit anti-NPS. After keeping the slides in a humid container at room temperature for 24 to 48hr precipitation lines developed, which could be observed by viewing against a dark background. The slides were preserved by drying the gel under absorbant paper. They were then soaked twice in 0.85% saline for 15min, washed in distilled water and stained with 0.5% Coomassie Brilliant Blue in distilled water. After 15min the slides were destained with a 4:5:1 mixture of 96% ethanol, glacial acetic acid and water.

e) Double gel diffusion

The method of Oudin (1946) modified by Ouchterlony (1948) was used. Plastic immunodiffusion plates (Miles Laboratories, UK) were filled with 10ml of molten 1% Noble agar. After allowing the gel to set at +4°C wells, 5mm in diameter, were cut to form a pattern of one central well surrounded by six others. Fifty microlitres of rabbit antiserum to pig IgG, IgM or IgA (Nordic Laboratories, UK) was placed in the central well and 50µL of various test samples were added to the outer wells. The plates were covered, incubated in a moist chamber at room temperature for 48hr and then examined for precipitation lines.

f) Sedimentation coefficient determination

Samples were dialysed for 72hr against frequently changed 0.85% saline and diluted in 0.85% saline to give a protein concentration of 1-2mg/ml. The sedimentation coefficients (S values) were determined in an analytical ultracentrifuge, at 115,000 x g and 20°C, fitted with a Schlieren optical system with wavelength 550nm, knife-edge angle 75° and transmission scale 100-0%. Isotonic saline was used in the reference cell and scans were performed at 10-minute intervals. The S values were calculated using the following equations:-

$$S = \frac{dr}{dt} \times \frac{1}{W^2 r} \quad \therefore \quad S.dt = \frac{dr}{r} \cdot \frac{1}{W^2}$$

where $W = \frac{2\pi \times \text{rotor speed in rpm}}{60}$

r = Distance of a moving peak from the centre of rotation.
t = Time at which the scan was taken.
$\frac{dr}{dt}$ = The rate of movement of the peak.

Integrating and substituting for W,

$$S = \frac{2.303 \times \log r}{t(\text{sec})} \times \frac{3600}{(2\pi\ \text{rpm})^2}$$

Thus, if log r was plotted against t a linear slope could be calculated and used in the following equation:-

$$S = \frac{2.303 \times 3600}{(2\pi\ \text{rpm})^2} \times \text{slope of graph}$$

This calculation was performed in a Wang 2200 desk-top computer by 'feeding in' speed in RPM, R1 to R2 distance off graph in millimetres, balance cell distance in millimetres, scan interval in minutes, number of scans, number of peaks and the R1 to peak distance in millimetres for successive scans and peaks.

In order to standardize results the observed sedimentation coefficients were corrected for water at 20°C using the following equation:-

$$S_{20w} = S_{obs} \times \frac{\text{Viscosity saline } 20°C}{\text{Viscosity water } 20°C} \times \frac{1-\bar{V}P \text{ water}}{1-\bar{V}P \text{ saline}}$$

where

S_{obs} = S value observed

$\bar{V}$ = Partial specific volume (Albert and Johnson, 1961)

P = Density

Thus

$$S_{20}W = S_{obs} \times \frac{1.07}{1.002} \times \frac{1-(0.725 \times 0.9982)}{1-(0.725 \times 1.007)}$$

$$S_{20}W = S_{obs} \times 1.0936$$

Worked examples and traces of analytical runs on purified preparations of pig IgG and IgM are given in Appendix 2.

g) Protein assay

The protein assay method used was based on the binding of an acidic solution of Coomassie Brilliant Blue G-250 to proteins. When this occurs there is a shift in the wavelength of the maximum adsorption from 465nm to 595nm which allows the concentration of proteins to be determined (Bradford, 1976).

One hundred microlitres of bovine serum albumin protein standards with concentrations ranging from 0 to 1.4mg/ml protein and of appropriately diluted test samples were dispensed into clean, dry test tubes. Five millilitres of diluted, and filtered, dye reagent (Bio Rad Laboratories, UK) was then added and the solutions were mixed in a

vortex mixer, taking care to avoid foaming. After 5min the optical density was measured at 595nm against a reagent blank. The optical densities of the protein standards at 595nm were plotted against their protein concentrations and the protein concentration test samples were then read from the standard slope.

2.12 PREPARATION OF FMD NEUTRALIZING IgG ANTIBODY CONCENTRATE FOR PASSIVE TRANSFER EXPERIMENT

Five Large White pigs were inoculated i/m at 0 and 28 days with 2ml of O_1BFS 1860/67 oil-emulsion vaccine. After a further 30 days and at weekly intervals thereafter for 2 months, 400 to 500ml of blood was collected from each animal. After a break of 60 days (i.e. 6 months after the intial vaccination) each pig was given a further vaccination and 400 to 500ml of blood was again collected weekly from 30 days after this vaccination for 2 months. Serum from the blood samples was titrated for FMD neutralizing activity and for the class of antibody responsible using the sucrose density gradient separation technique, described previously. If the FMD neutralizing titres proved to be satisfactory (>2.0 $\log_{10}SN_{50}$), and activity was restricted to the IgG fraction, the samples were pooled. This stock of antiserum was purified and concentrated using a modification of the methods described by Warrington and Morgan (1971); Inglot, Gadzinska and Chudzio (1975); Curling, Lindquist and Erikson (1977).

The optimal conditions for concentrating FMD neutralizing pig IgG antibodies were investigated by precipitating pig antiserum with a range of PEG 6,000 concentrations varying from 0 to 25% final concentration and the following technique was established.

A 50% w/v solution of PEG 6000 was added slowly whilst stirring to pig antiserum pools to give a final concentration of 10% PEG and the mixture was stirred overnight at +4°C. The precipitate was sedimented at 2,200 x g for 30min in an MSE 6L centrifuge and redissolved by adding 0.2M Tris buffer (pH 7.6) containing 1.5M NaCl to give one tenth of the original volume. After stirring for 4hr at +4°C the concentrates were treated with antibiotics (100 i.u./ml penicillin, 100 i.u./ml neomycin and 100 i.u./ml polymyxin). In order to monitor the concentration process samples of the original serum pool, PEG supernatant, redissolved precipitate and final concentrate were assayed for anti O_1BFS 1860 FMD neutralizing activity.

CHAPTER 3

VALIDATION OF TESTS

3.1 CORRELATION OF MICRO NEUTRALIZATION TEST WITH CHALLENGE RESULTS

Introduction

In order to evaluate the virus neutralizing antibody titres, reported in subsequent chapters, it is important to determine their correlation with protection from infection.

This study compares the neutralizing antibody titres ($\log_{10}SN_{50}$) determined, just prior to live virus challenge, with the results of that challenge. In this way, predicted $\log_{10}SN_{50}$ values (PA values) can be equated with percentage protection from infection.

Experimental design

Sera collected from 156 pigs prior to O_1BFS FMD virus challenge were tested on three separate occasions for *in vitro* FMD virus neutralizing activity using the micro test method described in Chapter 2. The data produced were used in a probit analysis.

Results

Figure 3.1(a) shows the $\log_{10}SN_{50}$ titres (mean of repeated *in vitro* tests) of the individual pigs and whether or not they were protected from O_1BFS challenge, while Figure 3.1(b) is the probit regression slope showing the percent protected against the serum titres (the confidence limits are also shown). Table 3.1 summarises the predicted $\log_{10}SN_{50}$ titres (PA values) of the probit analysis at selected 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

Figure 3.1: Correlation between O_1BFS neutralizing antibody titres and protection from experimental infection

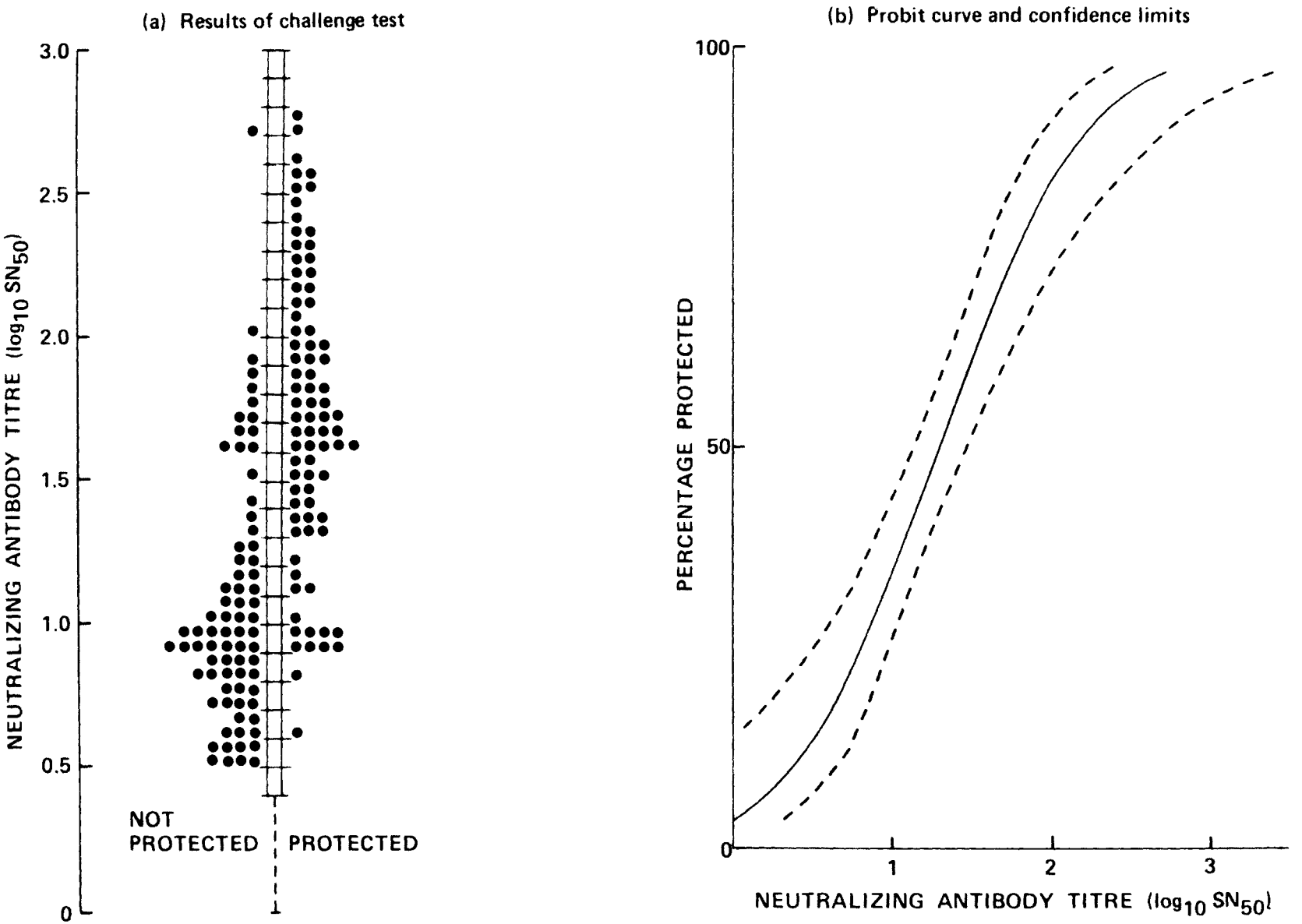


Table 3.1: Probit analysis of the O_1BFS neutralizing antibody titration and protection results for 156 O_1BFS challenged pigs

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

N.B. PA value for 62.5% protection is 1.53

interest since it corresponds to 5 out of 8 pigs protected which is the accepted passmark for oil-emulsion vaccines in pig potency tests.

Discussion

The probit analysis demonstrated that neutralizing antibody titres were correlated with protection from infection ($p<0.01$) and that the observed values were not significantly different from expected values in a chi-square test. Therefore, the results of this analysis can be used to predict the levels of protection that would be afforded by the $\log_{10}SN_{50}$ titres measured in the subsequent chapters of this thesis.

3.2 ANALYSIS OF GEL-FILTRATION FRACTIONS

Introduction

In order to study the immunoglobulin ,class-specific neutralizing activity of test samples after separation on a gel-filtration column it was necessary to characterize a sample of normal pig serum (NPS) and to identify the immunoglobulin-rich column fractions.

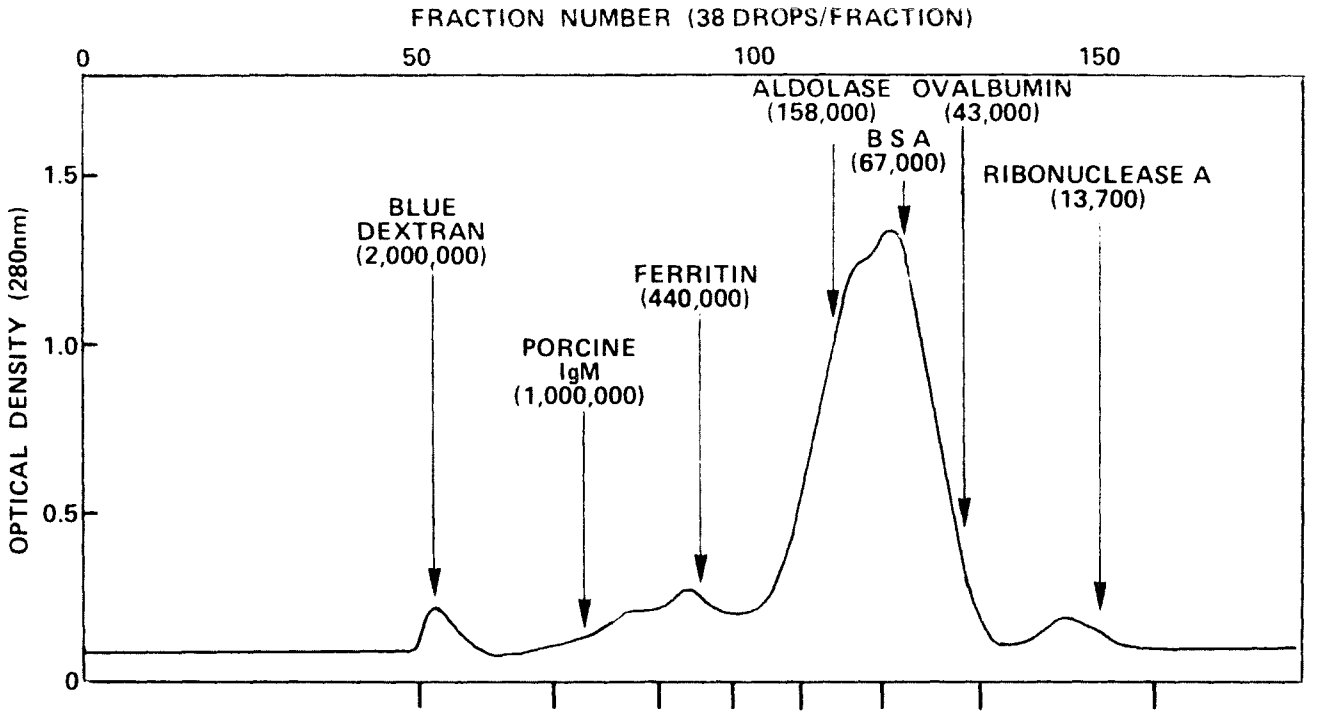
Experimental design

A Bio-Gel A-5M column was calibrated using seven different molecular weight markers. This column was then used to fractionate 2.5ml samples of NPS into either 54 x 90 drop fractions or 180 x 38 drop fractions. The 90-drop fractions were then tested for the presence of the three major serum immunoglobulin classes in an ELISA using anti-pig IgG, IgM or IgA conjugated to peroxidase. The 38-drop fractions were combined into 8 pools, using the 280nm elution profile (Figure 3.2) as a guideline, and tested for their sedimentation coefficient, precipitation in double gel diffusion, and, immunoelectrophoretic (IEP) characteristics.

Results

The 280nm elution profile for NPS and the elution sites for the molecular weight calibration markers are shown in Figure 3.2. This figure also gives the sedimentation coefficient, gel diffusion and IEP results of the 8 pooled fractions. The optical density values obtained by testing 30 of the 90-drop fractions in an ELISA are given in Table 3.2 and are presented graphically in Figure 3.3. From this figure the IgM, IgA and IgG-rich areas of the column could be identified and these corresponded with pooled fractions 2 to 3, 3 to 4 and 4 to 5 (Figure 3.2) respectively.

Figure 3.2: Separation of normal pig serum on a Bio-Gel A-5M gel filtration column



POOLS		①	②	③	④	⑤	⑥	⑦	⑧
SEDIMENTATION COEFFICIENT ($S_{20,w}$)		30.8	19.1	18.2	11.6	6.9	4.2	N.T.	N.T.
GEL DIFFUSION	IgM	+	+	+	–	–	–	–	–
	IgA	–	–	+	+	+	–	–	–
	IgG	–	–	–	+	+	+	–	–
I.E.P. (MAIN COMPONENT)		–	IgM	α2-Macro	IgA	IgG	Albumin	N.T.	N.T.

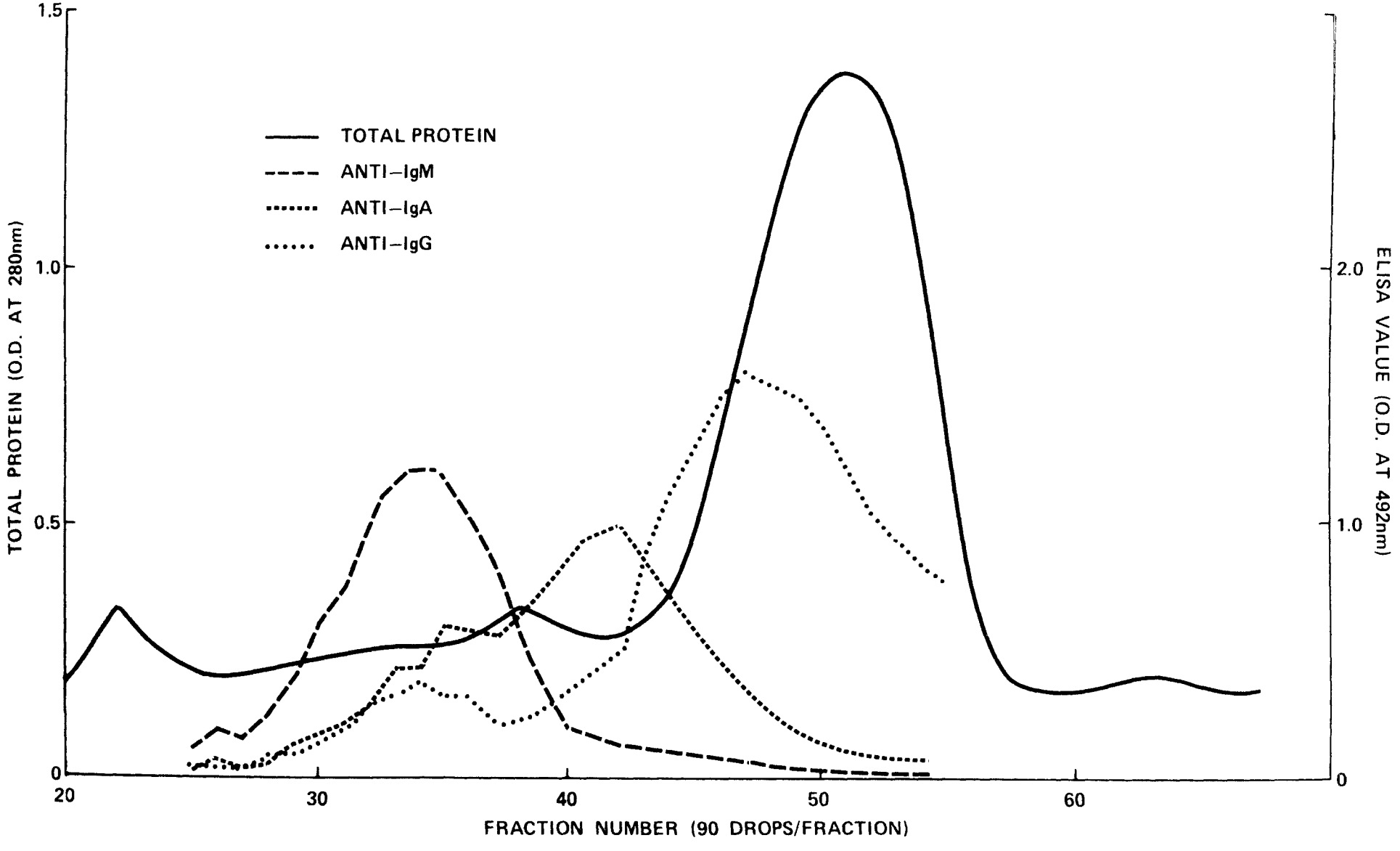
N.T. = NOT TESTED

Table 3.2: Characterization of gel-filtration fractions using immunoglobulin class-specific ELISA conjugates

*Fraction number (90 drops/fract.)	Conjugate specificity (OD at 492nm)			Fraction number (90 drops/fract.)	Conjugate specificity (OD at 492nm)		
	IgM	IgA	IgG		IgM	IgA	IgG
25	0.12	0.03	0.04	40	0.19	0.89	0.35
26	0.18	0.07	0.06	41	0.16	0.95	0.59
27	0.15	0.04	0.04	42	0.13	0.97	0.91
28	0.25	0.07	0.08	43	0.12	0.82	1.12
29	0.40	0.13	0.09	44	0.11	0.70	1.30
30	0.60	0.17	0.15	45	0.09	0.57	1.49
31	0.75	0.20	0.20	46	0.09	0.43	1.59
32	1.00	0.28	0.29	47	0.07	0.36	1.53
33	1.15	0.42	0.31	48	0.04	0.25	1.48
34	1.20	0.43	0.38	49	0.03	0.18	1.38
35	1.18	0.59	0.31	50	0.03	0.13	1.20
36	1.03	0.58	0.31	51	0.03	0.11	1.01
37	0.81	0.56	0.21	52	0.02	0.09	0.93
38	0.53	0.62	0.21	53	0.02	0.06	0.81
39	0.32	0.72	0.26	54	0.02	0.08	0.77

*Fractions diluted 1/100 prior to testing

Figure 3.3: Analysis of pig serum gel filtration fractions by enzyme linked imunosorbent assay (ELISA)



Discussion

The gel diffusion and IEP results identified pools 2, 4 and 5 as being rich in IgM, IgA and IgG respectively. The sedimentation coefficients (S_{20},w) for these pools (19.1, 11.6 and 6.9) agree fairly closely with published S_{20},w values for pig IgM, IgA and IgG (18.1, 9.5 and 6.6; Setcavage and Kim, 1976), despite the fact that these fractions contained some admixture of serum proteins. The calibration of the column with molecular weight markers could also be used to predict that IgM (MW 1,100,000), IgA (MW 330,000) and IgG (MW 170,000) would occur in pools 2, 4 and 5 respectively. Finally all these results were confirmed by ELISA analysis which identified three areas rich in IgM, IgA and IgG coinciding with pools 2, 4 and 5. There was some evidence of minor IgG activity in pool 2 (see ELISA profile, Figure 3.3), which agrees with previously published findings where an 18S IgG component was identified (Franek, Riha and Sterzl, 1961). However, activity was low in comparison to the main IgG peak in pool 5.

These results served to identify the IgM, IgA and IgG-rich areas of the gel filtration eluates, thus enabling neutralization results of column fractions to be broadly ascribed to the activity of individual immunoglobulin classes. They also confirmed that the conjugated antisera used in the ELISA were immunoglobulin class specific.

CHAPTER 4

RESPONSE OF PREGNANT SOWS TO VACCINATION

INTRODUCTION

The effectiveness of FMD oil-emulsion vaccines has been widely studied in fattening pigs from 1 to 2 up to 10 months old (McKercher and Giordano, 1967a; Anderson, 1969; Basarab and Pay, 1982; Ouldridge, Francis and 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 chapter will provide data on the level and persistence of FMD virus neutralizing antibody in the pregnant sow, the antibody classes involved and whether the nature of the antibodies changes with time after vaccination.

EXPERIMENTAL DESIGN

Ten pregnant Large White sows, never previously vaccinated against nor exposed to FMD, were divided into three groups of two and one group of four and vaccinated with FMD oil-emulsion vaccine according to the following schedule:

Group A: Sows NU02 and NU05 vaccinated once at 12 to 13 days before farrowing (dbf).

Group B: Sows NU01 and NU04 vaccinated once at 30 to 32dbf.

Group C: Sows NU00 and NU03 vaccinated twice at 51 to 52 and 31 to 32dbf.

Group D: Sows OE91, OE92, OE93, OE94 vaccinated twice at 87 to 89 and 30 to 32dbf.

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 80dpiv from sows NU00 to 05 and up to 90dpiv from 0E91 to 94 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 (NU02) and one doubly vaccinated sow (NU00) were further analysed by ELISA for IgG antibodies using (a) a titration method and (b) a logistic analysis (see appendix 2).

RESULTS

a) Neutralizing antibody response

The mean data of the repeated virus neutralization tests are shown in Table 4.1. These data are also presented graphically in Figures 4.1, 4.2 and 4.3. From Figures 4.1 and 4.3 it can be seen that neutralizing activity reached peak levels 2 to 4 weeks after primary vaccination and that this activity began to subside within 4 to 8 weeks. In those sows that did not receive a booster vaccination (Figure 4.1) the neutralizing activity returned to its original peak level about 14 to 35 weeks after vaccination. The level of neutralizing activity was then sustained or increased steadily (sows NU01 and NU05) until the end of the experiment (10 to 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

Table 4.1: Neutralizing antibody titres ($log_{10}SN_{50}$) of sows following FMD vaccination

Group A			Group B		
DPV	Tag no.		DPV	Tag no.	
	NU02	NU05		NU01	NU04
► 0	<0.46	<0.46	► 0	<0.47	<0.47
7	1.31	1.37	6	0.91	0.71
11	1.62	1.67	13	1.22	1.06
12-13	[1.67]	[1.67]	20	1.68	1.14
13-14	1.97	1.92	27	2.13	1.29
15-16	1.82	1.74	30	-	[1.44]
18	2.03	1.87	31	-	1.38
25	1.60	1.70	32	[2.07]	-
32	1.82	1.41	33	2.05	1.29
39	1.67	1.78	35	1.90	-
46	1.89	1.63	37-38	1.88	1.30
53	1.82	1.55	44-45	1.56	1.24
60	1.66	1.56	51-52	1.76	1.01
67	1.67	1.41	58-59	2.08	1.16
74	1.60	1.48	65-66	2.16	1.01
80	1.62	1.45	72-73	1.94	1.01
88	1.74	1.48	79-80	1.93	1.16
95	1.52	1.70	86-87	1.94	1.08
102	1.67	1.71	93-94	2.01	1.16
112	1.59	1.40	100-101	2.09	1.08
116	1.54	1.48	107-110	2.20	0.99
123	1.67	1.48	114-115	2.24	0.94
132	1.52	1.48	121-122	2.01	1.09
137	1.46	1.41	129	2.16	0.94
144	1.52	1.37	139	2.09	0.94
150	1.72	1.42	143	2.09	1.01
164	1.65	1.49	150	2.31	0.93
178	1.72	1.57	157	2.39	1.28
199	1.96	1.74	164	2.09	1.28
213	1.90	1.69	171	2.22	1.32
241	1.90	1.74	177	2.22	1.11
255	1.90	1.69	191	2.22	1.18
269	1.83	1.87	205	2.30	1.35
283	1.93	1.86	226	2.47	1.29
297	1.92	1.96	240	2.43	1.26
311	1.81	2.05	268	2.38	1.33
318	1.95	1.85	282	2.38	1.25
			296	2.41	1.35
			310	2.44	1.32
			324	2.47	1.26
			338	2.51	1.15
			345	2.61	1.29

[ ] = Farrow ► = Vaccination DPV = Days post vaccination

Table 4.1 contd: Neutralizing antibody titres ($log_{10}SN_{50}$) of sows following FMD vaccination

Group C			Group D				
DPV	Tag no.		DPV	Tag no.			
	NU00	NU03		OE91	OE92	OE93	OE94
► 0	<0.58	<0.58	► 0	<0.72	<0.72	<0.72	<0.72
8	0.88	0.82	3	<0.72	<0.72	<0.72	<0.72
12-14	1.05	1.64	10	1.04	1.26	0.98	1.42
► 20	1.13	1.71	17	1.62	1.76	1.42	1.87
27	2.32	2.91	24	1.72	2.07	1.72	2.17
34	2.39	2.62	31	1.67	2.02	1.77	2.03
41	2.17	2.69	38	1.62	1.72	1.46	1.87
48	1.94	2.39	45	1.51	1.72	1.62	1.92
51-52	[1.86]	[2.39]	► 57	1.42	1.51	1.66	1.87
52-53	1.85	1.80	60	1.51	1.61	1.67	1.77
54-55	1.88	1.95	66	2.93	2.79	2.73	2.78
58-59	1.91	1.95	78	2.68	2.42	2.43	2.42
65-66	1.83	1.81	87	2.47	2.31	2.28	[2.27]
72-73	1.58	1.63	88	-	[2.37]	-	-
79-80	1.63	1.80	89	[2.52]	-	[2.18]	-
86-87	1.73	1.96	90-92	2.48	2.07	2.08	2.37
93-94	1.53	1.65	94-96	2.52	2.28	2.17	2.23
100-101	1.68	2.11	101-103	2.37	2.27	2.12	2.17
106-107	1.63	1.88	108-110	2.12	2.28	1.97	2.07
114-115	1.58	2.19	115-117	2.22	2.17	1.92	2.22
120-121	1.54	2.22	122-124	2.22	2.12	2.22	2.07
128-129	1.68	1.93	129-131	2.17	2.02	2.18	2.22
135-136	1.63	2.03	136-138	2.23	2.12	1.97	2.12
143	1.68	1.96	143-145	2.22	2.02	1.92	2.12
153	1.58	2.11	156-158	2.32	2.12	1.77	2.08
157	1.68	2.11	170-172	2.17	2.17	2.00	1.82
164	1.54	2.34	184-186	2.37	2.17	1.74	2.05
171	1.83	2.34	198-200	2.12	2.17	1.97	2.02
178	1.58	2.38	212-214	2.52	2.43	2.09	1.98
185	1.74	2.44	226-228	2.22	2.28	1.78	2.09
191	1.70	2.24	240-242	2.47	2.32	1.82	2.02
205	1.59	2.30	254-256	2.33	2.52	1.67	2.07
219	1.79	2.32	268-270	2.38	2.23	1.72	2.07
240	1.93	2.47	281-284	2.48	2.02	1.93	2.02
254	1.87	2.50	295	2.58	2.17	1.83	2.27
282	1.93	2.59	312	2.34	2.22	1.92	2.22
296	1.78	2.56					
310	1.93	2.47					
324	1.99	2.44					
338	1.84	2.56					
352	2.11	2.41					
359	1.80	2.41					

[ ] = Farrow ► = Vaccination DPV = Days post vaccination

Figure 4.1: Response of sows to single vaccination at 12 to 13 or 30 to 32 dbf

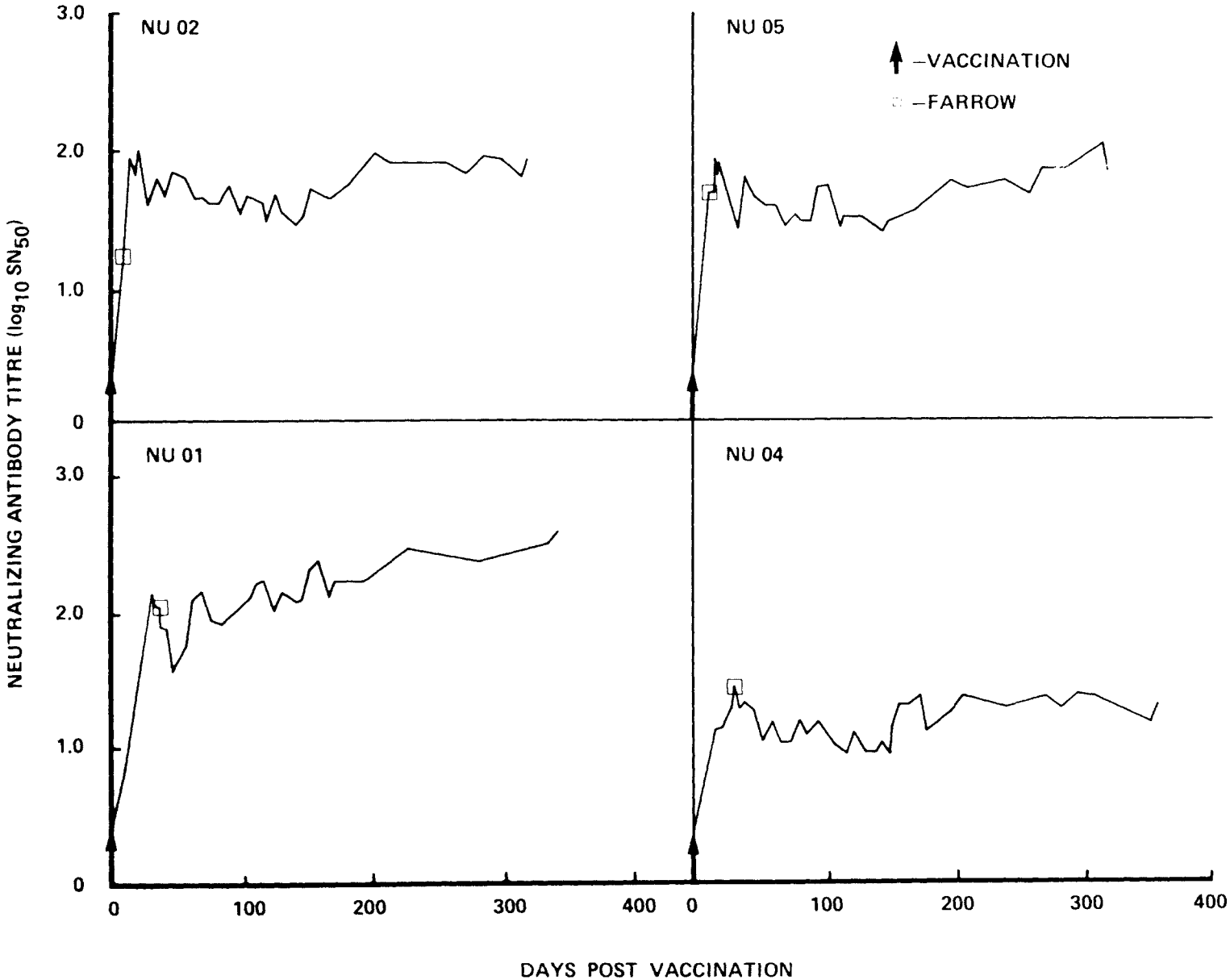


Figure 4.2: Response of sows to double vaccination at 51 to 52 and 31 to 32 dbf

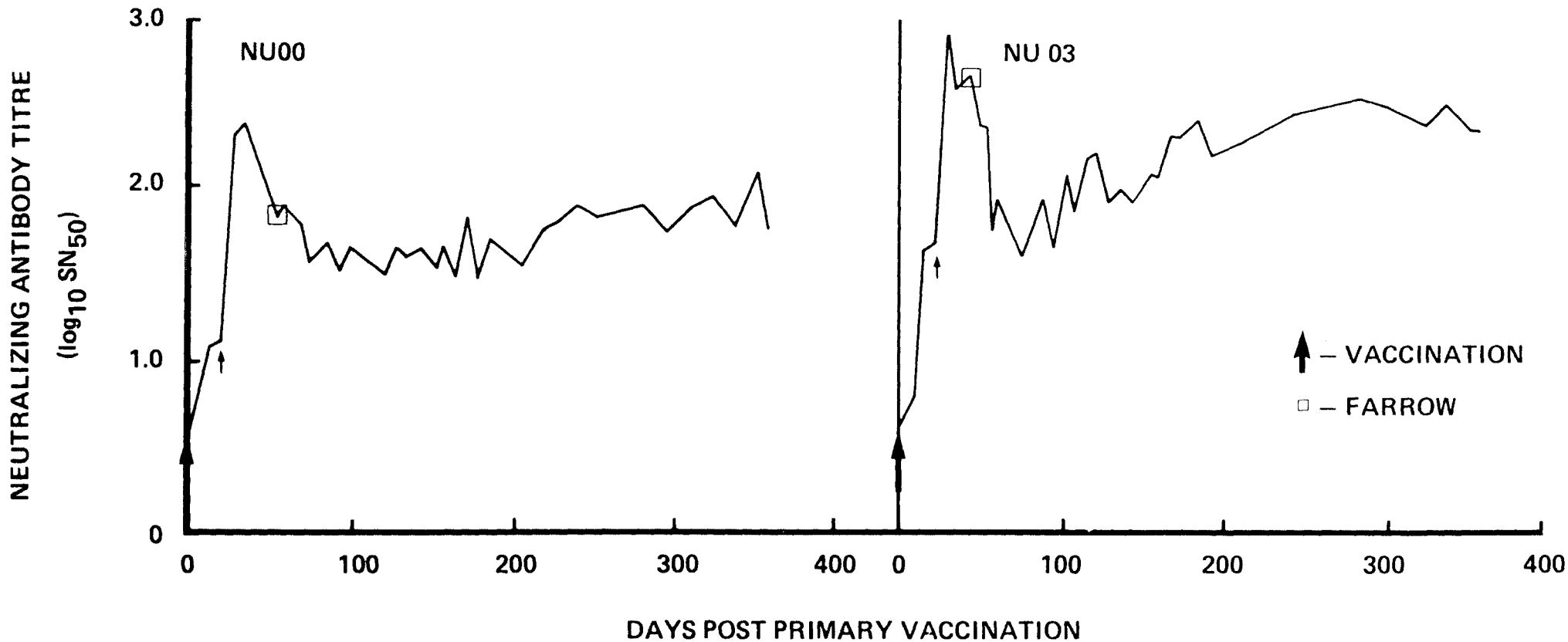
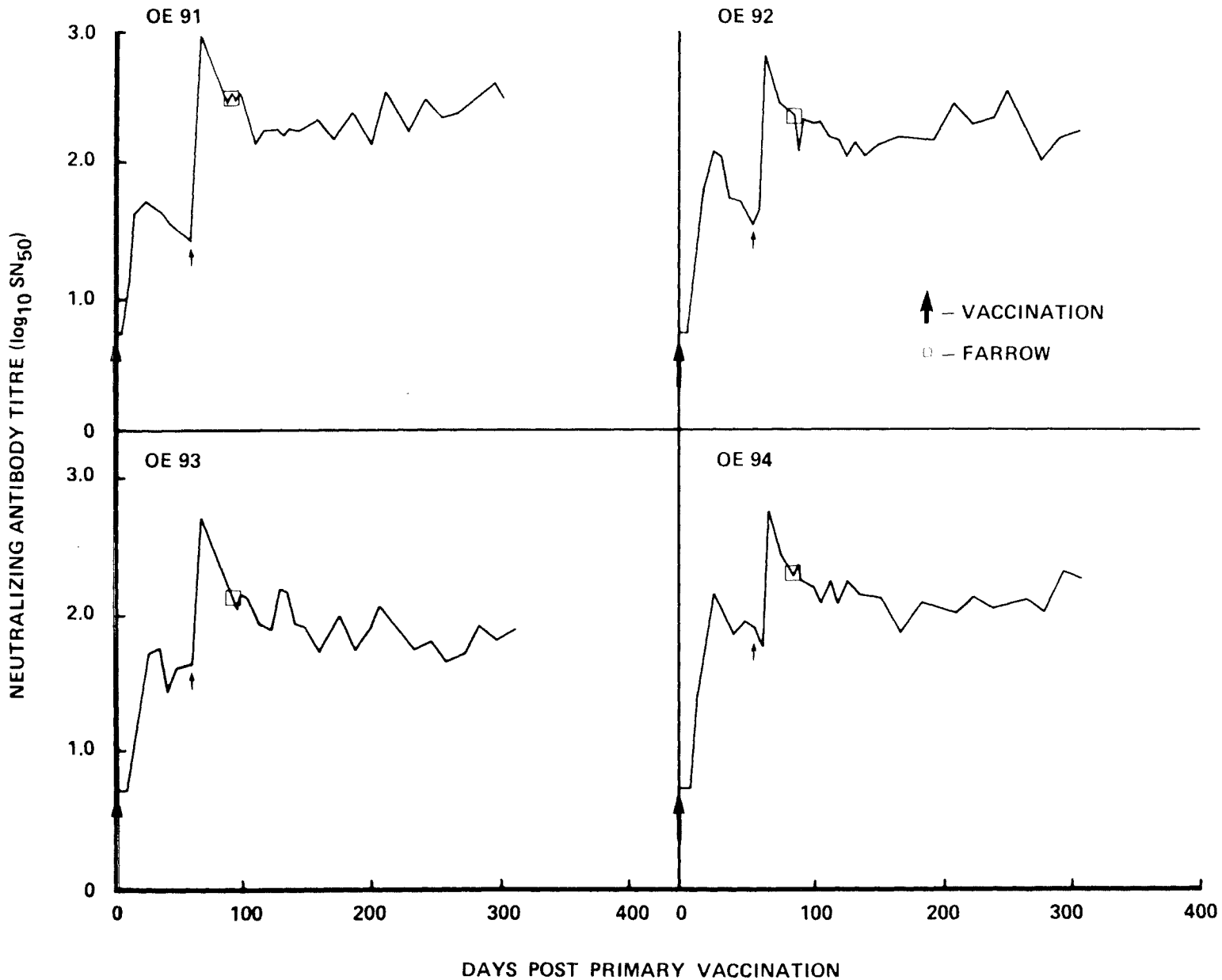


Figure 4.3: Response of sows to double vaccination at 87 to 89 and 30 to 32 dbf



after vaccination for more than 45 weeks. The exception was sow NU04 the neutralizing antibody titres of which remained at or just below the 50% protective level. Figures 4.2 and 4.3 show that revaccinating the sows at either 20 or 57dpiv boosted antibody levels from approximately 1.5 $\log_{10}SN_{50}$ to more than 2.2 $\log_{10}SN_{50}$ within 7 days. The peak of this activity subsided during the next 7 to 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% to 90% protection levels for the duration of the experiment (312 to 359dpiv).

It is interesting to note that at the time of revaccination (20dpiv) the serum antibody titre of sow NU00 (1.13 $\log_{10}SN_{50}$) was almost identical with that of sow NU04 (1.14 $\log_{10}SN_{50}$) and that revaccination boosted the antibody titres of NU00 to a 70 to 80% protection level for more than 10 months while those of NU04 remained at only a 40 to 50% protection level.

There was some indication of a limited fall in the SN_{50} titres immediately after farrowing (see Figures 4.1 to 4.3) but this was not severe and was only of short duration.

b) Comparative immunoglobulin-class activity

The mean optical density (OD) values from two immunoglobulin class-specific ELISA tests are summarised in Tables A3.1 and A3.2 (see appendix 3), and immunoglobulin class response curves for each animal are given in Figures 4.4 to 4.6. It is not possible to make direct comparisons between the magnitude of each immunoglobulin class response

Figure 4.4: Immunoglobulin class response of sows to single vaccination at 12 to 13 or 30 to 32 dbf

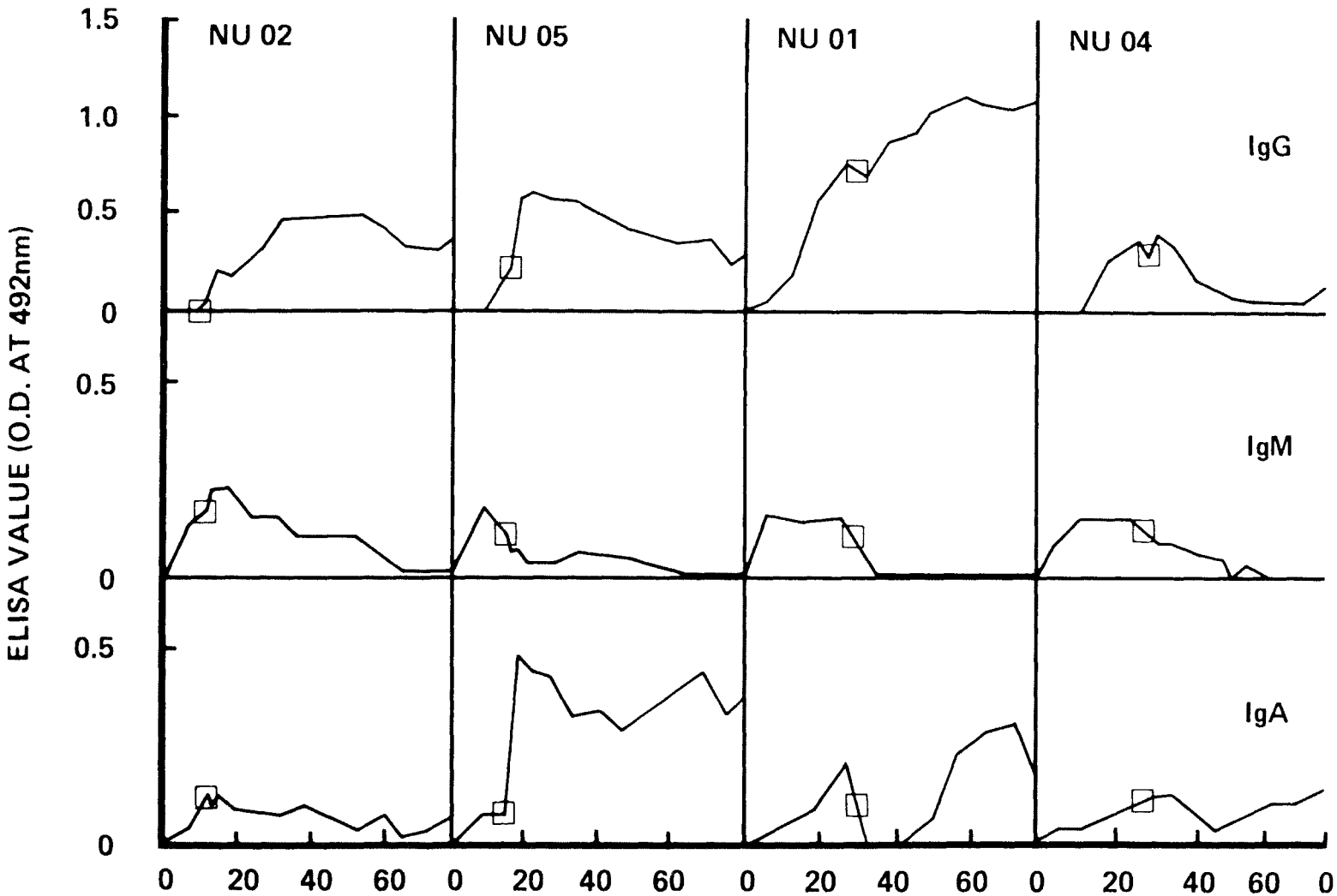


Figure 4.5: Immunoglobulin class response of sows to double vaccination at 51 to 52 and 31 to 32 dbf

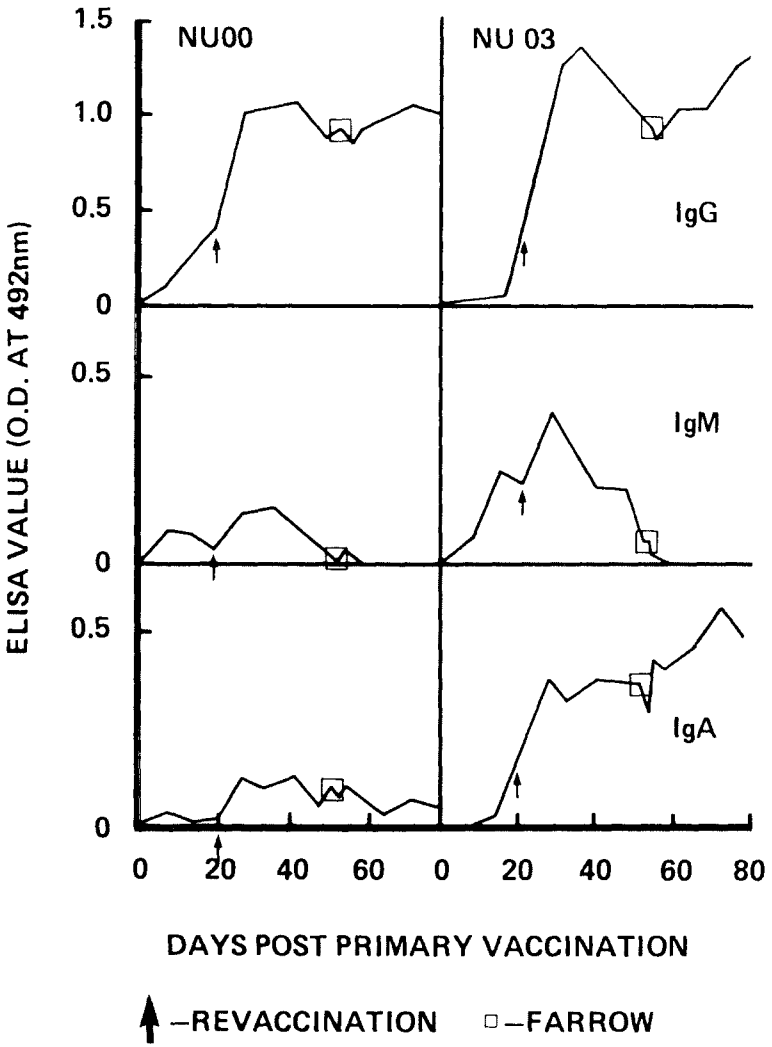
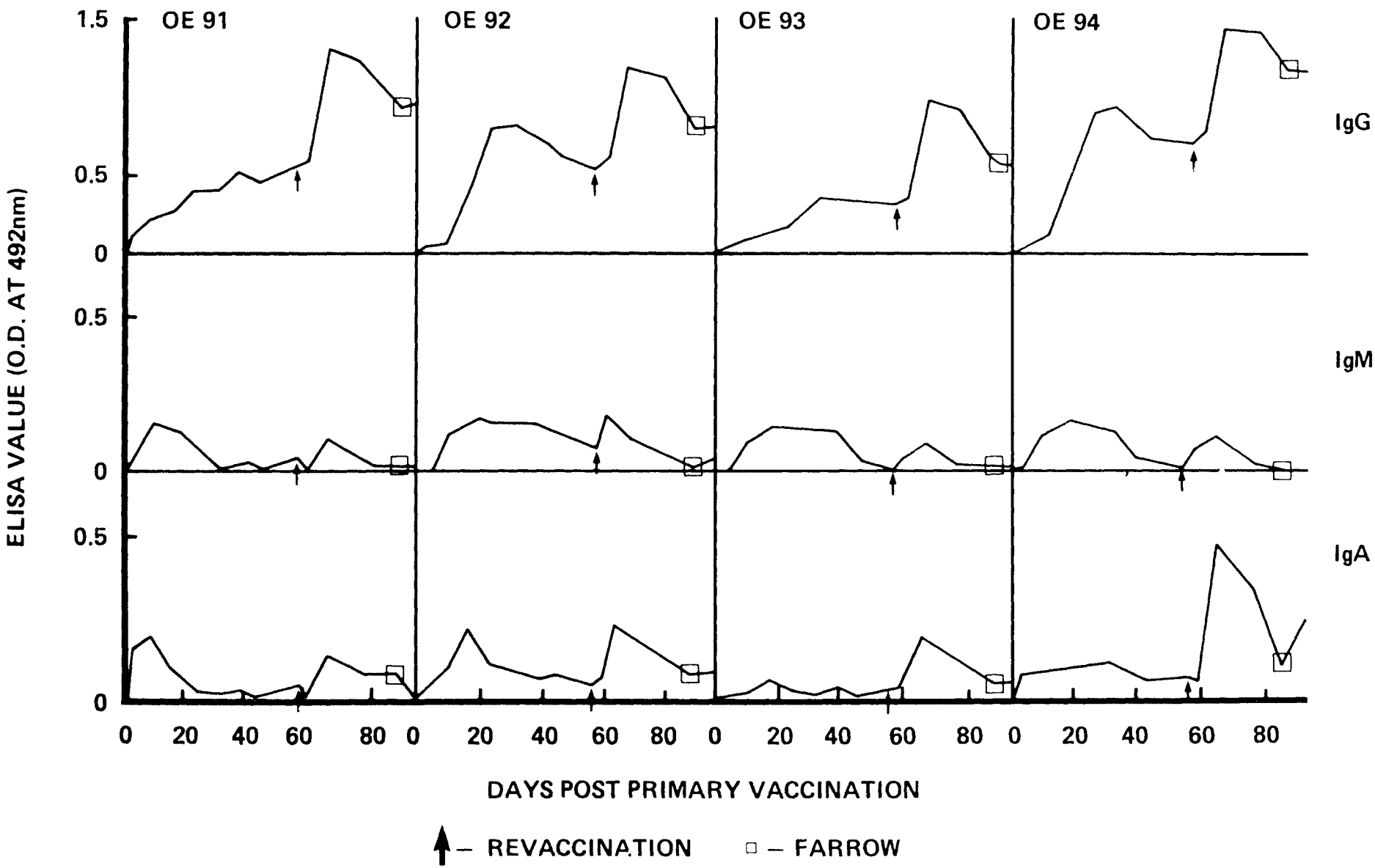


Figure 4.6: Immunoglobulin class response of sows to double vaccination at 87 to 89 and 30 to 32 dbf



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 (Figure 4.4) the IgM activity reached a peak within 1 to 2 weeks and then subsided to undetectable levels after 5 to 9 weeks. In contrast, the IgG response in the same sows did not reach a peak until 3 to 8 weeks after the primary vaccination and persisted for at least 11 weeks. The IgA response was generally more variable. For example, in sow NU05 the IgA response was especially pronounced and a peak of activity was observed 2 to 3 weeks after primary vaccination which persisted for more than 11 weeks. In sows NU02 and NU04 however, IgA activity was only present at very low levels. The remaining singly vaccinated sow (NU01) had a biphasic IgA response curve with peaks of activity at 27 and 73dpiv.

Revaccination of the sows (Figures 4.5 and 4.6) resulted in further IgM activity similar to that observed in the primary response. The IgG response, however, was significantly increased in magnitude to reach peak activity 1 to 2 weeks after revaccination. This effect was most apparent in sows revaccinated at 57dpiv (OE91 to 94) 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 Figure

4.5). 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.

c) The nature of the IgG antibodies involved in long-term immunity

The OD values obtained for IgG activity in an ELISA titration are presented in Table 4.2 and the calculated (see appendix 2b) IgG titres are shown in Table 4.3 and Figures 4.7 and 4.8. For comparison, neutralizing antibody titres for each sample are also given, showing that the ELISA IgG response profiles (Figures 4.7 and 4.8) for both sows were similar to the neutralizing antibody profiles, other than those from the 18dpv value for sow NU02 and the 20 and 51dpiv values for sow NUOC. 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.

The ELISA OD values used to produce computer fitted sigmoid curves for logistic analysis are provided in Table 4.4. From the curves estimates of $\bar{K}$, average intrinsic association, and $\underline{a}$, heterogeneity index, for IgG were established.

Although it is difficult to carry out reliable investigations into the functional affinity (avidity) of FMD virus/antibody interactions, due to the heterogeneity of the antigens and antibodies involved, the $\bar{K}$ value obtained by logistic analysis gives the association constant between the FMD antigen fixed to the ELISA plates and the IgG antibodies in the serum of the sows and thus an increase in $\bar{K}$ may be taken as an increase in the avidity of the antibody population being studied. The heterogeneity index ($\underline{a}$) is a measure of the number of different molecular species of IgG which can range from a value of 0 to 1; low

Table 4.2: Results of ELISA titration for IgG activity in sows following single or double vaccination

(a) Single vaccination (Sow NU02)

As dil (log_{10}) \ DPV	0	18	53	74	102	150	199	255	297	318
1.0	0.90*	1.10	1.24	1.15	1.15	1.25	1.20	1.19	1.17	1.17
1.5	0.64	0.79	0.95	0.86	0.90	0.97	0.92	0.89	0.93	0.92
2.0	0.42	0.52	0.57	0.51	0.57	0.63	0.57	0.52	0.54	0.53
2.5	0.23	0.26	0.26	0.22	0.28	0.31	0.26	0.22	0.24	0.27
3.0	0.13	0.10	0.10	0.09	0.14	0.14	0.11	0.10	0.11	0.13
3.5	0.07	0.06	0.04	0.05	0.06	0.07	0.06	0.04	0.05	0.07
4.0	0.04	0.03	0.03	0.03	0.04	0.03	0.02	0.02	0.02	0.03

(b) Double vaccination (Sow NU00)

As dil (log_{10}) \ DPV	0	20	51	72	100	157	205	254	296	324
1.0	0.75*	1.13	1.38	1.52	1.51	1.45	1.46	1.46	1.46	1.44
1.5	0.49	0.87	1.25	1.40	1.35	1.31	1.30	1.25	1.34	1.36
2.0	0.28	0.50	1.00	1.16	1.13	1.10	0.98	0.99	1.07	1.10
2.5	0.16	0.22	0.58	0.73	0.68	0.66	0.51	0.54	0.61	0.65
3.0	0.07	0.10	0.23	0.35	0.32	0.31	0.21	0.23	0.26	0.27
3.5	0.06	0.06	0.10	0.17	0.16	0.14	0.10	0.09	0.10	0.11
4.0	0.04	0.04	0.05	0.08	0.08	0.07	0.04	0.05	0.04	0.04

↑ Revaccination

As dil = Antiserum dilution DPV = Days post vaccination *Optical density at 492nm

Table 4.3: Results of ELISA titration and logistic analysis on sow serum

(a) Response of sow NUO2 to single FMD vaccination

Test	Days post vaccination									
	0	18	53	74	102	150	199	255	297	318
Neutralizing antibody ($log_{10}SN_{50}$)	<0.46	2.03	1.82	1.60	1.67	1.72	1.96	1.90	1.92	1.95
ELISA IgG titration (log_{10})	1.00	1.33	1.57	1.45	1.50	1.60	1.52	1.48	1.53	1.52
Corrected $\bar{K}$ value	0	0.19	0.20	-	0.20	-	0.49	-	-	0.64
Corrected $\underline{a}$ value	0	0.18	0.20	-	0.32	-	0.38	-	-	0.71

Table 4.3 (Contd.)

(b) Response of sow NU00 to double FMD vaccination

Test	Days post vaccination									
	0	20	51	72	100	157	205	254	296	324
Neutralizing antibody ($log_{10}SN_{50}$)	<0.58	1.13	1.86	1.58	1.68	1.68	1.59	1.87	1.78	1.99
ELISA IgG titration (log_{10})	1.00	1.68	2.30	2.50	2.43	2.40	2.23	2.26	2.34	2.39
Corrected $\overline{K}$ value	0	0.28	0.56	-	0.84	-	0.68	-	-	0.65
Corrected $\underline{a}$ value	0	0.21	0.38	-	0.36	-	0.28	-	-	0.35

▲ Revaccination

Figure 4.7: Nature of response to single vaccination (sow NU 02)

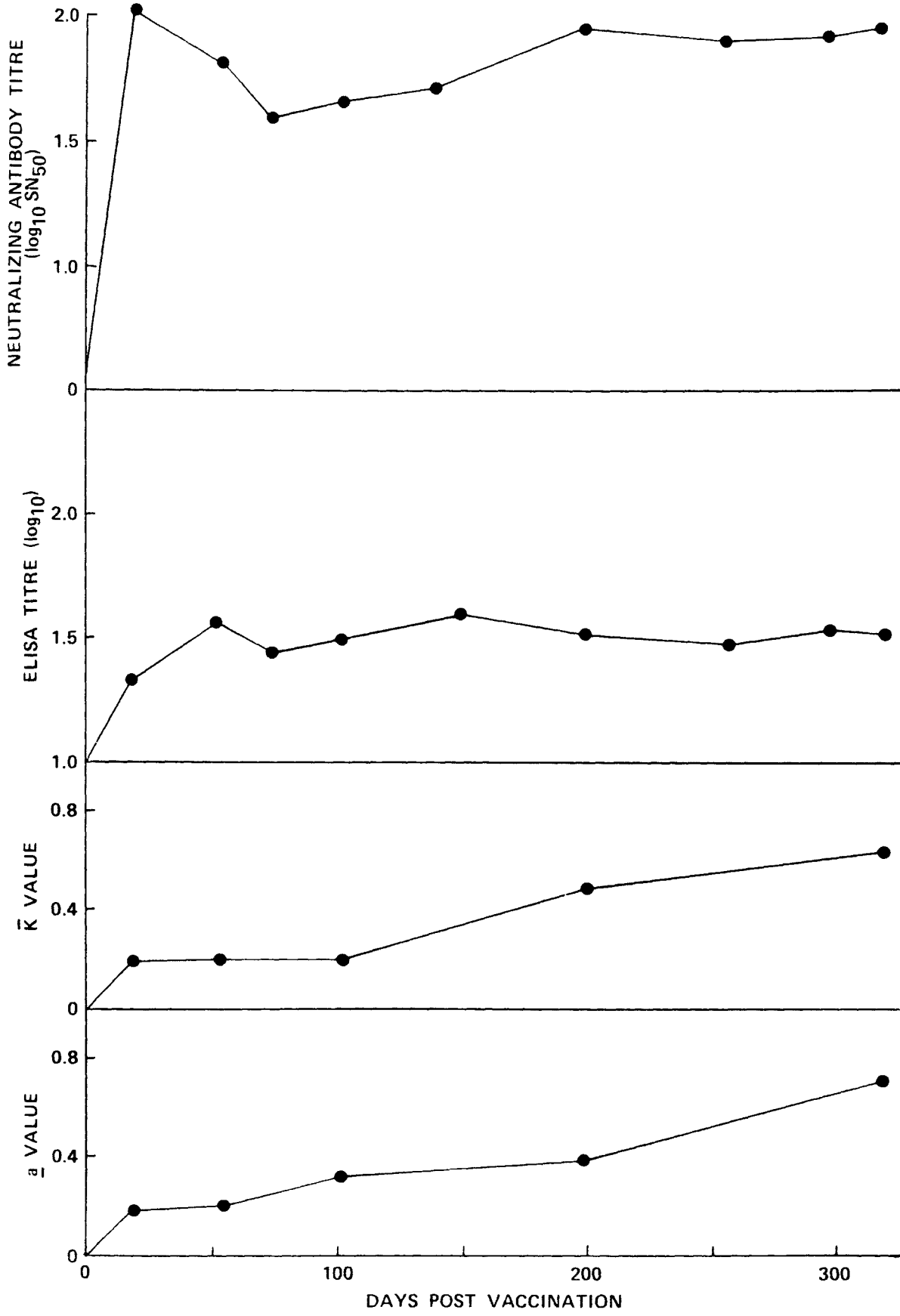


Figure 4.8: Nature of response to double vaccination (sow NU 00)

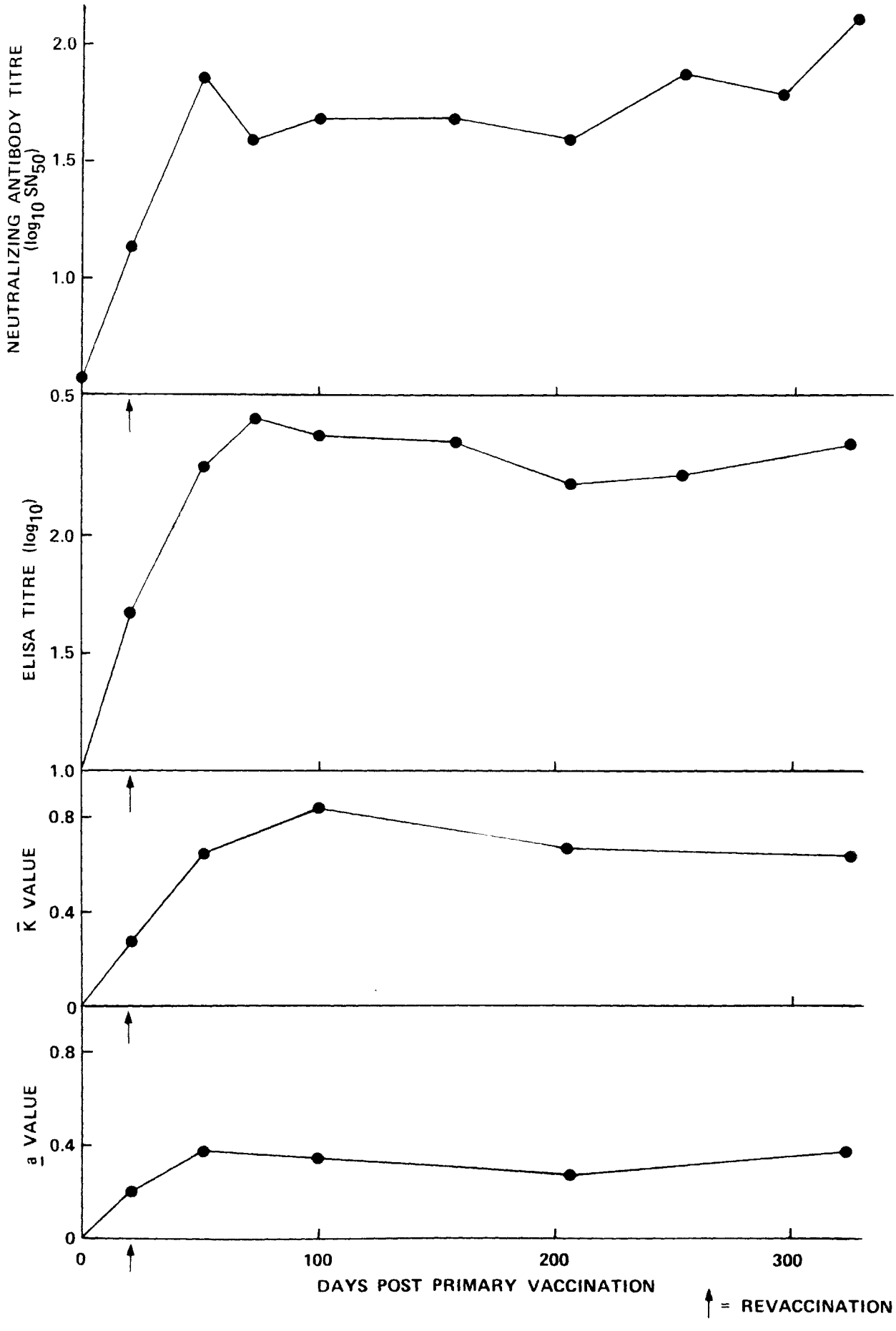


Table 4.4: ELISA data for logistic analysis of sera

(a) from a singly vaccinated sow (NU02)

140S antigen dose (μg/ml)	Days post initial vaccination					
	▲ 0	18	53	102	199	318
0	0.23*	0.19	0.20	0.14	0.18	0.14
0.25	0.39	0.48	0.45	0.39	0.49	0.43
0.50	0.57	0.65	0.64	0.53	0.68	0.61
1.00	0.67	0.82	0.92	0.76	0.99	0.96
2.00	0.90	1.22	1.13	1.19	1.44	1.46
4.00	1.08	1.60	1.65	1.55	1.72	1.71
8.00	1.26	1.67	1.67	1.66	1.79	1.75

(b) from a doubly vaccinated sow (NU00)

140S antigen dose (μg/ml)	Days post initial vaccination					
	▲ 0	▲ 20	51	100	205	324
0	0.16*	0.14	0.17	0.18	0.12	0.14
0.25	0.35	0.43	0.49	0.58	0.52	0.51
0.50	0.40	0.58	0.71	0.86	0.78	0.75
1.00	0.54	0.78	1.03	1.16	1.11	1.09
2.00	0.65	1.20	1.43	1.58	1.50	1.53
4.00	0.78	1.41	1.67	1.76	1.73	1.69
8.00	0.95	1.48	1.67	1.74	1.76	1.76

▲ = FMD vaccination

*Optical density at 492nm

values represent a large degree of heterogeneity, whereas monoclonal antibodies would have an index of 1. Table 4.3 and Figures 4.7 and 4.8 show that there were differences between the $\bar{K}$ and $\underline{a}$ curves of the two sows studied. Thus, in the serum samples from the singly vaccinated sow (NU02) the $\bar{K}$ and $\underline{a}$ values increased steadily for at least 300 days indicating that the IgG antibody population became more avid and less heterogeneous with time after vaccination, while in the doubly vaccinated sow (NU00) both $\bar{K}$ and $\underline{a}$ increased for 30 to 80 days after revaccination and then remained fairly constant for the next 200 to 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 (>1.53 $\log_{10}SN_{50}$) in three out of the four 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 (NU04) indicating that the duration of the response was not affected by the animal's poor initial response. These observations compare favourably with anti-FMD responses seen previously in young fattening pigs (McKercher and Giordano 1967(a); Graves et al, 1968; Wittman, Bauer and Mussgay, 1969; Anderson, 1969; Basarab, 1978; Ouldridge, Francis and 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 to 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 effect on the neutralizing antibody response which is in agreement with the findings of Morgan and 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 and Giordano, 1967(b); Anderson, Masters and Mowat, 1971(a); Ouldridge, Francis and 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 provide long-lasting immunity. The ELISA data obtained from pregnant sows supports these observations by demonstrating that IgM activity is transient, lasting for only 40 to 60 days after vaccination, and that it is the IgG antibodies that provide sustained levels of immunity. Although a clear 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.

The result that IgG antibody population becomes gradually less heterogeneous and more avid with time has been observed previously with antibodies against polio (Svehag, 1965), bacteriophage (Finkelstein and 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, valence and possibly molecular configuration of the antibody (Blank, Leslie and Clem, 1972). In the present study revaccination of the sows also increased the avidity and decreased the heterogeneity of the antibody population. However, the change was more rapid and levelled off after 30 to 80 days suggesting that a population of memory cells, produced as part of the animals' immune response to the primary vaccination, were being stimulated.

In conclusion it is apparent from these results that oil-emulsion vaccination provides pregnant sows with long lasting immunity to FMD virus infection equivalent to that previously observed in young fattening pigs.

CHAPTER 5

THE PASSIVE TRANSFER OF MATERNAL ANTIBODIES

INTRODUCTION

In order to design an optimal vaccination regimen for breeding sows it is essential to understand the factors affecting the transfer of immunity to the young. It is known that most, or all, absorption of colostral antibodies through the intestinal wall of the newborn piglet occurs during the first 24 to 48 hours after birth (Lecce and Morgan, 1962; Bourne, 1971; Dujin, 1971; Yabiki, Kashiwazaki and Namioka, 1974) and this raises the question as to what effect vaccination of the sow has on the passive transfer of maternally derived antibodies to the piglets. The proportion of the various antibody classes in the serum during the critical few days around farrowing and the predominant antibody class passed onto the piglets is of special importance since IgG, IgM and IgA vary in their decay rates (Porter and Hill, 1970; Curtis and Bourne, 1971). Hence inappropriate vaccination schedules for breeding sows may result in piglets which are either deficient in their initial antibody levels or conversely piglets which have satisfactory antibody titres initially but become susceptible to FMD at an early age due to the rapid decay rate of their maternally derived antibodies.

This chapter provides information about the class and titre of neutralizing antibodies transferred to piglets via the colostrum and the effect that the sows vaccination regimen has on this transfer.

EXPERIMENTAL DESIGN

The ten Large White pregnant sows, grouped and vaccinated as described in Chapter 4 were used to study the sow-to-piglet transfer of antibodies.

Blood was collected from groups A, B and C sows (NUOO to O5) at the time of farrowing and at 1, 3 and 5 to 7 days later. The piglets were bled just before suckling (groups B and C), or 1.5 to 2.5 hours after (group A) and again, 1, 3 and 5 to 7 days later. Blood samples were also collected from group D sows (OE91 to 94) at the time of farrowing and from their piglets at 3 days after farrowing.

Colostrum/milk samples were collected from group A, B and C sows at the time of farrowing and at 1, 3 and 5 to 7 days later. Samples taken on each occasion from two fore (one left and one right), two central and two hind teats were pooled. Colostrum/milk was also collected from group D sows at the time of farrowing and at 3 and 7 days later but in this case the teat samples were analysed separately.

All the serum and colostrum/milk samples were examined for neutralizing antibody and for immunoglobulin class activity.

RESULTS

a) Analysis of immunoglobulin class by ELISA

Table 5.1 summarises the results of the immunoglobulin class activity in sow serum and colostrum at farrowing, and in the pooled sera of 3-day-old piglet litters.

Table 5.1: Immunoglobulin class activity in sow serum and colostrum at farrowing and in the 3-day-old piglet serum as measured by ELISA

Group	Tag No.	IgM			IgA			IgG		
		Sow serum	Colostrum	Piglet serum	Sow serum	Colostrum	Piglet serum	Sow serum	Colostrum	Piglet serum
A (vaccinated 12/13dbf)	NU02	0.17*	0.27	0.38	0.14	0	0.34	0.03	0.04	0.19
	NU05	0.12	0.33	0.47	0.07	0.06	0.61	0.21	0.10	0.16
B (Vaccinated 30/32dbf)	NU01	0.06	0.07	0.10	0.10	0.02	0.29	0.70	1.15	0.74
	NU04	0.13	0	0.09	0.12	0.34	0.31	0.29	0.47	0.30
C (Vaccinated 51/52 and 31/32dbf)	NU00	0.01	0.10	0.15	0.10	0	0.20	0.92	1.09	0.90
	NU03	0.07	0.06	0.13	0.39	0	0.24	0.99	1.33	1.18
D (Vaccinated 87/89 and 30/32dbf)	OE91	0.03	0.05	0	0.08	0	0	0.95	1.45	1.27
	OE92	0.03	0	0.02	0.08	0.02	0.05	0.79	1.41	1.34
	OE93	0.03	0.07	0.10	0.06	0	0.05	0.63	1.41	1.35
	OE94	0	0.10	0.08	0.12	0.60	0.38	1.23	1.54	1.55

* OD at 492nm

dbf = Days before farrowing

The IgM concentration in group A sows at farrowing (where vaccination was close to farrowing) was higher than that in groups B, C or D sows (where the last vacination had been carried out at least one month before farrowing) whereas the IgG activity in groups B, C and D predominated over that in group A. The IgA activity, however, was variable and was not predominant in any single group.

The relative concentrations of each immunoglobulin class in the sows' serum at farrowing was also reflected in the colostrum and in the sera of their 3-day-old piglets. However, the levels of IgM activity in group A, and IgG activity in groups B, C and C, were relatively greater in the colostrum, and in the 3-day-old piglet sera, than those observed in the sows' serum at farrowing.

b) Neutralizing antibody activity

Tables 5.2 to 5.4 summarise the neutralizing antibody titres present in the sow serum, colostrum/milk and piglet serum of groups A, B and C at farrowing and for 144 to 168 hours after. Figure 5.1 shows the mean neutralizing activity within groups A, B and C in the form of a bar chart. At farrowing the mean serum titre of the sows vaccinated once at 12 to 13dbf (group A) was 1.67 $\log_{10}$ while that of the animals vaccinated 30 to 32dbf (group B) was 1.78 $\log_{10}$. The sows vaccinated twice (group C) had a mean titre of 2.13 $\log_{10}$ at farrowing. The serum titres of all four groups were correlated with colostral titres ($r=0.897$) as illustrated in Figure 5.2a. The interrelationship between the serum and colostrum titres was reflected by the slope of the regression line ($m=1.568$) which was in turn influenced by colostral titres which were lower than the serum titres at farrowing in group A, but higher than those in groups B, C and D.

Table 5.2: Neutralizing antibody titres in Group A sows (vaccinated 12 to 13dbf) and their piglets

Sow number		NU02				NU05			
		Time after farrow (hrs)				Time after farrow (hrs)			
		1.5	21	72	144	2.5	30	72	168
Sow serum		1.67	1.97	1.82	2.03	1.67	1.92	1.74	1.87
Piglet serum	1	1.01	1.87	1.49	1.33	1.11	1.32	1.13	1.07
	2	1.11	1.52	1.48	1.34	0.91	1.41	1.38	1.18
	3	1.36	1.67	1.58	1.46	1.72	1.52	1.44	1.34
	4	1.06	1.41	1.39	1.24	1.21	1.36	1.23	1.05
	5	<0.46	1.17	0.97	0.80	1.41	1.26	1.22	1.16
	6	1.26	1.62	1.45	1.21	1.06	1.10	0.90	0.95
	7	<0.52	Crushed	-	-	1.21	1.47	1.38	1.35
	8	1.06	1.62	1.45	1.12	1.46	1.51	Died on bleeding	-
	9	1.06	1.72	1.39	1.23	1.27	1.42	1.38	1.26
	10	0.71	1.67	1.43	1.27				
	11	1.21	1.67	1.39	1.30				
	12	<0.46	Died weak at birth	-	-				
	$\bar{x}$	0.94 ±0.31	1.59 ±0.18	1.40 ±0.15	1.23 ±0.17	1.26 ±0.23	1.37 ±0.13	1.26 ±0.18	1.17 ±0.13
Sow Colostrum/milk		1.64	1.77	1.51	0.97	1.56	1.62	1.56	1.35

dbf = Days before farrowing

Table 5.3: Neutralizing antibody titres in Group B sows (vaccinated 30 to 32dbf) and their piglets

Sow number		NU01				NU04			
		Time after farrow (hrs)				Time after farrow (hrs)			
		0	18	72	144	0	24	72	168
Sow serum		2.11	2.05	1.90	1.88	1.44	1.38	1.29	1.30
Piglet serum	1	<0.60	2.35	2.13	1.84	<0.47	1.36	1.29	0.96
	2	<0.60	2.05	2.13	2.14	<0.47	0.99	0.99	0.95
	3	<0.60	2.20	1.98	1.95	<0.47	0.84	0.62	0.61
	4	<0.60	1.45	1.29	1.31	<0.47	1.29	1.21	0.94
	5	<0.60	2.05	2.05	1.95	<0.47	1.21	1.14	1.00
	6	<0.60	1.90	1.75	1.65	<0.47	1.29	0.99	0.89
	7	<0.60	1.83	1.83	1.69	<0.47	1.07	0.84	0.74
	8	<0.60	2.28	1.98	1.84	<0.47	<0.56	<0.47	<0.48
	9	<0.60	2.35	2.28	1.99	<0.47	1.29	1.14	0.96
	10	<0.60	1.60	1.60	1.62	<0.47	0.99	0.92	0.86
	11					<0.47	1.07	0.99	0.89
	12					<0.47	1.29	Died on bleeding	-
	13					<0.47	1.14	1.07	1.07
	$\bar{x}$	<0.60	2.01 ±0.29	1.90 ±0.28	1.80 ±0.23	<0.47	1.11 ±0.22	0.97 ±0.22	0.86 ±0.17
Sow Colostrum/milk		2.41	2.13	1.52	1.11	1.42	0.91	<0.47	<0.48

dbf = Days before farrowing

Table 5.4: Neutralizing antibody titres in Group C sows (vaccinated 51 to 52 and 31 to 32dbf) and their piglets

Sow number		NU00				NU03			
		Time after farrow (hrs)				Time after farrow (hrs)			
		0	24	72	168	0	24	72	168
Sow serum		1.86	1.85	1.88	1.91	2.39	1.80	1.95	1.95
Piglet serum	1	<0.58	2.11	2.03	1.95	<0.58	1.55	1.94	1.73
	2	<0.58	2.11	2.15	2.18	<0.58	1.76	2.02	1.99
	3	<0.58	2.18	2.14	2.03	<0.58	2.03	1.87	2.02
	4	<0.58	1.91	2.15	2.00	<0.58	1.87	1.87	1.88
	5	<0.58	1.91	2.02	1.94	<0.58	1.83	2.02	1.80
	6	<0.58	1.78	1.54	1.67	<0.58	1.90	1.91	1.91
	7	<0.58	Stillborn	-	-	<0.58	Crushed	-	-
	8	<0.58	2.15	2.21	2.05	<0.58	2.04	1.95	2.05
	9	<0.58	1.72	2.00	1.76	<0.58	2.01	1.98	2.01
	10	<0.58	Stillborn	-	-	<0.58	1.73	1.91	1.64
	11	<0.58	1.96	2.00	2.00				
	$\bar{x}$	<0.58	1.98 ±0.16	2.02 ±0.19	1.95 ±0.14	<0.58	1.86 ±0.15	1.94 ±0.05	1.89 ±0.13
Sow Colostrum/milk		2.37	2.34	1.42	1.02	2.39	1.87	1.60	0.76

dbf = Days before farrowing

Figure 5.1: The mean neutralizing antibody titres of sow serum, colostrum/milk and piglet serum during the first week after farrowing

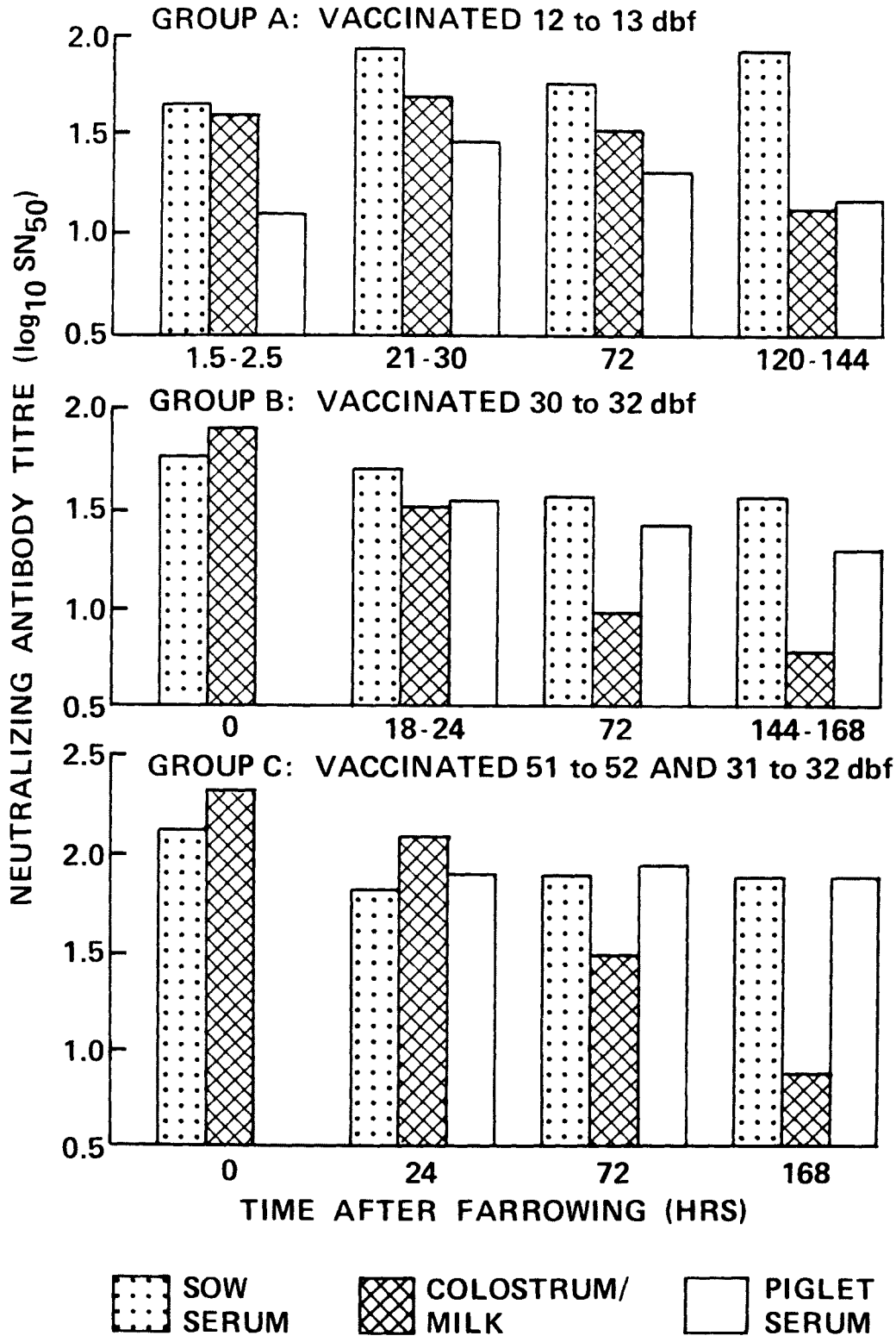
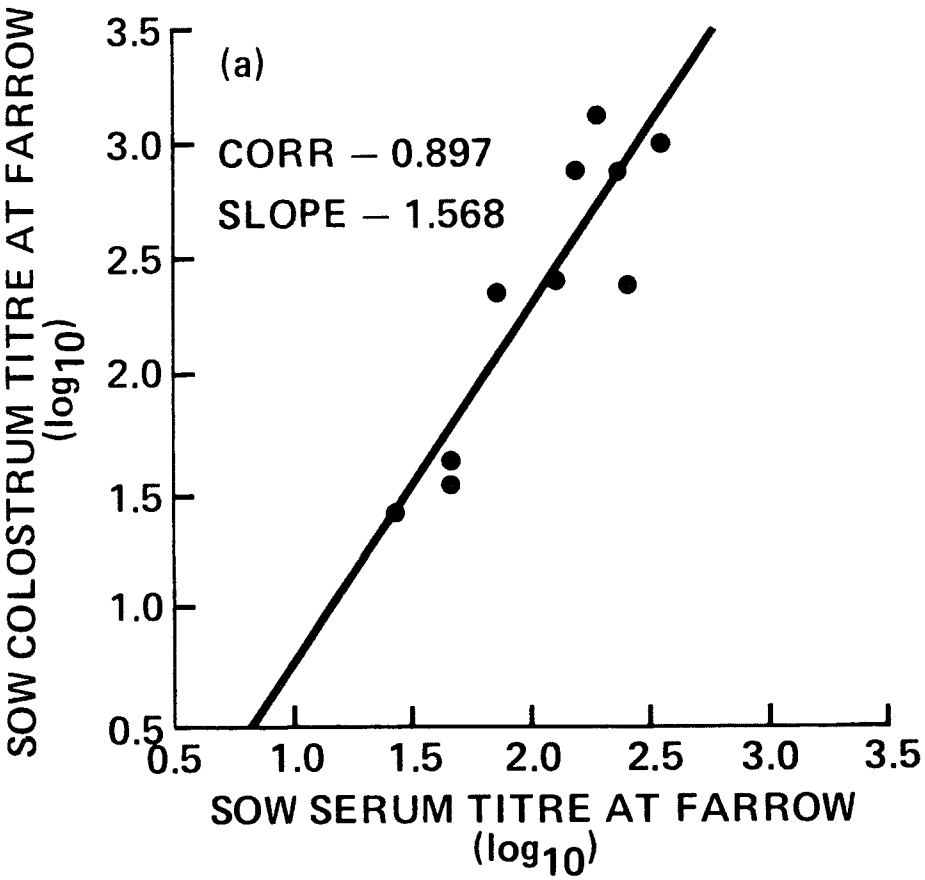
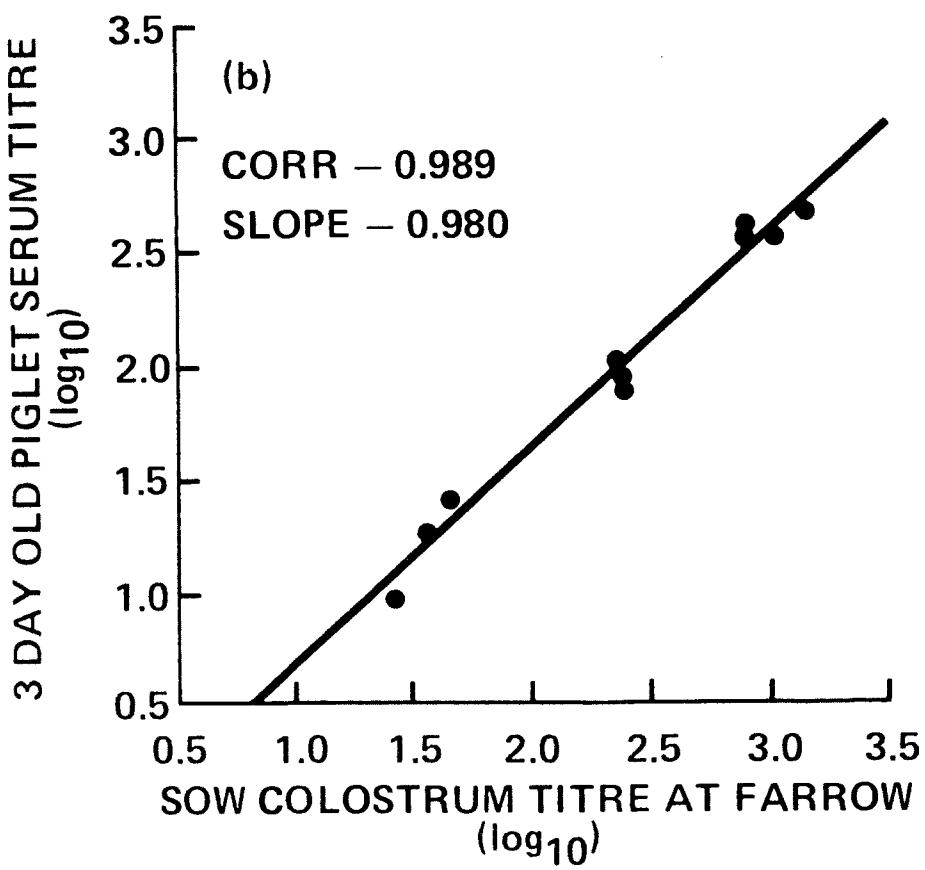


Figure 5.2: Regression analysis of relationship between sow serum, colostrum and 3 day old piglet serum titres





The serum titres of sows in groups B and C, all vaccinated at least a month before farrowing, declined during the first 24 hours after farrowing and then remained fairly constant for the next 5 to 6 days while the serum titres of group A sows (vaccinated 12dbf) increased during the first 21 to 30 hours after farrowing before declining somewhat. This result was also reflected in the colostral titres which increased during the first 30 hours after farrowing in group A but declined steadily in groups B and C.

The neutralizing activity in the colostrum/milk taken from the various teats of sows in group D (OE91 to 94) during the first week after farrowing is shown in Table 5.5, for comparison the sow serum titres are also given. The maximum deviation between titres from different teats occurred 3 days after farrowing and at this time there was significant ($P>0.005$) fore to hind variation as measured by a two way analysis of variance. The colostral titres at farrowing were also correlated ($r=0.989$) with the group mean 3-day-old piglet serum titres, for groups A to D, as illustrated in Figure 5.2b and in this case a direct relationship was suggested by the slope of the regression ($m=0.980$).

Sera collected from group B and C piglets prior to suckling had no detectable neutralizing activity whereas the mean titre of serum samples collected from group A piglets 1.5 to 2.5 hours after farrowing was 1.10 $\log_{10}$. Furthermore, individual piglets in these litters had titres as high as 1.36 and 1.72 $\log_{10}$. Peak antibody titres were observed in piglet groups A and B at 18 to 30 hours after farrowing and in group C at 72 hours after farrowing.

Table 5.5: Neutralizing activity in serum and colostrum/milk of group D sows (vaccinated 87 to 89 and 30 to 32dbf) for 1 week after farrowing

		0 Day		3 Day		7 Day	
Sow OE91		Left*	Right	Left	Right	Left	Right
Colostrum/milk titres	Fore*	2.90	2.94	1.89	1.65	1.43	1.49
	Central	2.99	2.94	1.74	1.79	1.54	1.54
	Hind	3.14	3.19	1.89	2.45	1.59	1.74
	Mean	3.02±0.12		1.90±0.28		1.56±0.11	
Serum titres		2.52		2.48		2.52	
Sow OE92							
Colostrum/milk titres	Fore	2.83	2.88	1.72	1.68	1.58	0.95
	Central	2.98	2.73	2.13	1.72	1.12	0.99
	Hind	3.03	2.93	2.28	2.48	1.68	1.37
	Mean	2.90±0.11		2.00±0.34		1.28±0.31	
Serum titres		2.37		2.07		2.28	
Sow OE93							
Colostrum/milk titres	Fore	2.55	2.90	1.20	1.25	0.66	0.80
	Central	2.80	3.10	1.19	2.15	0.62	1.55
	Hind	3.00	3.05	2.10	2.90	0.69	1.13
	Mean	2.90±0.20		1.80±0.70		0.91±0.36	
Serum titres		2.18		2.08		2.17	
Sow OE94							
Colostrum/milk titres	Fore	2.96	3.11	1.86	1.80	1.45	1.45
	Central	3.36	3.11	2.50	1.90	2.26	1.45
	Hind	3.06	3.31	2.86	2.36	1.90	2.06
	Mean	3.15±0.15		2.21±0.43		1.76±0.36	
Serum titres		2.27		2.37		2.23	

* Teat of origin for colostrum/milk samples

dbf = Days before farrowing

DISCUSSION

No FMD virus neutralizing antibodies were detectable in any piglet sampled at birth and prior to suckling which agrees with previous findings relating to the impermeability of the sow's epitheliochorial placenta (Brambell, 1958; Kim, Bradley and Watson, 1966(a); Porter, 1969; Dujin, 1971; Bourne et al, 1974). Nevertheless, group A piglets attained antibody levels as high as 1.36 $\log_{10}$ within 1.5 hours and 1.72 $\log_{10}$ within 2.5 hours of birth (Table 5.2) demonstrating the piglets' capacity to absorb protective antibodies from their mothers' colostrum. The peak of passive antibody activity was observed in the 18 to 30 hour samples collected from litters derived from singly vaccinated sows (groups A and B; Table 5.2 and Figure 5.1) which tends to support previous findings that antibody absorption ceases within 24 to 48 hours of suckling (Lecce and Morgan, 1962; Bourne, 1971; Dujin, 1971; Yabiki, Kashiwazaki and Namioka, 1974). However, the maximal level of passive antibody in piglets from revaccinated sows occurred between 24 and 72 hours after farrowing (Table 5.4 and Figure 5.1). It is therefore possible that the vaccination regimen of the sow may have influenced the rate at which the antibodies were absorbed by the piglets.

No significant teat-to-teat variation was detected in the colostral antibody titres at the time of farrowing, contrary to published data on the subject (Perry and Watson, 1967(b)). Therefore, any variation observed between the serum titres of individuals within a litter is not due to variation in the colostrum titres from different teats of the sow. It is possible that the greater degree of variation which occurred 3 days later was due to selective suckling by the young piglets. If this hypothesis is correct then the piglets displayed a preference for

suckling from the fore teats on the sow, as demonstrated by the significantly lower titres in samples collected from these teats 3 days after suckling.

There was a significant correlation (r=0.99) between the sows' colostral titres at farrowing and 3-day-old piglet serum titres (Figure 5.2) and since the regression slope was very close to 1.00 (m=0.98) a direct relationship between the two is indicated. This observation would imply that the piglet gut absorbs neutralizing antibodies of different immunoglobulin classes non-selectively. This is supported by previously published data (Bourne, 1971; Brown, 1976) and by the results of the immunoglobulin class analysis (Table 5.1) since the class of antibody predominating in the sows' colostrum at farrowing was directly related to the class predominating in the piglets' serum 3 days later. However, the class of immunoglobulin involved was dependent on the vaccination regimen employed since in group A (sows vaccinated 12 to 13dbf) the IgM activity was relatively greater than that of IgG while in groups B, C and D (sows vaccinated or revaccinated 30 to 32dbf) the opposite pertained and IgG activity was relatively greater than IgM. These findings were demonstrable in the sows' colostrum and were also reflected in the sera of their piglets. The relationship between IgA present in the sows' colostrum and that in piglet serum is less clear and does not appear to be related to the sows vaccination regimen.

There was also a good correlation between sow serum and colostrum titres at farrowing. However, the slope of the regression between the two (m=1.57) suggests that some mechanism of concentrating the antibodies occurred during the transfer of antibodies from the serum to the colostrum. This may have been either selective for immunoglobulin

class or influenced in some way by the sows' vaccination regimen. The increased concentration of antibodies in the colostrum over and above that in the serum has been observed by other workers (Curtis and Bourne, 1971; Jonsson, 1973).

The immunoglobulin class analysis also demonstrated that the class present in the sows' serum at farrowing was related to that in the sows' colostrum. However, the relative levels appeared to be governed by the period between the final vaccination and farrowing since the levels of IgG present in the colostrum of group A sows (vaccinated only 12 to 13dbf) were similar to or lower than the levels in the sows serum, while in groups B, C and D (vaccinated or revaccinated 30 to 32dbf) the IgG levels in the colostrum were invariably higher. Therefore, it appears likely that the active concentration of antibodies into the colostrum is influenced by the sows' vaccination regimen, in particular by the period between vaccination and farrowing.

CHAPTER 6

DECAY RATE OF MATERNALLY DERIVED ANTIBODIES IN PIGLETS

INTRODUCTION

Relatively little attention has been directed towards the FMD vaccination regimen of the sow and its effect on the class of antibody in the serum and colostrum at the time of farrowing. Since the antibody class influences the persistence of maternally derived antibodies in piglets (Porter and Hill, 1970; Curtis and Bourne, 1971) the experiments described here were designed to determine the effect of various sow vaccination regimens on the decay rate of maternal antibodies passed to their piglets and to investigate the influence of piglet growth rates on these results. The information obtained should facilitate predictions concerning the duration of colostral protection and any inhibitory activity (see chapter 7) in piglets born to FMD vaccinated sows. This would assist in the design optimal vaccination regimens for breeding stock.

EXPERIMENTAL DESIGN

The piglets used in this chapter for passive antibody decay rate studies were born to sows in groups A, B and C whose vaccination regimens have been described in Chapter 4.

Blood samples were collected from each piglet at 3 days old, 1 week and then weekly for 10 weeks. Immediately prior to each bleeding the weight of the piglets was recorded and converted to blood volume using the formula given in Chapter 2.

All sera were analysed for neutralizing antibody activity. In addition, sera from 3 to 21 day old piglets were analysed for class

activity by ELISA, and 3-day-old piglet sera were also subjected to gel filtration and the fractions were analysed for neutralizing antibody class activity. The percentage activity in each antibody class was calculated by taking the antilog of the neutralization titre for one class, dividing it by the sum of the antilog values for all three classes and multiplying that figure by one hundred. The observed half-lives of the maternally derived neutralizing antibodies in the piglet sera, before and after correction to take into account the expanding blood volume, were calculated according to the methods described in Chapter 2.

RESULTS

a) Antibody class analysis

The results of the neutralizing antibody class analysis (Table 6.1) demonstrated that in 3-day-old piglets born to group B and C sows vaccinated or revaccinated 30 to 32dbf, 60-91% of the neutralizing activity resided in the IgG fraction, while in piglets born to group A sows vaccinated 12 to 13dbf 100% of the neutralizing activity was in the IgM fraction. Results which tended to confirm this were obtained from the ELISA immunoglobulin class analysis (Table 6.2). At 3 days old the IgM activity was greatest in the piglets born to sows vaccinated 12 to 13dbf while the IgG activity was greatest in the sows vaccinated or revaccinated 30 to 32dbf. The ELISA results also indicated that IgA was present at similar levels in all the litters regardless of the sow vaccination regimen. The fact that the ELISA detected activity in immunoglobulin classes that did not display neutralizing activity may have been due to the greater sensitivity of the ELISA technique over to neutralizing antibody assay, or the fact that the antibodies detected were directed against non-neutralizing epitopes on the FMD virion.

Table 6.1: Neutralizing antibody class activity in 3-day-old piglet serum

Group No. (Vaccination)	Litter No.	Neutralizing antibody class activity ($Log_{10}SN_{50}$)			Percentage of total neutralization		
		IgM	IgA	IgG	IgM	IgA	IgG
A (12 to 13dbf)	NU02	1.75	0	0	100	0	0
	NU05	1.52	0	0	100	0	0
B (30 to 32dbf)	NU01	0.78	1.38	2.49	2	7	91
	NU04	0.85	0.85	1.32	20	20	60
C (51 to 52 and 31 to 32dbf)	NU00	0	1.96	2.77	0	13	87
	NU03	1.40	1.45	2.59	6	6	88

dbf = Days before farrowing

Table 6.2: Immunoglobulin class activity in the sera of piglets born to vaccinated sows (measured by ELISA)

Group No. (Vaccination)	Litter No.	Age of piglets (days)	Class specificity of conjugate		
			IgM	IgA	IgG
A (12 to 13dbf)	NU02	3	0.38*	0.34	0.19
		6	0.17	0.15	0.16
		13	0	0.02	0
		20	0	0	0
	NU05	3	0.47	0.61	0.16
		5	0.33	0.40	0.15
		12	0.01	0.09	0
		19	0	0	0
B (30 to 32dbf)	NU01	3	0.10	0.29	0.74
		6	0.05	0.13	0.60
		13	0	0.03	0.48
		20	0	0.01	0.39
	NU04	3	0.09	0.31	0.30
		7	0.04	0.06	0.22
		14	0	0.03	0.04
		21	0	0.01	0.03
C (51 to 52 and 31 to 32dbf)	NU00	3	0.15	0.20	0.90
		7	0.08	0.08	0.81
		14	0	0.02	0.66
		21	0	0.01	0.50
	NU03	3	0.13	0.24	1.18
		7	0.05	0.08	0.93
		14	0	0.02	0.80
		21	0	0	0.63

* O.D. values at 492nm

dbf = Days before farrowing

b) Observed half-lives of neutralizing antibodies

The neutralizing antibody titres (log_{10}) for each piglet, and the mean titres for each litter, at each sample point are shown in Tables 6.3 to 6.8. The observed half-lives of the neutralizing antibodies in each piglet, and each litter, calculated by linear regression analysis are provided in Table 6.9 and the decay rates are displayed graphically as linear regression slopes for each litter in Figures 6.1 to 6.3. The results were based on 7 to 8 piglets per litter except in litter NU04 where, due the sows' poor vaccination response, only 4 piglets met the first condition described in Chapter 2. The observed mean half-lives of maternally derived antibodies were 18.4 (range 12.4 to 23.6, n=8) and 7.4 (range 4.8 to 13.2, n=4) days in the piglets born to sows NU01 and NU04 vaccinated 32 and 30dbf respectively; 20.9 (range 16.9 to 25.6, n=8) and 20.6 (range 18.3 to 24.6, n=8) days in piglets born to sows NU00 and NU03 vaccinated at 51 and 31, and 52 and 32dbf respectively; and 4.2 (range 3.8 to 4.9, n=8) and 7.8 (range 6.0 to 9.8, n=7) days in piglets born to sows vaccinated 12 and 13dbf respectively. The fit of the linear regression slopes to individual piglet and litter mean data was highly significant ($r>0.9$, $P<0.001$) in all cases.

c) Corrected half-lives of neutralizing antibodies

The blood volume (ml) of each piglet, calculated from their body weights, and the litter mean blood volumes at each sample point are provided in Tables 6.10 to 6.15. These values were used to correct the antibody decay rate data for blood volume increase (see Chapter 2) and the corrected decay rates are shown in Table 6.16 and Figures 6.4a to 6.4f. The half-lives of antibodies in the serum of piglets from group A sows (NU02 and NU05), vaccinated 12 and 13dbf, corrected for blood volume increase were 6.9 and 18.5 days respectively (Figures 6.4a and 6.4b) while the corrected half-lives in piglets from group B and C sows (NU01,

Table 6.3: Serum titres ($\log_{10}$) of piglets from group A sow NUO2 vaccinated 12 days before farrowing

Days post farrowing	3	6	13	20	27	34	41	48	55	62	68
Piglet no.											
1	1.48	1.34	0.72	<0.61	<0.54	<0.54	<0.54	<0.54	<0.54	<0.54	<0.54
2	1.58	1.46	0.72	<0.54	<0.54	<0.54	-	-	-	-	-
3	1.39	1.24	0.78	0.61	<0.54	<0.54	<0.54	<0.54	<0.54	<0.54	<0.54
4	1.45	1.21	0.72	<0.54	<0.54	<0.54	<0.54	<0.54	<0.54	<0.54	<0.54
5	1.45	1.12	0.72	0.54	<0.54	<0.54	<0.54	<0.54	<0.54	<0.54	<0.54
6	1.39	1.23	0.72	<0.54	<0.54	<0.54	<0.54	<0.54	<0.54	<0.54	<0.54
7	1.43	1.27	0.78	0.54	<0.54	<0.54	<0.54	<0.54	<0.54	<0.54	<0.54
8	1.39	1.30	0.78	0.54	<0.54	<0.54	<0.54	<0.54	<0.54	<0.54	<0.54
$\bar{x}$	1.45	1.27	0.74	-	-	-	-	-	-	-	-
S.D. ±	0.06	0.10	0.31								

Table 6.4: Serum titres (log_{10}) of piglets from group A sow NU05 vaccinated 13 days before farrowing

Days post farrowing	3	5	12	19	26	33	41	47	54	63	69
Piglet no.											
1	1.13	1.07	0.74	0.66	<0.59	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50
2	1.38	1.18	0.81	0.74	0.68	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50
3	1.44	1.34	0.81	0.68	0.68	0.59	<0.50	<0.50	<0.50	<0.50	<0.50
4	1.23	1.05	0.95	0.68	<0.59	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50
5	1.22	1.16	1.07	0.69	0.59	0.54	<0.50	<0.50	-	-	-
6	1.38	1.35	1.10	0.68	0.59	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50
7	1.38	1.26	0.86	0.68	0.59	0.50	<0.50	<0.50	<0.50	<0.50	<0.50
$\bar{x}$	1.31	1.20	0.91	0.69	-	-	-	-	-	-	-
S.D. ±	0.11	0.12	0.14	0.02							

Table 6.5: Serum titres (log_{10}) of piglets from group B sow NU01 vaccinated 32 days before farrowing

Days post farrowing	3	6	13	20	27	34	41	48	55	62	69
Piglet no.											
1	2.13	1.84	1.47	1.35	1.02	1.18	1.28	1.05	1.04	0.94	0.80
2	2.13	2.14	1.64	1.50	1.48	1.48	0.95	0.73	0.74	0.67	0.61
3	1.98	1.95	1.47	1.47	1.32	1.32	1.25	1.03	1.02	1.02	0.87
4	2.05	1.95	1.64	1.18	1.49	1.16	1.25	1.25	1.02	0.95	0.62
5	1.75	1.65	1.55	1.54	1.42	1.13	1.18	1.16	1.03	0.87	0.75
6	1.83	1.69	1.62	1.56	1.49	1.41	1.41	1.17	0.88	0.95	0.87
7	2.28	1.99	1.64	1.50	1.48	1.43	1.34	1.25	1.10	1.09	0.89
8	1.69	1.62	1.40	1.25	1.63	1.18	1.25	1.10	1.03	0.95	0.67
$\bar{x}$	1.98	1.85	1.55	1.42	1.42	1.29	1.24	1.09	0.98	0.93	0.76
S.D. ±	0.21	0.19	0.10	0.14	0.18	0.14	0.14	0.17	0.12	0.12	0.12

Table 6.6: Serum titres (log_{10}) of piglets from group B sow NU04 vaccinated 30 days before farrowing

Days post farrowing	3	7	14	21	28	35	42	50	56	63	70
Piglet no.											
1	1.29	0.96	0.58	<0.49	<0.49	<0.49	<0.49	<0.49	<0.49	<0.49	<0.49
2	1.21	0.94	0.93	0.67	0.58	<0.49	<0.49	<0.49	<0.49	<0.49	<0.49
3	1.14	1.00	0.79	0.58	<0.49	<0.49	<0.49	<0.49	<0.49	<0.49	<0.49
4	1.14	0.96	0.73	<0.58	<0.49	<0.49	<0.49	<0.49	<0.49	<0.49	<0.49
$\bar{x}$	1.20	0.97	0.76	-	-	-	-	-	-	-	-
S.D. ±	0.07	0.03	0.15								

Table 6.7: Serum titres (log_{10}) of piglets from group C sow NUOO vaccinated 51 and 31 days before farrowing

Days post farrowing	3	7	14	21	28	35	43	49	56	63	70
Piglet no.											
1	2.03	1.95	1.89	1.89	1.73	1.68	1.48	1.43	1.36	1.33	1.30
2	2.15	2.18	1.73	1.83	1.64	1.58	1.53	1.27	1.27	1.18	1.18
3	2.14	2.03	1.88	1.89	1.68	1.68	1.53	1.35	1.34	1.43	1.23
4	2.15	2.00	1.78	1.63	1.64	1.53	1.48	1.22	1.44	1.17	1.00
5	2.02	1.94	1.78	1.63	1.68	1.73	1.45	1.43	1.29	1.17	1.08
6	1.54	1.67	1.37	1.12	1.17	1.12	1.02	0.92	0.83	0.65	0.68
7	2.21	2.05	1.89	1.78	1.74	1.58	1.53	1.38	1.28	1.11	1.07
8	2.00	1.76	1.54	1.38	1.43	1.02	0.98	0.92	0.87	0.69	0.82
$\bar{x}$	2.03	1.95	1.73	1.64	1.59	1.49	1.38	1.24	1.21	1.09	1.05
S.D. ±	0.21	0.16	0.19	0.27	0.19	0.27	0.23	0.21	0.23	0.28	0.21

Table 6.8: Serum titres (log_{10}) of piglets from group C sow NU03 vaccinated 52 and 32 days before farrowing

Days post farrowing	3	7	14	21	28	35	42	49	56	63	69
Piglet no.											
1	1.94	1.73	1.58	1.32	1.31	1.26	1.25	1.17	1.11	1.07	0.97
2	2.02	1.99	1.51	1.58	1.34	1.31	1.27	1.27	1.12	1.12	0.91
3	1.87	2.02	1.58	1.43	1.34	1.34	1.27	1.04	1.04	0.97	0.73
4	1.87	1.88	1.34	1.50	1.20	1.27	1.34	1.04	1.00	0.90	0.67
5	2.02	1.80	1.43	1.43	1.35	1.34	1.27	1.27	1.04	0.91	0.85
6	1.91	1.91	1.51	1.43	1.40	1.36	1.34	1.27	1.19	1.05	0.91
7	1.95	2.05	1.65	1.66	1.51	1.43	1.43	1.27	1.19	1.12	0.98
8	1.98	2.01	1.66	1.43	1.66	1.43	1.27	1.12	1.04	1.05	0.85
$\bar{x}$	1.95	1.92	1.53	1.47	1.39	1.34	1.31	1.18	1.09	1.02	0.86
S.D. ±	0.06	0.11	0.11	0.11	0.14	0.06	0.06	0.10	0.07	0.09	0.11

Table 6.9: Observed half-lives of neutralizing antibodies in piglets calculated by regression analysis

Piglet no.	Group A (Sows vaccinated 12 to 13dbf)		Group B (Sows vaccinated 30 to 32dbf)		Group C (Sows vaccinated 51 to 52 and 31 to 32dbf)	
	Litter NU02	Litter NU05	Litter NU01	Litter NU04	Litter NU00	Litter NU03
1	3.85	9.67	19.37	4.75	25.58	24.60
2	3.37	7.64	12.40	13.24	19.92	20.86
3	4.87	5.99	19.67	9.52	23.68	18.26
4	4.15	9.83	17.36	8.19	20.67	19.35
5	4.29	9.64	21.17	-	23.06	20.71
6	4.42	6.86	20.85	-	21.57	23.20
7	4.57	6.75	17.86	-	17.88	20.89
8	4.75	-	23.64	-	16.87	18.29
Litter* Mean	4.19	7.82	18.44	7.74	20.92	20.56

*Calculated from the regression slope of the litter mean antibody titres

dbf = Days before farrowing

Figure 6.1: Decay rate of maternally derived neutralizing antibodies in individual piglets from group A litters

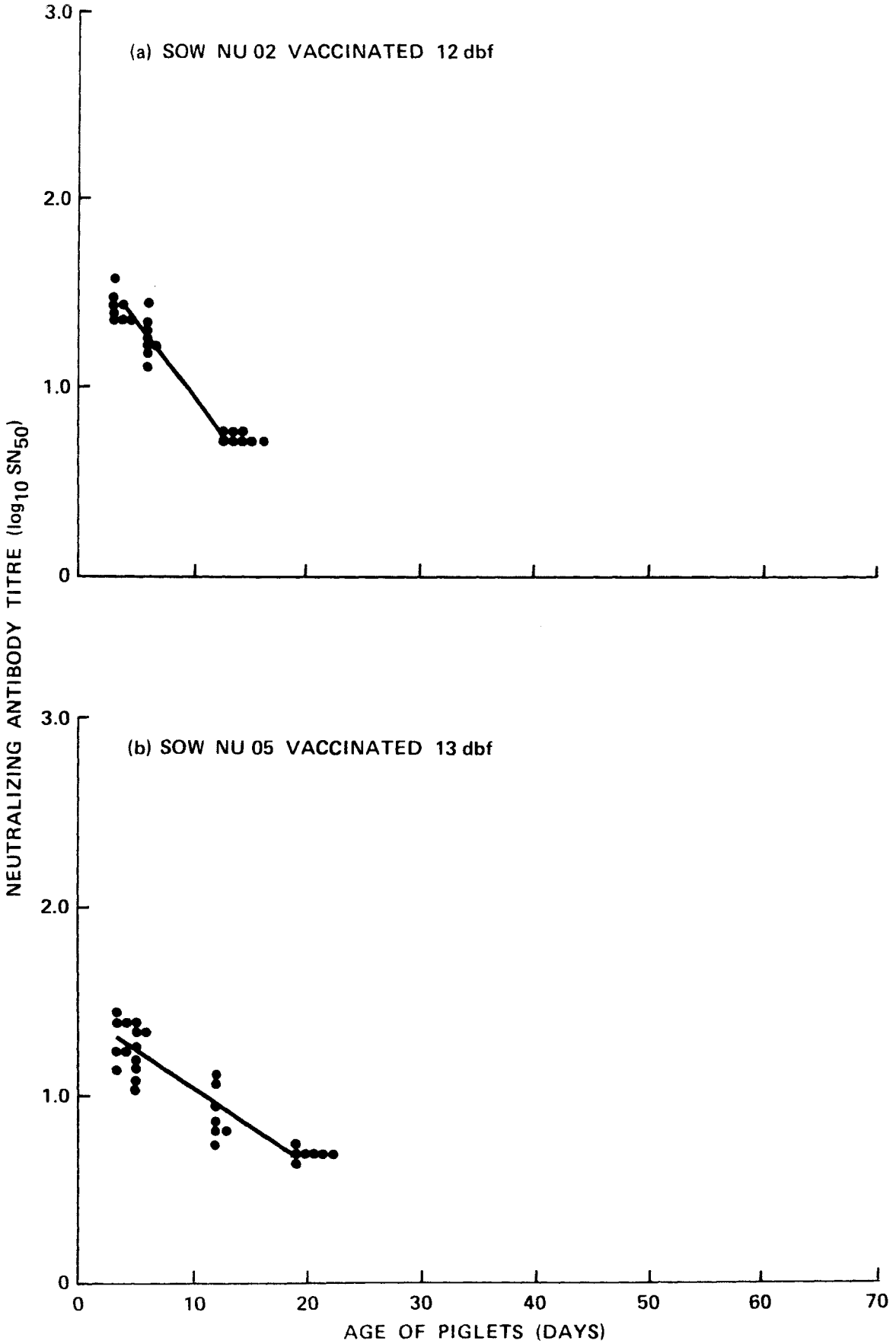


Figure 6.2: Decay rate of maternally derived neutralizing antibodies in individual piglets from group B litters

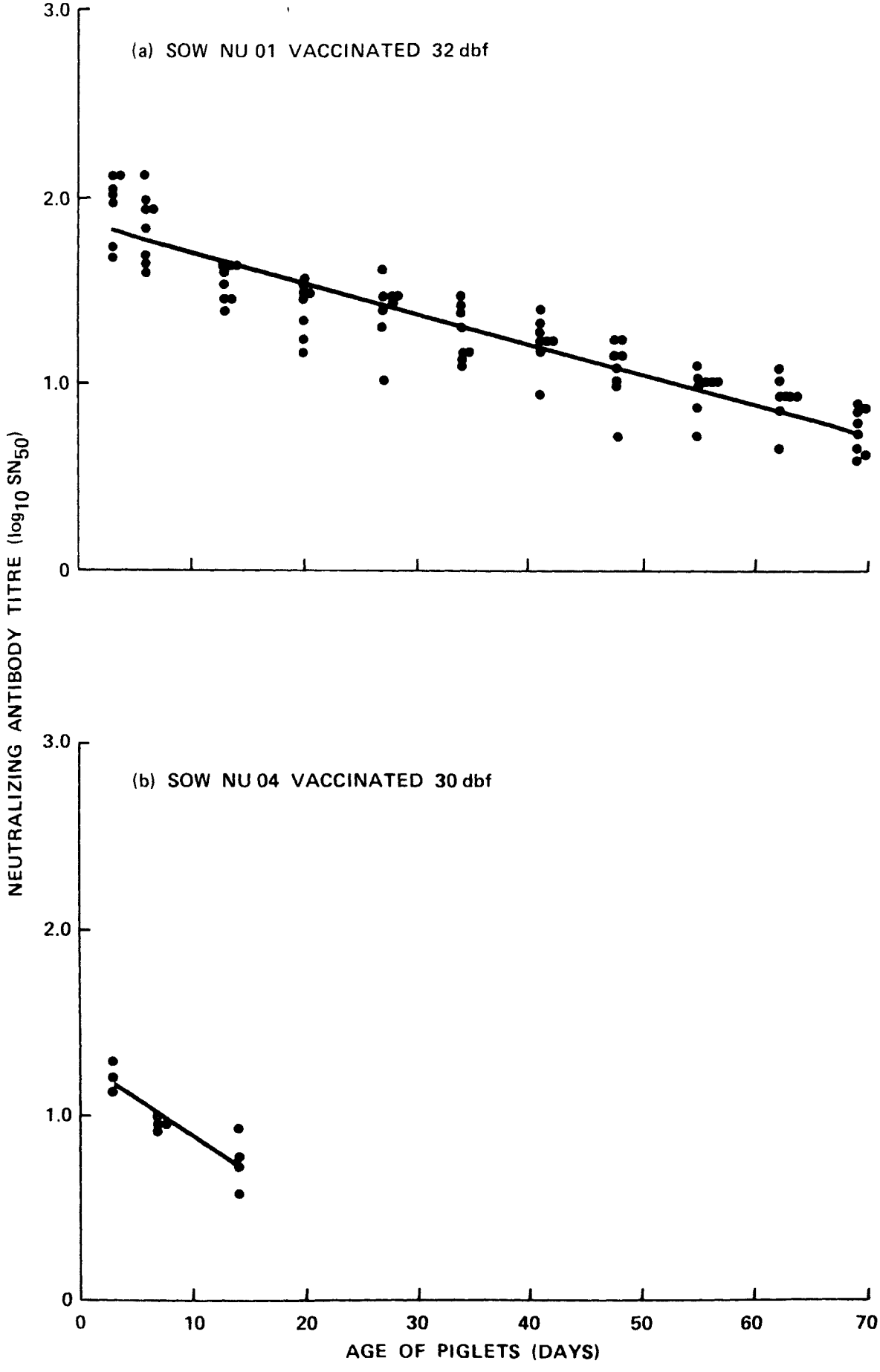


Figure 6.3: Decay rate of maternally derived neutralizing antibodies in individual piglets from group C litters

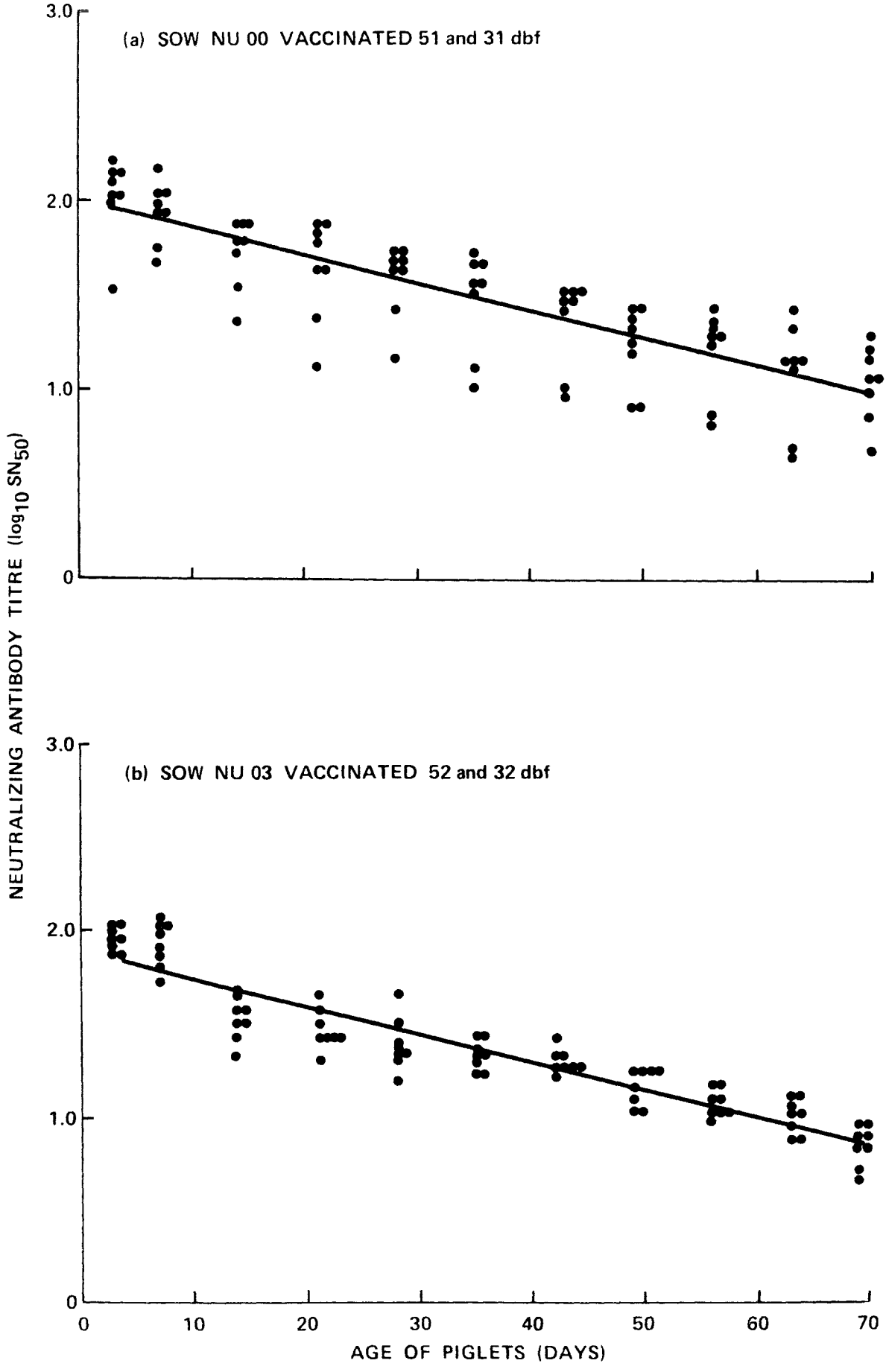


Table 6.10: Blood volume in millilitres of piglets from group A sow NUO2 vaccinated 12 days before farrowing

Days post farrowing	3	6	13	20	27	34	41	48	55	62	68
Piglet no.											
1	146.36	213.99	298.02	389.93	532.78	829.71	1185.33	1385.22	1451.37	1713.16	1843.30
2	166.00	185.47	325.63	470.83	639.01	914.82	-	-	-	-	-
3	166.00	194.73	298.02	371.53	532.78	812.31	1050.71	1252.22	1219.18	1484.00	1680.88
4	185.47	251.31	389.93	532.78	708.85	794.89	949.30	1185.33	1152.16	1385.22	1549.83
5	175.33	223.15	242.23	316.72	639.01	639.01	1017.24	1219.18	982.94	1252.22	1484.00
6	136.90	185.47	242.23	479.49	568.60	691.25	982.94	1185.33	1285.20	1417.96	1778.32
7	146.36	185.47	316.72	407.48	639.01	812.31	1034.36	1318.84	1185.33	1219.18	1549.83
8	156.63	223.15	371.53	497.56	691.25	881.00	1152.16	1318.84	1285.20	1417.96	1615.45
$\bar{x}$	159.88	207.84	310.54	433.29	618.91	796.91	1053.15	1266.43	1223.05	1412.82	1643.09
$\log_{10}\bar{x}$	2.20	2.32	2.49	2.64	2.79	2.90	3.02	3.10	3.09	3.15	3.22
$\log_{10}(Dx) - \log_{10}(D_3)$	0	0.12	0.29	0.44	0.59	0.70	0.82	0.90	0.89	0.95	1.02

Dx = Mean blood volume at sample point D_3 = Mean blood volume at 3 days old

Table 6.11: Blood volume in millilitres of piglets from group A sow NU05 vaccinated 13 days before farrowing

Days post farrowing	3	5	12	19	26	33	41	47	54	63	69
Piglet no.											
1	242.23	242.23	316.72	461.37	673.61	812.31	982.94	1152.16	1118.17	1118.17	1484.00
2	166.00	175.33	264.48	306.97	568.60	743.20	881.00	1050.71	778.19	1017.24	1185.33
3	166.00	203.96	353.06	532.78	656.71	914.82	1185.33	1451.37	1484.00	1713.16	1843.30
4	166.00	185.47	242.23	316.72	497.56	708.85	1000.10	1118.17	1152.16	1417.96	1484.00
5	175.33	194.73	213.99	353.06	515.58	656.71	881.00	-	-	-	-
6	185.47	203.96	232.29	334.52	532.78	691.25	847.09	1017.24	1084.85	1285.20	1285.20
7	156.63	156.63	232.29	532.78	778.19	914.82	1185.33	1417.96	1583.02	1746.12	1901.65
$\bar{x}$	179.67	194.62	265.00	405.46	603.29	777.42	994.68	1201.27	1200.07	1382.98	1530.58
$\log_{10}\bar{x}$	2.25	2.29	2.42	2.61	2.78	2.89	3.00	3.08	3.08	3.14	3.18
$\log_{10}(Dx)$ $-\log_{10}(D_3)$	0	0.04	0.17	0.36	1.53	1.64	1.75	1.83	1.83	1.89	1.93

Dx = Mean blood volume at sample point D_3 = Mean blood volume at 3 days old

Table 6.12: Blood volume in millilitres of piglets from group B sow NU01 vaccinated 32 days before farrowing

Days post farrowing	3	6	13	20	27	34	41	48	55	62	69
Piglet no.											
1	126.53	242.23	371.53	532.78	682.82	812.31	881.00	1017.24	1285.20	1484.00	1517.30
2	126.53	185.47	289.05	389.93	497.56	812.31	1101.15	1252.22	1484.00	1615.45	1549.83
3	126.53	203.96	316.72	443.98	532.78	691.25	1118.17	1252.22	1549.83	1615.45	1778.32
4	223.15	279.25	434.48	647.48	717.26	1017.24	1202.26	1349.51	1583.02	1876.08	1999.42
5	213.99	279.25	407.48	568.60	778.19	1084.85	1269.08	1517.30	1746.12	1876.08	1901.65
6	87.01	185.47	279.25	443.98	568.60	743.20	982.94	1219.18	1549.83	1583.02	1746.12
7	126.53	167.70	371.53	524.18	647.48	778.19	1017.24	1417.96	1713.16	1876.08	1713.16
8	126.53	213.99	425.76	568.60	743.20	914.82	1067.79	1219.18	1517.30	1484.00	1746.12
$\bar{x}$	144.59	219.66	361.97	514.94	645.99	856.77	1079.96	1280.60	1553.56	1676.27	1743.99
$\log_{10}\bar{x}$	2.16	2.34	2.56	2.71	2.81	2.93	3.03	3.11	3.19	3.22	3.24
$\log_{10}(Dx) - \log_{10}(D_3)$	0	0.18	0.40	0.55	0.65	0.77	0.87	0.95	1.03	1.06	1.08

Dx = Mean blood volume at sample point D_3 = Mean blood volume at 3 days old

Table 6.13: Blood volume in millilitres of piglets from group B sow NU04 vaccinated 30 days before farrowing

Days post farrowing	3	7	14	21	28	35	42	50	56	63	70
Piglet no.											
1	107.34	203.96	325.63	497.56	639.01	794.89	1084.85	1318.84	1615.45	1713.16	1876.08
2	126.53	186.32	334.52	434.48	515.58	734.81	949.30	1219.18	1417.96	1549.83	1517.30
3	96.77	203.96	334.52	479.49	586.46	812.31	949.30	1417.96	1926.12	1975.28	1901.65
4	136.90	242.23	361.90	515.58	682.82	1000.10	1252.22	1517.30	1811.19	1876.08	1999.42
$\bar{x}$	116.88	209.11	339.14	481.78	605.97	835.53	1058.42	1368.32	1692.68	1778.59	1823.61
$\log_{10}\bar{x}$	2.07	2.32	2.53	2.68	2.78	2.92	3.02	3.14	3.23	3.25	3.26
$\log_{10}(Dx)$ $-\log_{10}(D_3)$	0	0.25	0.46	0.61	0.71	0.85	0.95	1.07	1.16	1.18	1.19

Dx = Mean blood volume at sample point D_3 = Mean blood volume at 3 days old

Table 6.14: Blood volume in millilitres of piglets from group C sow NUOO vaccinated 51 and 31 days before farrowing

Days post farrowing	3	7	14	21	28	35	43	49	56	63	70
Piglet no.											
1	213.99	298.02	470.83	603.49	778.19	982.94	1219.18	1451.37	1680.88	1713.16	1901.65
2	185.47	279.25	443.98	603.49	778.19	1000.10	1252.22	1417.96	1549.83	1745.40	2047.91
3	166.00	242.23	371.53	434.48	639.01	829.71	1084.85	1252.22	1417.96	1615.45	1778.32
4	203.96	232.29	334.52	443.98	603.49	812.31	1017.24	1285.20	1385.22	1517.30	1746.12
5	203.96	279.25	434.48	568.60	743.20	982.94	1118.17	1285.20	1876.08	1582.30	1811.19
6	166.00	242.23	371.53	524.18	725.66	881.00	1084.85	1385.22	1517.30	1680.88	1901.65
7	213.99	289.05	434.48	550.71	847.08	1000.10	1219.18	1385.22	1417.96	1680.88	1901.65
8	166.00	223.15	353.06	524.18	673.61	863.68	1118.17	1285.20	1647.83	1517.30	1746.12
$\bar{x}$	189.92	260.68	401.80	531.64	723.56	919.10	1139.23	1343.45	1561.63	1631.59	1854.32
$\log_{10}\bar{x}$	2.28	2.42	2.60	2.73	2.86	2.96	3.06	3.13	3.19	3.21	3.27
$\log_{10}(Dx)$ $-\log_{10}(D_3)$	0	0.14	0.32	0.45	0.58	0.71	0.78	0.85	0.91	0.93	0.99

Dx = Mean blood volume at sample point D_3 = Mean blood volume at 3 days old

Table 6.15: Blood volume in millilitres of piglets from group C sow NUO3 vaccinated 52 and 32 days before farrowing

Days post farrowing	3	7	14	21	28	35	42	49	56	63	69
Piglet no.											
1	107.34	156.63	251.31	353.06	532.78	743.96	1252.22	1417.96	1583.02	1451.37	1901.10
2	126.53	194.73	306.97	479.49	639.01	778.19	1017.24	1285.20	1417.96	1451.37	1713.16
3	203.96	298.02	443.98	497.56	656.71	778.19	1050.71	1252.22	1451.37	1583.02	1713.16
4	126.53	203.96	316.72	589.46	778.19	982.94	1185.33	1417.96	1615.45	1778.18	1901.65
5	136.90	242.23	389.93	550.71	794.89	982.94	1252.22	1385.22	1451.37	1680.88	1778.32
6	156.63	289.05	461.37	708.85	829.71	1050.71	1285.20	1484.00	1746.12	1811.19	2047.91
7	136.90	213.99	316.72	476.34	708.85	812.31	982.93	1118.17	1050.71	1252.22	1484.00
8	203.96	289.05	462.15	639.01	779.19	1084.85	1318.84	1484.00	1713.16	1713.16	2047.91
$\bar{x}$	149.84	235.96	368.64	536.43	714.79	901.76	1168.09	1355.59	1503.65	1590.19	1823.40
$\log_{10}\bar{x}$	2.18	2.37	2.57	2.73	2.85	2.96	3.07	3.13	3.18	3.20	3.26
$\log_{10}(Dx) - \log_{10}(D_3)$	0	0.19	0.39	0.55	0.67	0.78	0.89	0.95	1.00	1.02	1.08

Dx = Mean blood volume at sample point D_3 = Mean blood volume at 3 days old

Figure 6.4: The mean observed (——) and corrected (▪▪▪▪) decay rates of maternally derived antibodies in piglets

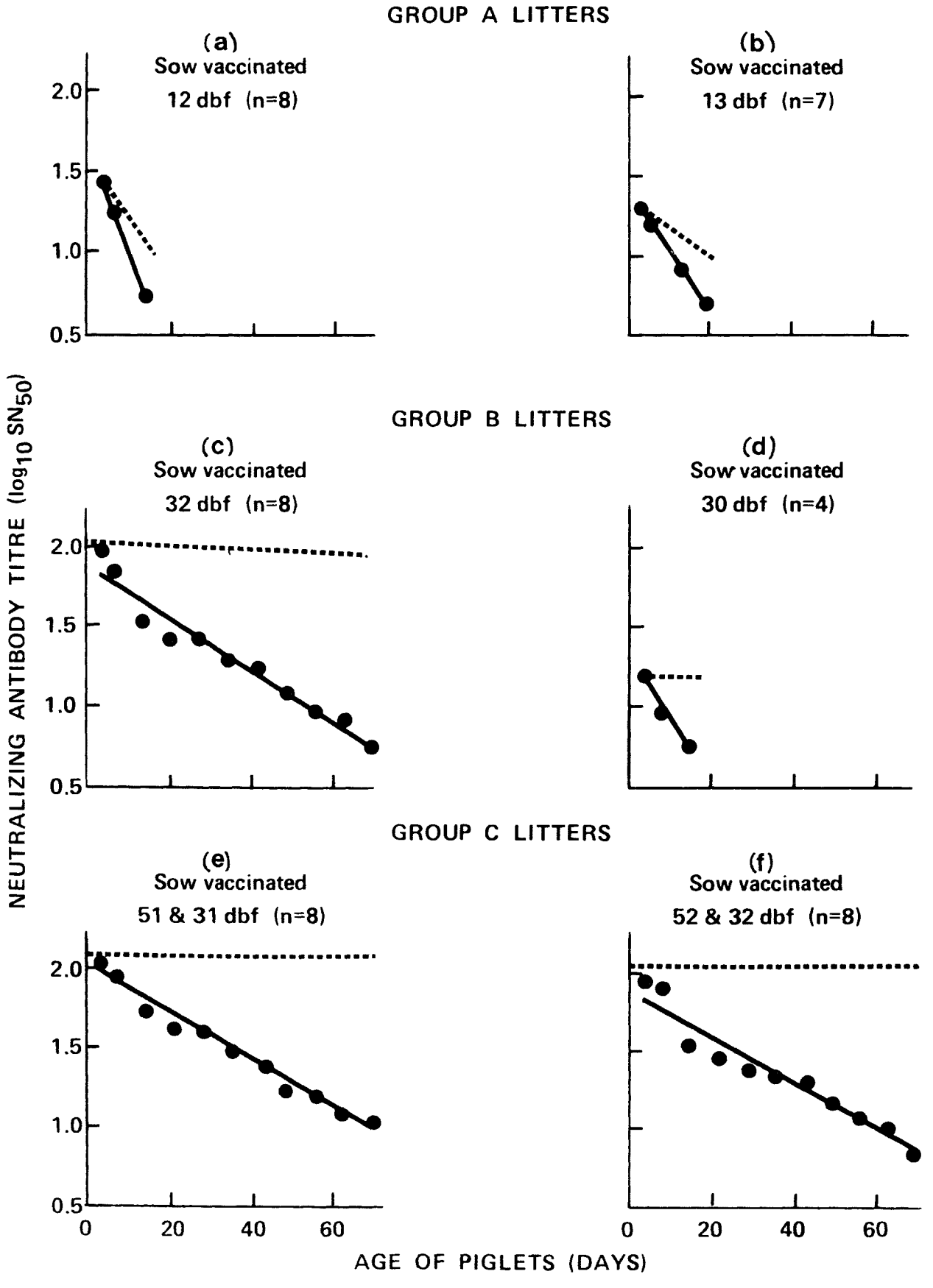


Table 6.16: Comparison between the observed half-lives of neutralizing antibodies and those corrected for blood volume increase

Group no.	Litter no.	Neutralizing antibody half-life (days)	
		Observed	Corrected
A (Sows vaccinated 12 to 13dbf)	NU02	4.19	6.89
	NU05	7.82	18.45
B (Sows vaccinated 30 to 32dbf)	NU01	18.44	428.62
	NU04	7.74	Infinity
C (Sows vaccinated 51 to 52 and 31 to 32dbf)	NU00	20.92	Infinity
	NU03	20.56	Infinity

dbf = Days before farrowing

Table 6.17: The mean body weight, blood volume and blood volume doubling over the first 70 days of life

Age of piglets (days)	Mean body weight (Kg)	Mean blood volume (ml)	Blood volume doubling time from day 3 (days)
3	1.75	160.04	-
5-7	2.50	223.15	7.02
12-14	3.97	343.40	8.68
19-21	5.77	486.56	10.81
26-28	7.92	653.64	12.19
33-35	10.52	851.61	13.39
41-43	13.64	1084.85	14.49
47-49	16.60	1302.76	15.58
54-56	18.67	1453.55	16.92
62-63	20.79	1606.81	18.31
69-70	22.91	1759.00	19.80

NOO4, NUOO and NUO3) which were vaccinated or revaccinated 30 to 32dbf ranged from greater than 408 days to infinity (Figures 6.4c to 6.4f).

The mean increase in body weight (Kg) and blood volume (ml) for all 43 piglets from the six litters is given in Table 6.17 along with the blood volume doubling time from 3 days of age onwards calculated by linear regression of the mean $\log_{10}$ values.

DISCUSSION

The vaccination schedules used in this experiment were based on previously published observations (Ouldridge, Francis and Black, 1982) and resulted in sows with principally either IgG or IgM neutralizing antibodies in their sera at the time of farrowing (see Chapters 4 and 5). The results described here show that the litters from these sows also had the corresponding predominance of IgG or IgM in their sera 3 days after birth.

The group A litters which received principally neutralizing IgM antibodies had observed mean half-lives of 4.2 and 7.8 days. These results, based on neutralizing activity, were somewhat higher than published values of 1.3 to 4.5 days which were based on the decay of normal serum IgM (Porter and Hill, 1970; Curtis and Bourne, 1971; 1973; Klobasa et al, 1981). However, although only neutralizing IgM activity could be detected in the 3-day-old piglet serum pools of these litters, it seems possible that low levels of neutralizing IgG were present at farrowing which were not detectable by the technique employed but nonetheless lengthened the observed half-lives of maternally derived antibodies in the piglet sera. This is supported by the ELISA test results which demonstrated low levels of IgG activity in the sera of these piglets. The observed mean half-lives in piglet litters from

groups B and C which received principally IgG antibodies were generally longer (7.4, 18.4, 20.6 and 20.9 days). A similar range of passive IgG half-life values (7.5 to 19.7 days) appears in the literature (Porter and Hill, 1970: Curtis and Bourne, 1971; 1973; Lannais, Aynaud and Corthier, 1978; Frenyo et al, 1980/81; Klobasa et al, 1981; Paul et al, 1982).

In seeking an explanation for the wide variation in the passive antibody half-lives observed in this and other studies, an attempt was made to identify the true half-lives due to catabolism/excretion. This was done by taking into account the effect of dilution, produced by increased blood volume with size, on the decay rate of the neutralizing antibodies. Although previous authors have drawn attention to the effect that the rapid growth rate of young pigs might have on their observed serum antibody half-life values (Porter and Hill, 1970; Curtis and Bourne, 1971; 1973) and used this to explain litter to litter variation (Curtis and Bourne, 1971; 1973) few have attempted to quantify this effect or correct the antibody decay rate for blood volume increase. The corrected half-lives of passive IgM antibodies in the litters due to catabolism were calculated to be between 6.9 and 18.4 days. However, with regard to the IgG antibodies the results suggested that there was little or no IgG catabolism/excretion during the first 10 weeks of the pigs life and that all, or almost all, the fall off in the piglets' neutralizing IgG titres was due to the increase in the vascular compartment volume.

In a recent study (Paul et al, 1982) on Porcine Parvovirus antibody neutralization the half-life was corrected for body weight increase in 16 to 24 week old piglets and a mean corrected half-life value of 29 days was obtained. It seems possible, therefore, that the low catabolic

rate observed for the IgG antibodies in this study was due to the age of the piglets used.

That the control mechanism for IgG catabolism/excretion rate is mediated by the total serum IgG concentration has been reported in other species (Fahey and Robinson, 1963; Sell, 1964; Waldmann and Strober, 1969). If a similar mechanism exists in young piglets then the results observed here may be explicable since the piglets used in this study were less than 10 weeks old and young pigs do not attain adult IgG levels until they are 12 to 16 weeks old (Curtis and Bourne, 1971; Frenyo et al, 1980/81). It follows therefore that no catabolism/excretion of IgG would be expected at an early age but that catabolism/excretion might be expected to occur to an increasing extent as the vascular volume expansion slowed down and as adult immunoglobulin levels were attained. Furthermore, IgM and IgA catabolism has been shown to be independent of serum concentration (Waldmann and Strober, 1969) which would explain why catabolism was observed in group A piglets.

The results obtained here also showed that the doubling time of the vascular volume depended largely on the age and period over which the observations were made. For example, when measured in 3 to 7-day-old piglets the doubling time for blood volume was approximately 7 days but when measured in the same piglets over 3 to 70 days the blood volume doubling time was almost 20 days. Thus, although a linear regression provides a good fit to the data ($r>0.95$ in all 6 litters), the observed half-life is not strictly linear and is influenced by the rapid blood volume doubling time in the first few weeks of the piglets' life. The influence of these findings on the half-life of the maternally derived IgG antibodies is apparent in the case of litter NU04 where the relatively short half-life of 7.4 days can be ascribed to the fact that

the titres of the maternal antibodies were low and could only be measured for 13 days after farrowing. After that time the more gradual decrease which could be expected for IgG antibodies remaining in the sera could not be measured as it fell below the threshold of sensitivity of the neutralization titration method employed. During the first 13 days the doubling time of the blood volume was correspondingly short (8.7 days). Therefore the observed half-life value for neutralizing antibodies may be affected by the piglets' age and the period over which observations are made. This is supported by the work of Paul et al (1982) who noted that the half-life of parvovirus antibodies varied with the age of the piglets.

It is possible to account for some of the differences in previously published IgG half-life values by relating them to the observation period used in each instance. For example, an IgG half-life of 12 to 14 days (Curtis and Bourne, 1971) was calculated over the first 4 weeks of life during which time the blood volume doubled, on average calculated from the population of 54 piglets used in this study, every 12.2 days while an IgG half-life of 17 days (Lannais, Aynaud and Corthier, 1978) was calculated over the first 10 weeks of life during which time the mean blood volume doubled every 19.8 days.

For practical purposes a linear regression drawn through the observed neutralizing antibody titres of all the piglets in a litter is useful to predict the time at which the titres will drop below protective levels (Black et al, 1984) and also when any vaccination inhibiting activity (see Chapter 7) subsides below acceptable limits.

CHAPTER 7

THE RESPONSE OF YOUNG PIGS TO VACCINATION IN THE PRESENCE AND ABSENCE OF MATERNALLY DERIVED NEUTRALIZING ANTIBODIES

INTRODUCTION

Chapters 5 and 6 described the transfer of neutralizing antibodies from the sow to her litter and some of the factors effecting the persistence of these antibodies in the young. The results indicated that maternally derived antibodies subside well before the piglets were 6 months old, the marketing age required by the bacon industry. Consequently in areas where FMD is endemic the young pigs need to be actively immunized at as early an age as possible.

Some published work claims that oil-emulsion vaccines are capable of inducing immunity to FMD in one-month-old piglets irrespective of whether they are derived from vaccinated or unvaccinated sows (Giraud et al, 1969) and that FMD oil vaccines produce a 'meaningful response' in piglets regardless of the immune status of their mothers (Morgan and McKercher, 1980). However, other workers have shown, to the contrary, that passively acquired maternal antibodies interfere with the active immune responses of pigs. Examples include studies using aqueous FMD vaccines (van Bekkum, Frenkel and Nathans, 1963; Nathans, 1965; Sidorov, 1974), artificial antigens (Hoerlein, 1957; Muscoplat, Setcavage and Kim, 1977), bacterial antigens (Watson, Bennell and Chaniago, 1979; Pedersen and Jensen, 1980; Senft and Heckelmann, 1980; Hoerlein, 1957) and also viral antigens other than FMDV (Coggins, 1964; Renshaw, 1975; Mensik et al, 1976; Corthier, 1976; Mierzejewska et al, 1977; Launais, Aynaud and Corthier, 1978). Similarly, inhibition by maternal antibodies has been reported in calves using aqueous FMD vaccines (Graves, 1963; van Bekkum, 1966; Srubar, 1966; Wisniewsky and Jankowska,

1972; Mayr and Thein, 1972; Nicholls et al, 1984) and oil-emulsion FMD vaccines (Brun et al, 1977).

In addition, the results of recent work (Black et al, 1982), indicate that some depression of the oil emulsion vaccination response in pigs by maternally derived FMD antibodies might be expected. This chapter of the thesis is designed to establish the earliest age at which young pigs are capable of responding to FMD vaccination, the nature of the response, whether early vaccination has any adverse effects on the piglets' weight gain and what effect the maternally derived antibodies have on the immune response, in terms of the neutralizing antibodies and antibody classes involved.

EXPERIMENTAL DESIGN

Eight pregnant Large White sows, never previously vaccinated against or exposed to FMD virus, were divided into 2 groups of 4. One group (group D from Chapter 4) was vaccinated with O_1BFS vaccine batch O-121 at 87-89 and again 30-32dbf. The other group was left unvaccinated. At farrowing the piglets were divided into 4 groups of 6 to 8 piglets from the vaccinated sows and 4 groups from the unvaccinated sows. Most groups contained representatives of the piglets from either the four vaccinated or four unvaccinated sows. One group of piglets from the vaccinated and one group from unvaccinated sows were subsequently vaccinated against FMD when they were 1, 2, 4 or 8 weeks old and all were challenged with live FMD virus when they were approximately 7 months old. A plan of the piglet distribution and vaccination schedule is shown in Table 7.1.

Table 7.1: Grouping of piglets and vaccination plan

	Vaccinated sows				Unvaccinated sows			
	OE 91	OE 92	OE 93	OE 94	OE 95	OE 96	OE 97	OE 98
Piglets vaccinated at 1 week old	- -	92/01 92/09	93/01 93/02	94/01 94/09	- -	96/01 96/02	97/01 97/12	98/01 98/02
Piglets vaccinated at 2 weeks old	91/03 91/04	93/02 92/04	93/03 93/04	94/03 94/04	- -	96/03 96/04	97/03 97/04	98/03 98/04
Piglets vaccinated at 4 weeks old	91/05 91/06	92/05 92/06	93/05 93/06	- 94/06	- - 95/07	96/05 96/06 96/07	97/05 97/06 97/11	98/05 - -
Piglets vaccinated at 8 weeks old	91/07 91/08	92/07 92/08	93/07 -	94/07 -	- 95/09	96/08 96/09 96/10	97/07 97/08 97/10	- -

Blood samples were collected from the piglets at 3 days old, at the time of vaccination and then at 3 days, 7 days and weekly until they were challenged.

All the piglets were weighed at birth, at 3 days old and then weekly for 10 weeks. Weights were again recorded for 3 to 4 weeks before challenge.

RESULTS

a) Effect of FMD oil emulsion vaccination on piglet growth rate

The weights of individual piglets at each sample point are shown in Tables 7.2 to 7.9, and the mean increase in body weight for piglets from either vaccinated or unvaccinated sows are presented graphically in Figures 7.1(a) and 7.1(b) respectively. Pooled data for piglets from both vaccinated and unvaccinated sows is given in Figure 7.2. Examination of these figures shows that the only group that appeared to exhibit any deviation from the general trend was the piglets from unvaccinated sows vaccinated at 1 week old. In order to determine whether this group was significantly different a two-way analysis of variance was carried out between piglet vaccination age and individual day weights. Where the ages were matched up to 69 days the weights of piglets from vaccinated and unvaccinated sows were grouped together (Table 7.10(a)). However, since the weights of piglets from unvaccinated sows at 179-209 days old and from vaccinated sows at 195-226 days old were not matched they were analysed separately (Tables 7.10(b) and (c)). These calculations showed that no significant differences were demonstrable in the weights of piglets vaccinated at 1, 2, 4 or 8 weeks old either for the first 10 weeks of life or just prior to challenge at 6 to 7 months old.

Table 7.2: Weights (Kg) of piglets vaccinated at 1 week old (born to vaccinated sows)

Piglet Numbers	Age (Days)															
	0	3	► 7	14	21	28	35	42	49	56	63	69	195	206	223	226
OE 92/01	1.59	2.38	3.40	5.67	7.71	9.53	15.88	16.78	18.60	22.23	24.49	28.58	72.57	76.20	78.47	80.28
OE 92/09	1.36	1.81	2.15	3.06	5.44	6.80	9.98	12.70	14.51	15.88	18.14	20.87	74.84	80.29	83.91	86.18
OE 93/01	2.04	2.61	3.86	6.24	8.62	12.25	15.42	18.14	20.18	23.59	27.22	30.84	79.83	81.65	83.46	86.18
OE 93/02	1.70	2.61	3.86	6.12	8.16	11.79	14.51	19.05	20.41	22.23	26.31	32.66	81.65	83.91	85.73	87.54
OE 94/01	1.59	1.59	1.93	4.54	6.80	9.52	12.25	13.61	15.88	22.23	24.04	29.48	84.37	86.64	89.36	92.53
OE 94/09	2.04	2.27	2.38	4.54	5.90	9.07	11.79	14.51	16.33	22.23	24.04	28.12	92.53	94.80	97.07	98.43
$\bar{x}$	1.72	2.21	2.93	5.03	7.11	9.83	13.31	15.30	17.65	21.40	24.04	28.43	80.97	83.92	86.33	88.52
S.D.	0.27	0.42	0.88	1.22	1.27	1.98	2.32	2.57	2.44	2.76	3.17	4.05	7.14	6.38	6.34	6.23

► = Vaccination

Table 7.3: Weights (Kg) of piglets vaccinated at 2 weeks old (born to vaccinated sows)

Piglet Numbers	Age (Days)															
	0	3	7	► 14	21	28	35	42	49	56	63	69	195	206	223	226
OE 91/03	1.59	1.81	2.83	4.54	6.80	9.07	11.79	15.88	17.69	20.41	22.23	24.95	89.35	89.35	92.53	102.96
OE 91/04	1.25	1.36	1.93	2.95	4.54	6.35	8.62	11.79	13.38	16.78	19.96	28.58	91.63	93.89	96.16	97.52
OE 92/03	1.81	2.38	3.40	5.33	7.71	11.34	15.42	13.14	19.96	22.32	28.58	29.48	80.74	85.73	89.36	90.72
OE 92/04	1.47	2.27	3.52	5.56	8.16	9.07	12.25	16.33	18.14	21.77	26.76	28.58	81.65	87.09	90.26	92.99
OE 93/03	1.81	2.38	3.52	5.33	7.17	11.34	13.15	16.78	19.05	20.41	29.03	35.38	79.83	81.65	83.46	85.73
OE 93/04	1.81	2.38	3.74	6.01	8.62	10.43	15.42	17.69	19.96	23.59	29.94	32.66	83.46	84.82	86.64	88.45
OE 94/03	1.59	2.04	2.61	3.74	5.10	7.71	11.79	16.78	18.60	25.85	27.22	30.39	86.18	90.26	94.80	98.88
OE 94/04	1.81	1.81	2.15	2.83	4.54	6.35	9.53	12.25	14.06	19.05	20.87	25.40	87.54	88.90	90.72	92.53
$\bar{x}$	1.64	2.05	2.96	4.54	6.65	8.96	12.25	15.71	17.61	21.26	25.57	29.43	85.05	87.71	90.49	93.72
S.D.	0.21	0.37	0.69	1.23	1.68	2.02	2.45	2.39	2.53	2.78	3.95	3.47	4.30	3.75	4.15	5.72

► = Vaccination

Table 7.4: Weights (Kg) of piglets vaccinated at 4 weeks old (born to vaccinated sows)

Piglet Numbers	Age (Days)															
	0	3	7	14	21	► 28	35	42	49	56	63	69	195	206	223	226
OE 91/05	0.91	1.59	2.38	3.74	6.35	7.71	10.89	13.38	15.88	19.05	20.86	27.67	88.45	89.36	90.72	92.53
OE 91/06	1.13	1.36	1.70	2.38	3.63	5.44	8.62	10.43	11.79	12.70	14.97	21.32	74.39	76.66	78.47	80.74
OE 92/05	1.59	2.38	3.18	4.65	7.26	10.43	14.51	17.24	19.05	24.49	28.58	31.75	86.18	91.63	93.89	95.25
OE 92/06	1.59	2.15	3.52	4.88	7.26	10.43	12.47	15.88	17.69	19.05	22.23	24.49	68.04	72.57	75.30	77.11
OE 93/05	1.59	1.59	2.59	3.97	7.03	9.07	13.15	14.97	16.33	17.24	20.41	25.40	68.05	72.57	74.84	77.11
OE 93/06	2.04	2.72	4.08	6.58	9.07	12.70	16.33	19.05	22.68	26.31	29.94	35.83	90.72	93.89	95.25	97.07
OE 94/06	1.13	1.25	1.70	2.61	3.86	5.44	7.71	9.07	11.34	15.42	17.69	19.96	83.91	88.45	92.53	96.16
$\bar{x}$	1.42	1.86	2.74	4.12	6.35	8.75	11.95	14.29	16.39	19.18	22.10	26.63	79.96	83.59	85.86	88.00
S.D.	0.39	0.56	0.90	1.44	1.96	2.72	3.10	3.59	3.98	4.81	5.45	5.64	9.64	9.30	9.20	9.24

► = Vaccination

Table 7.5: Weights (Kg) of piglets vaccinated at 8 weeks old (born to vaccinated sows)

Piglet Numbers	Age (Days)															
	0	3	7	14	21	28	35	42	49	► 56	63	69	195	206	223	226
OE 91/07	1.13	1.36	2.15	3.63	5.44	7.26	9.07	11.11	13.15	17.24	21.32	24.50	74.39	76.20	78.02	79.83
OE 91/08	1.02	1.25	2.15	3.63	5.90	8.16	10.43	12.47	16.33	19.96	25.40	32.20	86.18	88.45	90.72	92.99
OE 92/07	1.70	2.15	3.18	4.76	7.48	9.52	13.15	17.24	20.41	22.23	26.76	28.58	70.31	73.48	79.38	81.19
OE 92/08	1.70	2.04	2.95	4.65	7.03	10.66	14.06	17.24	20.41	24.04	26.31	29.03	66.22	71.68	73.03	74.84
OE 93/07	1.93	2.49	3.86	6.01	9.07	12.70	14.06	16.33	19.05	25.40	28.12	33.57	90.72	95.25	97.07	99.34
OE 94/07	1.81	2.27	2.73	5.67	8.16	9.98	11.79	14.06	15.88	22.23	28.12	32.66	89.36	90.72	93.44	96.16
$\bar{x}$	1.55	1.93	2.84	4.73	7.18	9.71	12.09	14.74	17.54	21.85	26.01	30.09	79.53	80.11	85.28	87.39
S.D.	0.38	0.51	0.65	0.99	1.36	1.92	2.04	2.50	2.91	2.92	2.53	3.40	10.53	8.84	9.72	10.04

► = Vaccination

Table 7.6: Weights (Kg) of piglets vaccinated at 1 week old (born to unvaccinated sows)

Piglet Numbers	Age (Days)														
	0	3	►7	14	21	28	35	42	49	56	63	69	179	196	209
OE 96/01	1.36	1.47	2.61	4.54	6.35	8.16	10.43	11.79	13.61	16.33	19.05	21.77	84.37	92.99	95.71
OE 96/02	1.59	1.93	3.18	4.54	5.90	8.16	9.98	11.57	12.25	15.42	16.78	18.82	68.04	76.20	81.65
OE 97/01	1.13	2.04	3.18	6.35	7.94	9.53	11.34	13.15	14.97	18.14	19.95	22.00	83.91	85.73	96.16
OE 97/12	1.36	2.49	3.63	6.58	8.85	9.75	11.34	13.15	15.42	18.14	20.41	22.91	86.18	88.45	97.52
OE 98/01	1.47	1.59	2.04	4.08	5.67	7.26	9.07	10.89	12.70	15.42	17.69	19.96	68.04	72.57	77.11
OE 98/02	1.81	2.04	2.38	3.17	5.44	7.26	9.75	10.89	12.25	15.42	17.69	19.73	70.31	73.94	79.38
$\bar{x}$	1.45	1.93	2.84	4.88	6.69	8.35	10.32	11.91	13.53	16.48	18.60	20.87	76.81	81.65	87.92
S.D.	0.23	0.36	0.59	1.33	1.38	1.08	0.91	1.03	1.39	1.33	1.43	1.59	8.85	8.52	9.49

► = Vaccination

Table 7.7: Weights (Kg) of piglets vaccinated at 2 weeks old (born to unvaccinated sows)

Piglet Numbers	Age (Days)														
	0	3	7	►14	21	28	35	42	49	56	63	69	179	196	209
OE 96/03	1.81	2.04	2.95	5.90	7.48	9.03	11.11	13.15	15.88	18.60	20.87	22.79	88.45	96.61	98.43
OE 96/04	1.47	1.70	2.83	5.44	7.03	8.62	10.43	12.25	14.06	16.33	19.05	21.55	73.48	81.65	85.73
OE 97/03	1.36	1.36	2.49	5.22	7.26	8.85	10.89	12.70	14.97	17.01	19.50	22.00	76.66	80.29	89.36
OE 97/04	1.13	2.04	2.72	5.44	7.71	9.53	11.34	12.70	15.42	17.69	20.41	23.02	77.11	78.92	89.36
OE 98/03	1.81	1.93	3.18	5.90	7.94	11.79	14.06	15.88	19.05	21.32	23.81	26.19	81.65	86.18	90.72
OE 98/04	1.93	2.27	2.95	5.90	8.62	9.53	13.15	16.33	19.05	21.77	24.00	26.42	95.25	97.52	99.34
$\bar{x}$	1.59	1.66	2.85	5.63	7.67	9.56	11.83	13.34	16.41	18.79	21.27	23.66	82.10	86.86	92.16
S.D.	0.31	0.35	0.23	0.30	0.56	1.15	1.44	1.79	2.14	2.27	2.14	2.12	8.27	8.28	5.48

► = Vaccination

Table 7.8: Weights (Kg) of piglets vaccinated at 4 weeks old (born to unvaccinated sows)

Piglet Numbers	Age (Days)														
	0	3	7	14	21	► 28	35	42	49	56	63	69	179	196	209
OE 95/07	1.59	2.04	4.08	5.90	7.26	9.07	12.70	14.06	15.08	17.24	20.75	23.81	72.57	78.02	81.19
OE 96/05	1.93	2.27	3.06	4.54	7.26	9.53	12.02	13.61	15.88	18.14	21.77	24.95	92.99	97.98	98.88
OE 96/06	1.36	1.70	2.49	4.76	6.35	7.94	9.53	11.34	13.15	15.88	19.05	22.00	90.72	93.44	97.07
OE 96/07	1.59	1.70	2.72	4.54	7.03	9.07	11.34	13.61	16.78	18.60	20.87	22.91	74.39	78.47	79.83
OE 97/05	1.59	2.27	4.54	5.67	8.16	9.75	13.15	14.51	16.78	18.82	21.09	13.13	83.91	86.18	97.07
OE 97/06	1.47	1.81	4.54	5.67	7.71	9.07	12.70	14.06	14.97	17.24	20.41	22.23	65.77	66.68	75.29
OE 97/11	1.47	2.27	4.42	5.44	8.62	9.98	12.25	13.15	14.51	16.78	19.96	21.77	78.02	80.29	88.90
OE 98/05	1.81	2.15	3.52	5.44	8.62	11.79	14.97	18.14	22.68	25.40	28.35	31.52	82.55	88.45	94.80
$\bar{x}$	1.60	2.03	3.67	5.25	7.63	9.53	12.33	14.06	16.23	18.51	21.53	24.40	80.12	83.69	89.13
S.D.	0.19	0.25	0.84	0.55	0.80	1.10	1.55	1.91	2.87	2.95	2.87	3.20	9.25	9.92	9.22

► = Vaccination

Table 7.9: Weights (Kg) of piglets vaccinated at 8 weeks old (born to unvaccinated sows)

Piglet Numbers	Age (Days)														
	0	3	7	14	21	28	35	42	49	► 56	63	69	179	196	209
OE 95/09	1.59	2.04	3.18	5.67	7.71	9.53	13.15	14.97	17.69	19.96	23.59	26.76	73.48	78.47	82.10
OE 96/08	1.25	1.70	2.61	5.90	7.26	9.07	11.34	13.61	15.42	18.14	22.23	25.85	88.00	92.99	97.52
OE 96/09	1.70	3.52	3.40	5.90	8.16	10.43	12.47	14.51	17.24	19.50	23.13	26.31	92.98	97.07	98.43
OE 96/10	1.36	1.81	2.72	4.54	6.35	8.62	9.98	11.79	14.97	17.69	20.87	24.04	78.47	84.82	87.09
OE 97/07	1.25	2.04	3.18	5.44	7.71	9.75	11.34	13.61	16.33	19.05	22.68	25.40	68.95	71.21	77.11
OE 97/08	1.59	2.04	3.06	4.54	4.99	6.35	10.43	12.70	15.42	17.92	21.77	24.49	77.11	79.83	86.18
OE 97/10	1.47	2.04	2.95	4.99	7.71	9.53	10.43	12.70	14.97	17.92	20.64	23.81	78.92	81.65	88.90
$\bar{x}$	1.46	2.17	3.01	5.28	7.13	9.04	11.31	13.41	16.01	18.60	22.13	25.24	79.70	83.72	88.19
S.D.	0.18	0.61	0.28	0.60	1.10	1.31	1.16	1.11	1.10	0.90	1.11	1.15	8.25	8.84	7.72

► = Vaccination

Figure 7.1: Effect of vaccination on the growth rate of piglets

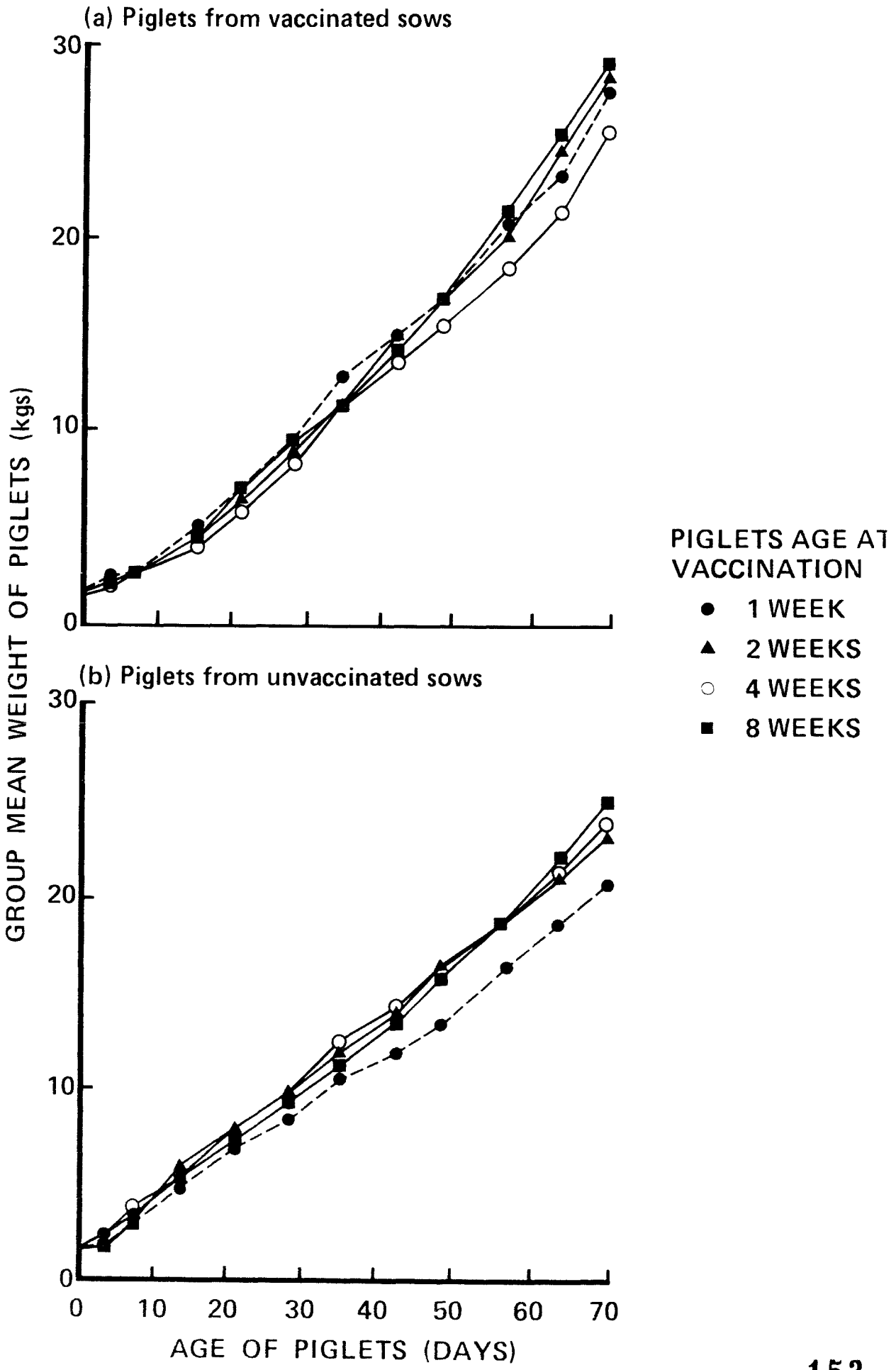


Figure 7.2: Effect of vaccination on the growth rate of piglets; pooled data from vaccinated and unvaccinated sows' litters

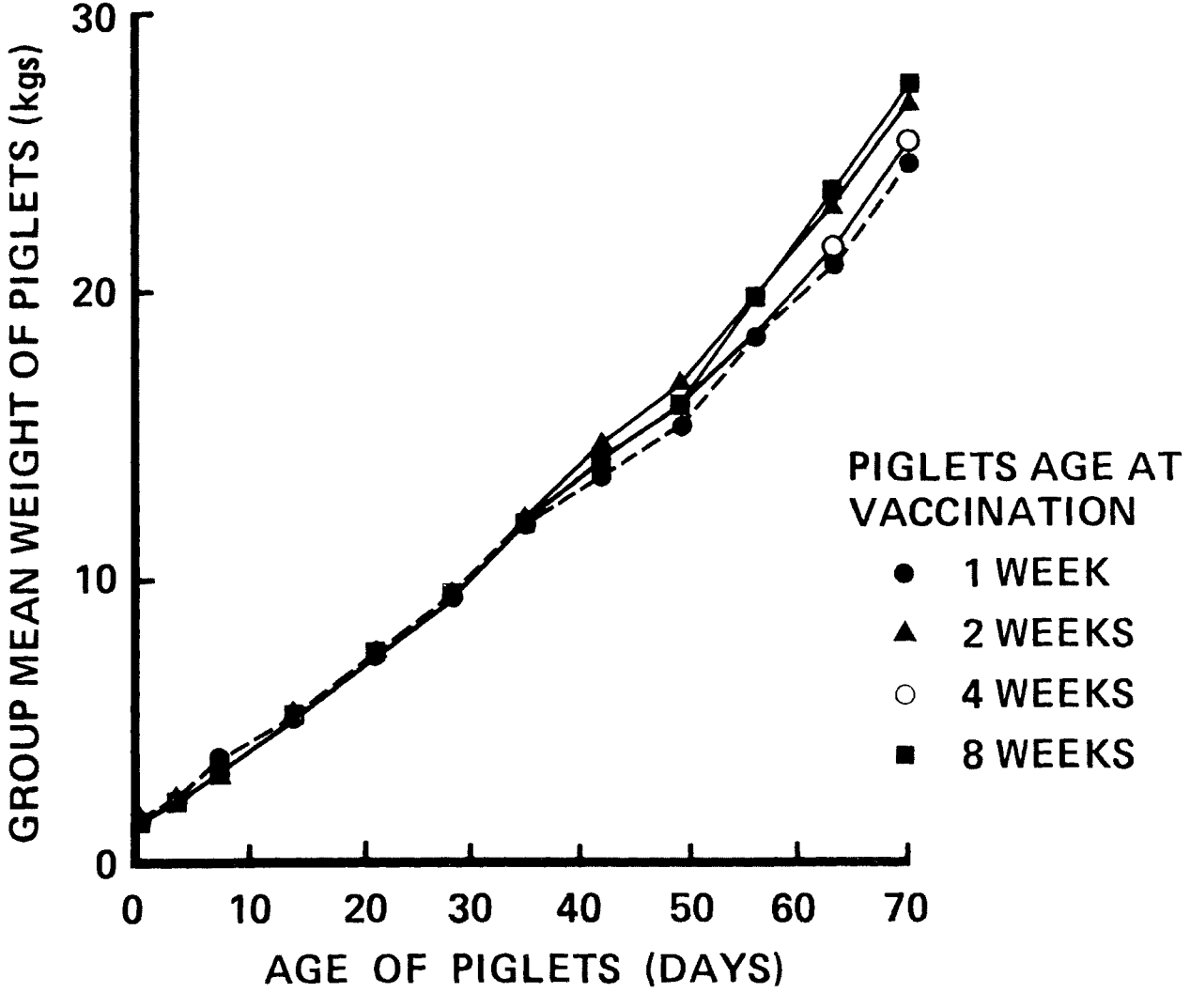


Table 7.10: Results of a two way analysis of variance to study the effect that the vaccination age has on the growth rate of piglets

a) Grouped data of litters from vaccinated and unvaccinated sows

Variable (day)	'F' value	Degrees of freedom	Significance (P)
0	0.53	3, 46	N.S.
3	0.25	3, 46	N.S.
7 ◄	0.54	3, 46	N.S.
14 ◄	0.41	3, 46	N.S.
21	0.10	3, 46	N.S.
28 ◄	0.07	3, 46	N.S.
35	0.14	3, 46	N.S.
42	0.39	3, 46	N.S.
49	0.73	3, 46	N.S.
56 ◄	0.85	3, 46	N.S.
63	2.27	3, 46	N.S.
69	1.98	3, 46	N.S.

b) Data of litters from vaccinated sows

Variable (day)	'F' value	Degrees of freedom	Significance (P)
179	0.39	3, 22	N.S.
196	0.34	3, 22	N.S.
209	0.29	3, 22	N.S.

c) Data of litters from vaccinated sows

Variable (day)	'F' value	Degrees of freedom	Significance (P)
195	0.73	3, 23	N.S.
206	0.64	3, 23	N.S.
223	0.74	3, 23	N.S.
226	1.01	3, 23	N.S.

◄ = day a piglet group was vaccinated

N.S. = Not significant

b) Neutralizing antibody response

(i) Piglets from unvaccinated sows - The neutralizing serum antibody titres ($\log_{10}SN_{50}$) for individual piglets derived from unvaccinated sows and vaccinated at 1, 2, 4 or 8 weeks old are given in Tables 7.11 to 7.14. The mean titres are plotted graphically in Figure 7.3.

Piglets vaccinated at 1 or 2 weeks old showed a rapid production of neutralizing antibody which reached peak levels of >2.4 $\log_{10}SN_{50}$ 3 to 4 weeks after vaccination. The levels then gradually declined over the next 6 months to final pre-challenge titres of between 1.9 and 2.0 $\log_{10}SN_{50}$. However, while the piglets vaccinated when 4 to 8 weeks old showed initial peak titres which were lower than in the groups vaccinated when 1 or 2 weeks old, (1.9 to 2.0 $\log_{10}SN_{50}$), their neutralizing antibody levels then remained stable, or gradually increased, over the next 5 to 6 months. The final titres in 7 month old piglets were between 2.0 and 2.4 $\log_{10}SN_{50}$.

(ii) Piglets from vaccinated sows - The neutralizing antibody titres ($\log_{10}SN_{50}$) for individual piglets derived from vaccinated sows and vaccinated themselves at 1, 2, 4 or 8 weeks old are given in Tables 7.15 to 7.18. The mean titres are plotted graphically in Figure 7.4 and a comparison between these responses and those of litters derived from unvaccinated sows appears in Figures 7.5(a) to 7.5(d).

Piglets vaccinated at 1, 2 or 4 weeks old did not appear to show any response to vaccination and the maternally derived passive antibodies continued to decline (see Figures 7.5(a) to 7.5(c)) with half-lives of 23.4, 19.7 and 23.0 days from 3 to 69 days old, and 44.3, 42.8 and 48.7 days from 3 to 226 days old respectively.

Table 7.11: Neutralizing antibody titres of piglets vaccinated at 1 week old (born to unvaccinated sows)

Piglet no.	Age of piglets (Days)												
	3	► 7	10	14	21	28	35	42	49	56	63	69	76
96/01	<0.47*	<0.47	<0.53	1.17	1.17	2.38	2.43	2.48	2.18	2.08	2.08	2.23	2.18
96/02	<0.47	<0.47	<0.47	1.48	1.93	1.93	2.18	2.44	2.28	2.28	2.13	1.98	2.33
97/01	<0.47	<0.65	<0.65	1.45	1.61	1.91	2.21	2.11	2.04	1.96	1.91	1.95	2.16
97/12	<0.47	<0.65	<0.65	1.66	1.61	2.41	2.46	2.41	2.36	2.26	2.36	2.66	2.51
98/01	<0.47	<0.47	<0.47	1.67	1.93	2.83	2.73	2.73	2.78	2.48	2.58	2.41	2.24
98/02	<0.47	<0.47	<0.53	1.57	1.98	3.68	3.53	3.43	3.38	3.13	3.14	3.03	2.88
$\bar{x}$	<0.47	<0.53	<0.55	1.50	1.71	2.52	2.59	2.60	2.50	2.37	2.37	2.38	2.38

Piglet no.	Age of piglets (Days)												
	83	90	97	104	111	118	125	129	153	166	179	196	209
96/01	2.28	2.13	2.23	1.97	2.13	2.03	2.03	1.88	1.92	1.93	1.98	1.93	1.93
96/02	2.08	2.28	1.93	2.08	2.13	2.18	2.38	2.28	2.03	2.28	2.13	2.41	2.33
97/01	2.01	2.26	2.01	2.06	2.06	1.86	1.90	1.86	1.76	1.75	1.56	1.66	1.55
97/12	2.21	2.36	2.61	2.56	2.56	2.23	2.81	2.41	2.60	2.61	2.56	2.51	2.46
98/01	2.23	2.18	1.98	1.93	2.03	2.03	2.18	1.98	1.92	1.64	1.53	1.67	1.47
98/02	2.77	2.63	2.58	2.67	2.53	2.28	2.53	2.43	2.18	2.08	2.18	2.03	1.87
$\bar{x}$	2.26	2.31	2.22	2.21	2.24	2.10	2.31	2.14	2.07	2.05	1.99	2.04	1.94

► = Vaccination

*$\log_{10}SN_{50}$ (mean of 3 tests)

Table 7.12: Neutralizing antibody titres of piglets vaccinated at 2 weeks old (born to unvaccinated sows)

Piglet no.	Age of piglets (Days)												
	3	► 14	17	21	28	35	42	49	56	63	69	76	83
96/03	<0.47*	0.47	0.53	1.62	2.03	2.03	2.03	2.23	2.08	1.88	1.78	2.08	1.83
96/04	<0.47	<0.47	<0.53	1.32	1.77	2.28	2.33	2.28	2.43	2.28	2.08	2.23	2.03
97/03	<0.65	<0.65	<0.65	1.25	1.35	1.95	2.06	1.85	1.52	2.31	2.51	2.61	2.56
97/04	<0.65	<0.65	<0.65	1.50	1.71	1.91	2.06	2.01	2.01	1.91	1.86	1.91	1.90
98/03	<0.47	<0.47	<0.47	1.42	2.23	3.31	3.18	3.18	2.98	3.00	2.93	2.78	2.48
98/04	<0.47	<0.47	<0.47	2.03	2.93	2.98	3.19	2.78	2.98	2.80	2.77	2.73	2.73
$\bar{x}$	<0.53	<0.53	<0.55	1.52	2.00	2.38	2.47	2.39	2.33	2.36	2.32	2.39	2.26

Piglet no.	Age of piglets (Days)											
	90	97	104	111	118	125	139	153	166	179	196	209
96/03	2.03	1.78	2.03	1.92	2.18	2.13	2.28	2.08	2.23	1.98	2.22	1.87
96/04	2.08	2.02	1.93	2.08	1.93	2.03	1.83	1.98	1.98	1.83	1.87	1.72
97/03	2.79	2.86	2.96	2.91	2.76	2.86	2.91	2.81	2.96	2.76	2.96	2.86
97/04	2.21	2.31	2.36	2.26	2.26	1.90	2.21	1.86	2.11	1.66	1.71	1.66
98/03	2.78	2.53	2.58	2.43	2.48	2.43	2.53	2.48	2.23	2.33	2.18	2.13
98/04	2.83	2.58	2.67	2.63	2.58	2.53	2.53	2.53	2.33	2.28	2.03	2.23
$\bar{x}$	2.45	2.35	2.42	2.37	2.37	2.31	2.38	2.29	2.31	2.14	2.16	2.08

► = Vaccination

*$\log_{10} SN_{50}$ (mean of 3 tests)

Table 7.13: Neutralizing antibody titres of piglets vaccinated at 4 weeks old (born to unvaccinated sows)

Piglet no.	Age of piglets (Days)											
	3	► 28	31	35	42	49	56	63	69	76	83	90
95/07	<0.47*	<0.47	<0.59	1.88	1.53	1.77	1.93	1.88	1.87	1.88	2.08	2.08
96/05	<0.47	<0.53	<0.63	1.88	2.43	2.18	1.98	2.07	2.03	1.97	2.23	2.22
96/06	<0.47	<0.47	<0.53	1.88	1.88	1.93	1.57	1.52	1.63	1.53	1.53	1.47
96/07	<0.47	<0.47	0.57	1.37	1.78	1.88	1.83	1.87	1.72	1.82	1.67	1.73
97/05	<0.65	<0.65	0.71	1.75	2.31	1.90	1.71	1.65	1.71	1.65	1.81	1.81
97/06	<0.65	<0.65	<0.65	1.91	1.90	1.85	1.81	1.71	1.91	1.65	1.76	1.71
97/11	<0.65	<0.65	<0.65	1.70	1.91	1.61	1.76	1.65	1.75	1.55	1.66	1.76
98/05	<0.47	<0.47	<0.53	2.23	2.38	2.47	2.53	2.36	2.14	2.51	2.28	2.18
$\bar{x}$	<0.54	<0.55	<0.61	1.83	2.02	1.95	1.89	1.84	1.85	1.82	1.88	1.87

Piglet no.	Age of piglets (Days)										
	97	104	111	118	125	139	153	166	179	196	209
95/07	2.38	2.13	2.13	2.18	2.48	2.68	2.43	2.58	2.38	2.33	2.23
96/05	2.43	2.13	2.38	2.38	2.73	2.43	2.78	2.78	2.73	2.58	2.88
96/06	1.47	1.62	1.67	1.72	1.78	1.73	1.52	1.62	1.73	1.77	1.67
96/07	1.63	1.77	1.97	1.98	1.83	1.93	1.82	1.73	1.63	1.57	1.67
97/05	1.75	1.91	1.91	2.01	1.86	1.96	1.91	1.70	1.45	1.51	1.71
97/06	1.75	1.81	1.81	1.81	1.80	1.80	1.86	1.55	1.80	1.55	1.61
97/11	1.71	1.61	1.81	1.65	1.81	1.71	1.90	1.45	1.65	1.50	1.61
98/05	2.03	2.23	2.43	2.43	2.48	2.68	2.68	2.48	2.43	2.58	2.88
$\bar{x}$	1.89	1.90	2.01	2.02	2.10	2.12	2.11	1.86	1.98	1.92	2.03

► = Vaccination

*$\log_{10}SN_{50}$ (mean of 3 tests)

Table 7.14: Neutralizing antibody titres of piglets vaccinated at 8 weeks old (born to unvaccinated sows)

Piglet no.	Age of piglets (Days)										
	3	► 56	59	63	69	76	83	90	97	104	111
95/09	<0.47*	<0.53	0.63	1.62	1.22	1.47	1.72	1.99	1.63	1.93	1.73
96/08	<0.47	<0.53	<0.62	1.93	1.83	1.92	2.03	1.77	1.67	1.73	1.77
96/09	<0.47	<0.47	<0.62	1.47	1.73	2.48	2.38	2.03	1.73	2.03	2.33
96/10	<0.47	<0.53	<0.62	1.63	1.71	2.23	2.08	1.88	1.73	1.88	1.98
97/07	<0.47	<0.71	0.77	1.51	1.61	1.75	1.81	1.86	1.95	1.80	2.01
97/08	<0.47	<0.65	<0.65	1.46	1.29	1.76	1.50	1.61	1.51	1.55	1.75
97/10	<0.47	<0.65	<0.65	1.50	1.25	1.56	1.85	1.81	1.65	1.75	1.81
$\bar{x}$	<0.55	<0.58	<0.65	1.59	1.52	1.88	1.91	1.85	1.70	1.81	1.91

Piglet no.	Age of piglets (Days)							
	118	125	139	153	166	179	196	209
95/09	2.13	2.48	2.73	2.77	2.78	2.83	2.86	2.96
96/08	1.67	1.67	1.67	1.72	1.68	1.63	1.73	1.77
96/09	2.18	2.53	2.33	2.78	2.78	2.78	2.58	2.58
96/10	2.43	2.43	2.63	2.63	2.58	2.58	2.63	2.38
97/07	1.91	2.06	2.16	2.46	2.41	2.41	2.36	2.26
97/08	1.91	1.96	2.26	2.36	2.21	2.36	2.56	2.36
97/10	1.86	1.71	1.86	1.96	2.21	2.31	2.31	2.46
$\bar{x}$	2.01	2.12	2.23	2.38	2.41	2.41	2.43	2.40

► = Vaccination *$\log_{10}SN_{50}$ (mean of 3 tests)

Figure 7.3: Vaccination response of piglets born to unvaccinated sows

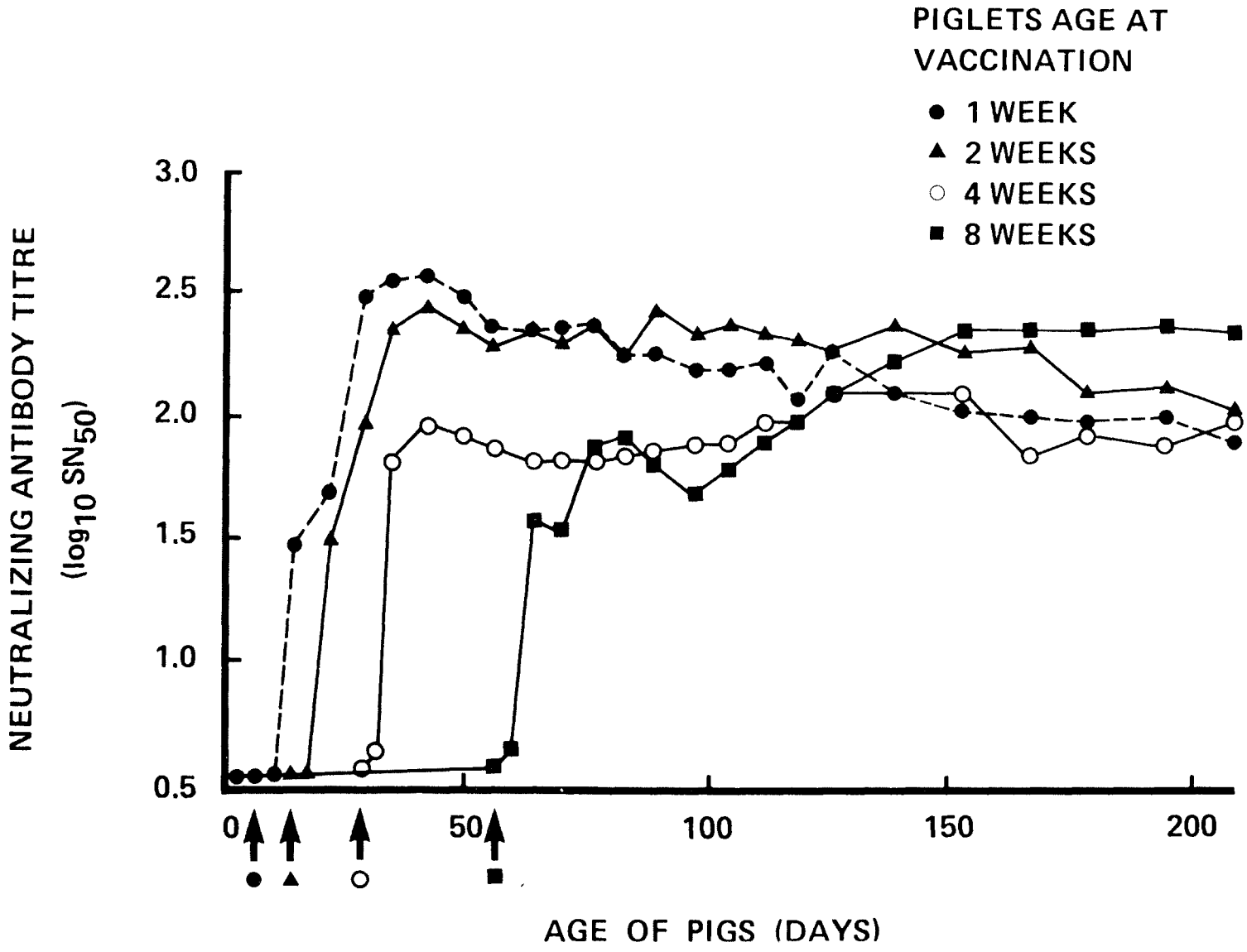


Table 7.15: Neutralizing antibody titres of piglets vaccinated at 1 week old (born to vaccinated sows)

Piglet no.	Age of piglets (Days)										
	3	►7	10	14	21	28	35	42	49	56	63
92/01	2.68*	2.18	2.28	2.28	2.07	1.88	1.88	1.93	1.73	1.62	1.83
92/09	2.58	2.38	2.48	2.43	2.23	2.28	2.07	1.93	2.03	1.78	1.72
93/01	2.65	2.45	2.35	2.40	2.10	2.05	2.00	2.25	1.85	1.75	1.69
93/02	2.60	2.60	2.45	2.30	2.45	2.15	2.10	1.95	1.85	1.80	1.85
94/01	2.96	2.66	2.61	2.61	2.46	2.41	2.56	2.16	2.05	1.95	2.16
94/09	2.66	2.56	2.61	2.61	2.31	2.21	2.21	2.01	1.90	1.90	1.86
$\bar{x}$	2.69	2.47	2.46	2.44	2.27	2.16	2.13	2.04	1.90	1.80	1.85

Piglet no.	Age of piglets (Days)										
	69	76	83	90	97	104	111	118	125	139	153
92/01	1.47	1.62	1.83	1.37	1.32	1.52	1.22	1.32	1.27	1.32	1.42
92/09	1.98	1.83	1.58	1.58	1.47	1.36	1.47	1.27	1.32	1.32	1.32
93/01	1.80	1.55	1.55	0.99	1.44	1.65	1.40	1.40	1.24	1.14	1.09
93/02	1.75	1.60	1.70	1.49	1.45	1.34	1.44	1.34	1.25	1.14	1.24
94/01	1.95	2.01	1.70	1.75	1.60	1.65	1.70	1.60	1.55	1.45	1.54
94/09	1.86	1.80	1.76	1.60	1.76	1.65	1.65	1.60	1.55	1.29	1.40
$\bar{x}$	1.80	1.74	1.69	1.50	1.51	1.47	1.48	1.42	1.36	1.28	1.34

Piglet no.	Age of piglets (Days)					
	167	181	195	206	223	226
92/01	1.32	1.07	1.07	1.02	0.88	0.94
92/09	1.13	1.17	1.07	1.12	1.13	1.02
93/01	0.89	1.30	1.24	1.08	1.19	1.19
93/02	1.18	1.04	0.80	1.04	0.84	0.99
94/01	1.35	1.21	1.30	1.35	1.15	1.09
94/09	1.20	1.19	1.09	1.01	0.97	0.86
$\bar{x}$	1.18	1.16	1.10	1.10	1.03	1.02

► = Vaccination *$\log_{10}SN_{50}$ (mean of 3 tests)

Table 7.16: Neutralizing antibody titres of piglets vaccinated at 2 weeks old (born to vaccinated sows)

Piglet no.	Age of piglets (Days)										
	3	► 14	17	21	28	35	42	49	56	63	69
91/03	2.95*	2.64	2.35	2.19	2.14	2.24	2.04	1.84	1.95	1.65	1.65
91/04	2.40	2.25	2.20	2.19	1.94	1.69	1.78	1.59	1.39	1.59	1.34
92/03	2.78	2.38	2.38	2.28	2.23	2.13	1.88	1.83	1.78	1.57	1.83
92/04	2.78	2.38	2.43	2.33	2.28	1.98	2.03	1.83	1.88	1.88	1.83
93/03	2.60	2.45	2.40	2.35	2.20	2.05	1.80	2.00	1.90	1.75	1.85
93/04	2.60	2.30	2.05	2.25	2.00	2.10	1.80	1.90	1.75	1.69	1.65
94/03	2.71	2.56	2.61	2.46	2.21	2.20	2.26	2.00	1.90	1.81	1.70
94/04	3.00	2.56	2.76	2.66	2.56	2.31	2.16	1.96	1.90	1.75	1.80
$\bar{x}$	2.73	2.44	2.40	2.34	2.20	2.09	1.97	1.87	1.81	1.72	1.71

Piglet no.	Age of piglets (Days)										
	76	83	90	97	104	111	118	125	139	153	167
91/03	1.54	1.74	1.59	1.49	1.39	1.28	1.18	1.18	1.23	1.03	1.01
91/04	1.33	1.23	1.04	0.98	1.03	0.94	1.04	1.18	1.25	1.33	1.18
92/03	1.83	1.68	1.52	1.47	1.52	1.46	1.53	1.32	1.42	1.47	1.13
92/04	1.72	1.63	1.42	1.47	1.37	1.36	1.53	1.52	1.32	1.47	1.37
93/03	1.50	1.65	1.55	1.30	1.47	1.34	1.29	1.30	1.20	1.19	1.09
93/04	1.44	1.44	1.50	1.40	1.34	1.29	1.25	1.30	1.14	1.08	0.98
94/03	1.80	1.55	1.70	1.50	1.50	1.35	1.40	1.44	1.35	1.20	1.09
94/04	1.80	1.86	1.55	1.60	1.55	1.45	1.61	1.40	1.40	1.39	1.25
$\bar{x}$	1.62	1.60	1.48	1.40	1.39	1.31	1.35	1.30	1.29	1.27	1.14

Piglet no.	Age of piglets (Days)				
	181	195	206	223	226
91/03	0.99	0.90	0.78	0.94	1.03
91/04	1.18	1.29	1.04	0.94	0.98
92/03	1.08	1.02	1.07	0.90	0.94
92/04	1.32	1.07	1.22	1.32	1.16
93/03	0.75	0.94	0.99	0.74	0.75
93/04	0.94	0.79	0.89	0.80	0.79
94/03	1.15	1.10	1.05	0.82	0.82
94/04	1.25	1.25	1.16	1.01	0.96
$\bar{x}$	1.08	1.04	1.03	0.93	0.93

► = Vaccination *$\log_{10}SN_{50}$ (mean of 3 tests)

Table 7.17: Neutralizing antibody titres of piglets vaccinated at 4 weeks old (born to vaccinated sows)

Piglet no.	Age of piglets (Days)												
	3	► 28	31	35	42	49	56	63	69	76	83	90	97
91/05	2.29*	1.94	1.79	1.75	1.64	1.43	1.33	1.59	1.33	1.39	1.54	1.34	1.2
91/06	2.69	2.30	2.29	2.24	1.99	2.09	1.85	1.84	1.84	1.74	1.64	1.43	1.3
92/05	2.68	1.83	2.08	1.83	1.98	1.75	1.88	1.88	1.77	1.62	1.63	1.58	1.4
92/06	2.63	2.13	2.03	1.97	2.03	1.83	1.88	1.78	1.68	1.68	1.52	1.47	1.6
93/05	2.70	2.20	2.20	2.15	1.95	1.80	1.75	1.70	1.80	1.75	1.49	1.34	1.5
93/06	2.50	2.30	2.10	2.10	1.75	1.85	1.80	1.90	1.80	1.80	1.65	1.45	1.5
94/06	2.66	2.36	2.21	2.21	2.00	1.95	1.81	1.85	1.80	1.60	1.70	1.55	1.5
$\bar{x}$	2.59	2.15	2.10	2.04	1.91	1.81	1.76	1.79	1.72	1.65	1.60	1.45	1.4

Piglet no.	Age of piglets (Days)											
	104	111	118	125	139	153	167	181	195	206	223	226
91/05	1.29	1.18	1.28	1.23	1.29	1.13	1.18	1.28	1.18	1.00	1.04	1.14
91/06	1.33	1.24	1.13	1.08	0.90	0.80	0.86	0.86	1.04	1.13	0.94	0.94
92/05	1.27	1.27	1.37	1.37	1.27	1.47	1.32	1.26	1.18	1.27	1.12	1.03
92/06	1.37	1.32	1.53	1.32	1.37	1.36	1.26	1.22	1.12	1.07	0.98	0.99
93/05	1.55	1.40	1.34	1.24	1.24	1.14	1.20	1.09	1.08	1.19	0.85	0.84
93/06	1.50	1.40	1.24	1.34	1.25	1.14	1.09	0.94	0.79	0.94	0.89	0.84
94/06	1.50	1.54	1.14	1.25	1.44	1.20	1.05	1.02	0.97	0.88	<0.82	0.88
$\bar{x}$	1.40	1.34	1.29	1.26	1.25	1.18	1.14	1.10	1.05	1.07	0.95	0.95

► = Vaccination *$\log_{10}SN_{50}$ (mean of 3 tests)

Table 7.18: Neutralizing antibody titres of piglets vaccinated at 8 weeks old (born to vaccinated sows)

Piglet no.	Age of piglets (Days)										
	3	► 56	59	63	69	76	83	90	97	104	111
91/07	2.79*	1.79	1.84	1.40	1.69	1.68	1.33	1.49	1.39	1.38	1.18
91/08	1.84	1.00	1.03	1.13	1.69	2.40	2.14	2.25	2.15	2.25	1.95
92/07	2.48	1.88	1.83	1.72	2.13	2.33	2.23	1.83	1.78	1.62	1.43
92/08	2.53	1.83	1.98	1.88	1.97	2.03	1.98	1.98	1.87	1.46	1.47
93/07	2.55	1.65	1.95	1.85	1.65	1.75	1.59	1.50	1.60	1.50	1.50
94/07	2.61	2.11	2.01	2.11	1.76	1.65	1.65	1.76	1.61	1.61	1.61
$\bar{x}$	2.47	1.71	1.77	1.68	1.82	1.97	1.82	1.80	1.73	1.64	1.52

Piglet no.	Age of piglets (Days)									
	118	125	139	153	167	181	195	206	223	226
91/07	1.04	1.18	1.08	0.98	0.90	0.98	1.49	1.18	1.00	0.90
91/08	1.84	1.75	1.95	1.79	1.79	1.79	1.84	1.79	1.79	1.74
92/07	1.57	1.52	1.58	1.78	1.73	1.62	1.73	1.62	1.78	1.77
92/08	1.47	1.62	1.73	1.72	1.47	1.37	1.37	1.35	1.47	1.42
93/07	1.40	1.30	1.30	1.25	1.24	1.25	0.79	1.34	1.30	1.30
94/07	1.40	1.70	1.61	1.29	1.15	1.24	1.05	1.00	0.82	0.82
$\bar{x}$	1.45	1.51	1.54	1.47	1.38	1.38	1.38	1.38	1.36	1.33

► = Vaccination *$\log_{10}SN_{50}$ (mean of 3 tests)

Figure 7.4: Vaccination response of piglets born to FMD vaccinated sows

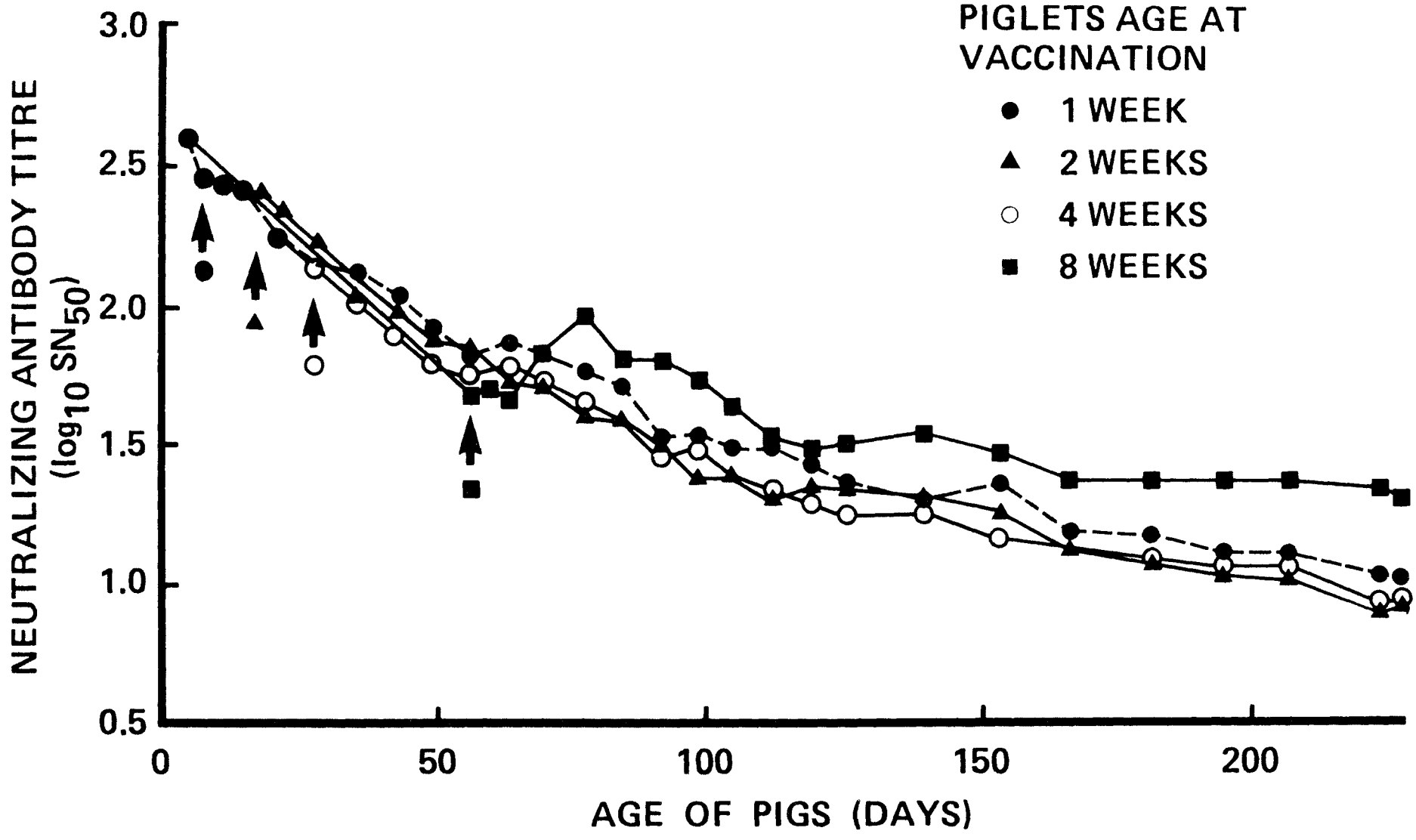
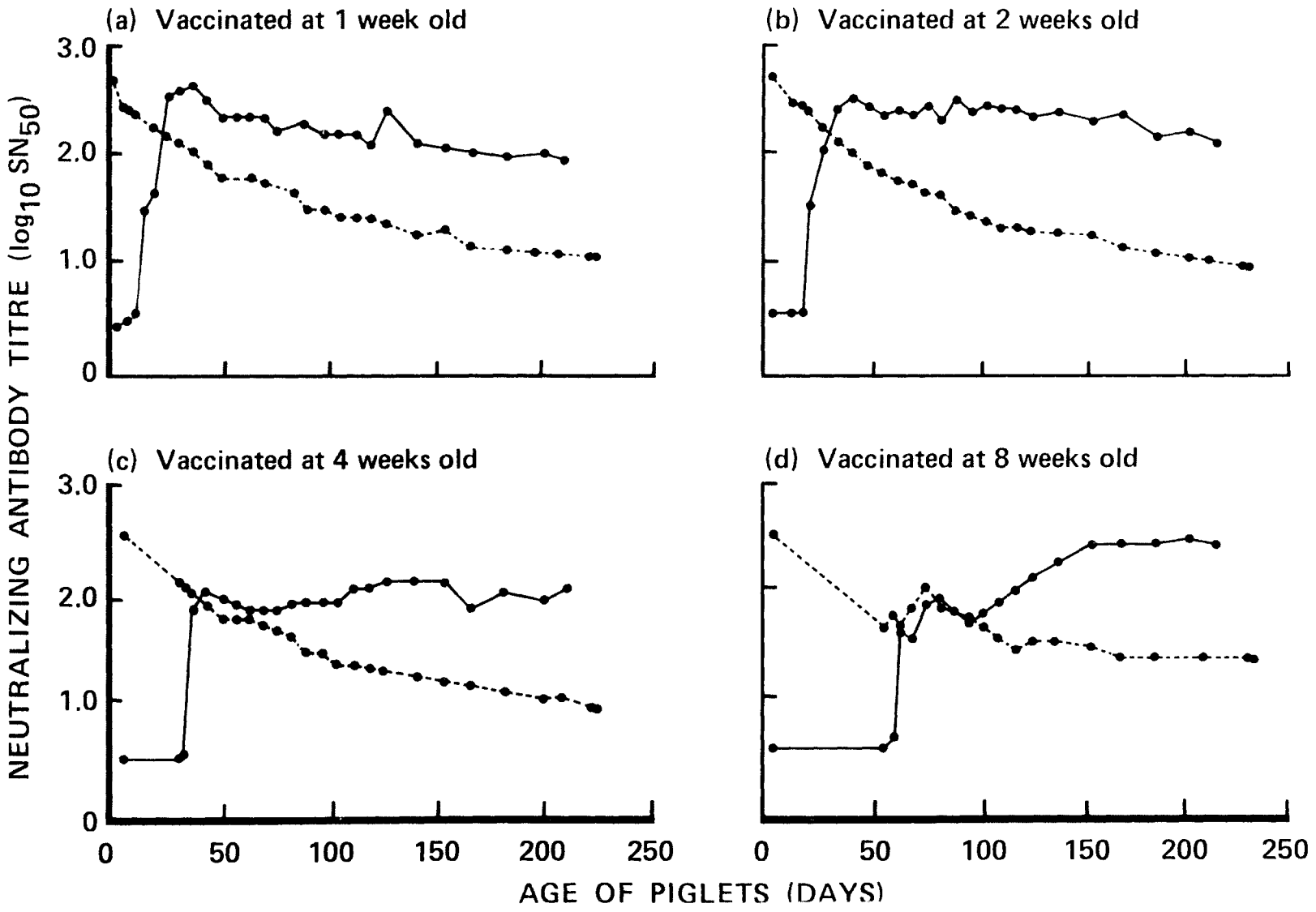


Figure 7.5: Comparison between the vaccination responses of piglets born to vaccinated (•·····•) and unvaccinated (•——•) sows



Vaccination of piglets at 8 weeks old (by which time the passive antibodies had declined to a mean titre of 1.71 $\log_{10}SN_{50}$) appeared to evoke some active immunity. However, this result was dependent on the level of passive antibody present in the individual piglet at the time of vaccination. For instance, piglet 91/08 which had the lowest titre (1.00 $\log_{10}SN_{50}$) at vaccination produced the best vaccination response with a titre of 2.40 $\log_{10}SN_{50}$ at 21dpv while piglet 94/07 which had the highest titre (2.11 $\log_{10}SN_{50}$) at vaccination produced the worst vaccination response with a titre of 1.65 $\log_{10}SN_{50}$ at 21dpv. The half-life of neutralizing antibodies in these piglets was 25.7 days from 3 to 69 days old and 78.3 days from 3 to 226 days old.

The effect of passive antibody level at the time of vaccination on the piglets responses at 21dpv (increase or decrease in the serum antibody titre) has been plotted in Figure 7.6. A regression line drawn through the points has a slope of -0.82 and a correlation coefficient of -0.78 (which is significant at a 1% level) confirming that the inhibiting activity of maternally derived antibodies was directly proportional to the titres in the piglets at time of vaccination.

c) Immunoglobulin class activity

(i) Piglets from unvaccinated sows - The mean OD values from repeat ELISA tests employing class-specific anti-IgG, anti-IgM and anti-IgA conjugates were established for the piglets vaccinated between 1 and 8 weeks old. These are presented as immunoglobulin response curves in Figure 7.7, the data for which is given in appendix 3 (Table A3.4). By comparing the response profiles of each immunoglobulin class it appears that while IgG activity was similar between all groups (peak OD 1.2 to 1.4) the IgM and IgA activity was lower in piglets vaccinated at 1 or 2 weeks old than in those vaccinated when 4 or 8 weeks old.

Figure 7.6: The effect of maternally derived antibodies on piglets 21 day post vaccinal titres

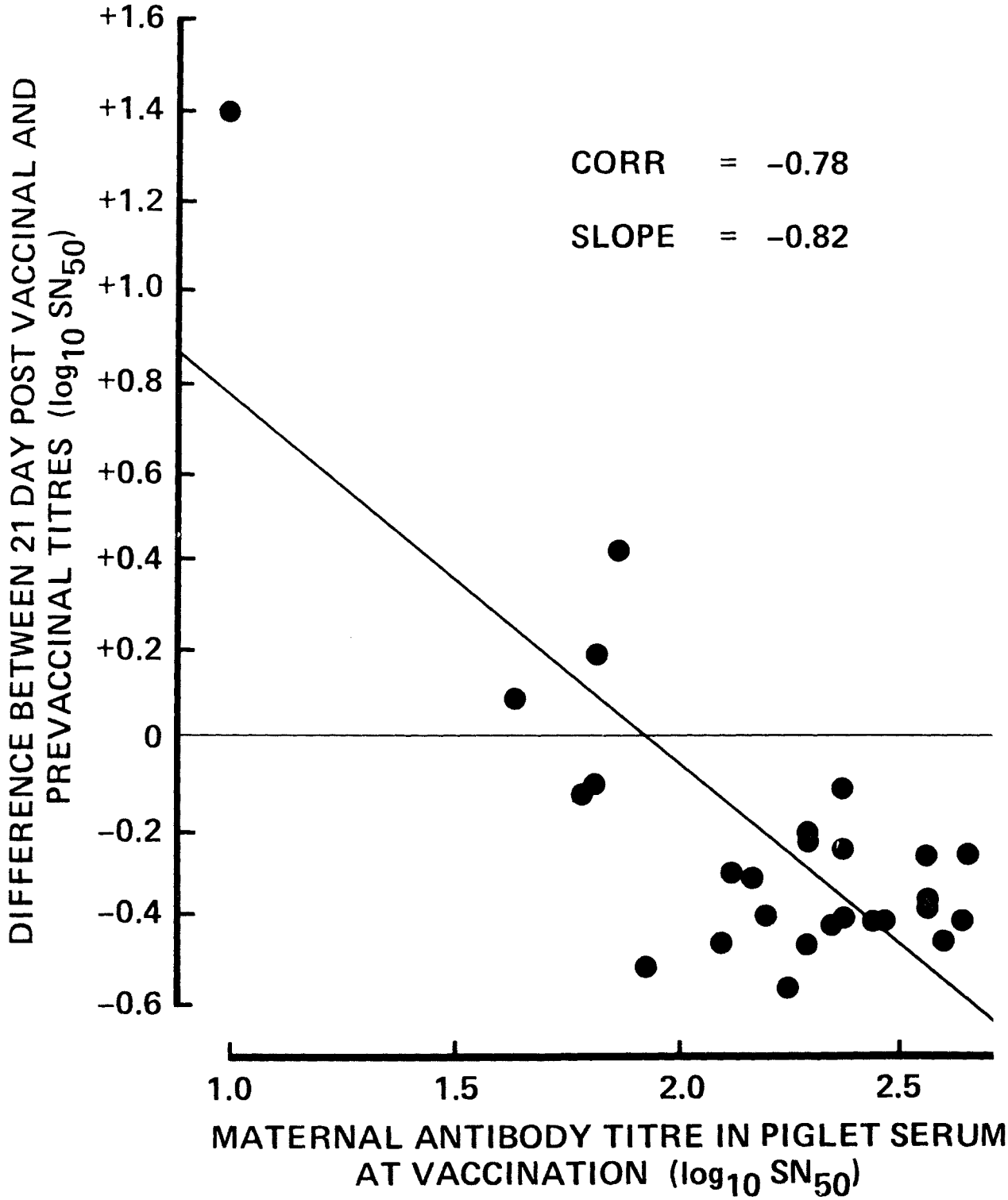
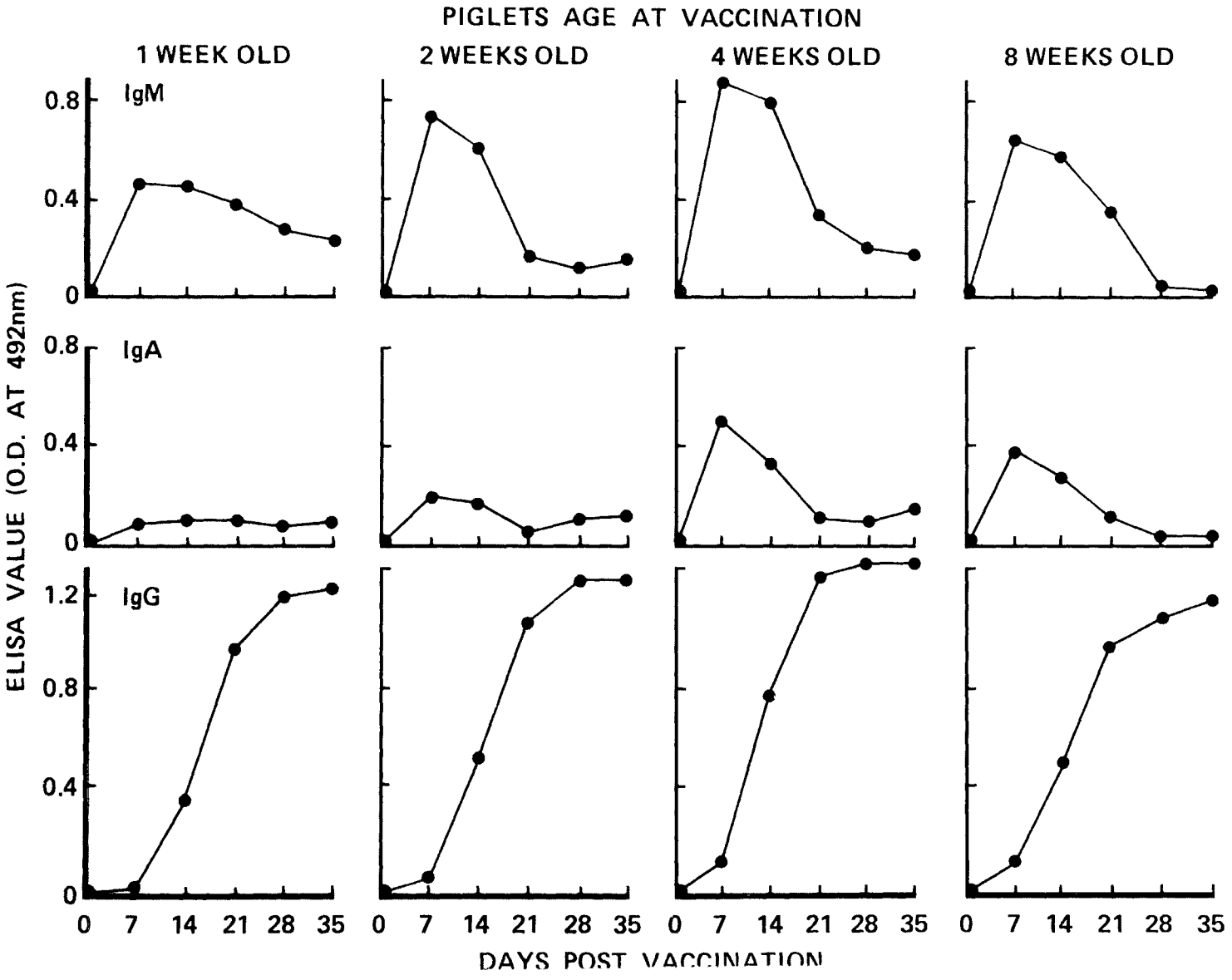


Figure 7.7: Immunoglobulin class response of piglets born to unvaccinated sows



(ii) Piglets from vaccinated sows - The mean OD values from repeat immunoglobulin class specific ELISA tests, on piglets vaccinated at between 1 and 8 weeks old, are presented as immunoglobulin response curves in Figure 7.8, the data for which is given in appendix 3 (Table A3.5). All the activity present at the time of vaccination in the 1 to 8 week old piglets, that is as a result of maternally derived antibody, was due to IgG antibodies. This was expected as the sows had been vaccinated twice with the last vaccination being given more than a month before farrowing.

In the piglets vaccinated when 1 and 2 weeks old there was a slight IgM response, no IgG or IgA response and the levels of maternally derived IgG continued to steadily subside. Vaccination at 4 weeks old produced a more clearcut IgM response. However, the IgA and IgG responses were still poor. Vaccination of the 8-week-old piglets did provoke IgG, IgM and IgA responses, although the levels were invariably lower than those observed in piglets born to unvaccinated sows.

d) Protection from FMD infection

The results of tests carried out to challenge the immunity of 6-7 month-old piglets from unvaccinated and vaccinated sows are given in Tables 7.19 and 7.20 respectively and are summarised in Table 7.21.

Piglets from unvaccinated sows vaccinated at 1 to 8 weeks old were between 33.3 and 87.5% protected against FMD virus infection at 6 to 7 months old while piglets from FMD vaccinated sows vaccinated at 1 to 8 weeks old were all unprotected at 6 to 7 months old.

Figure 7.8: Immunoglobulin class response of piglets born to FMD vaccinated sows

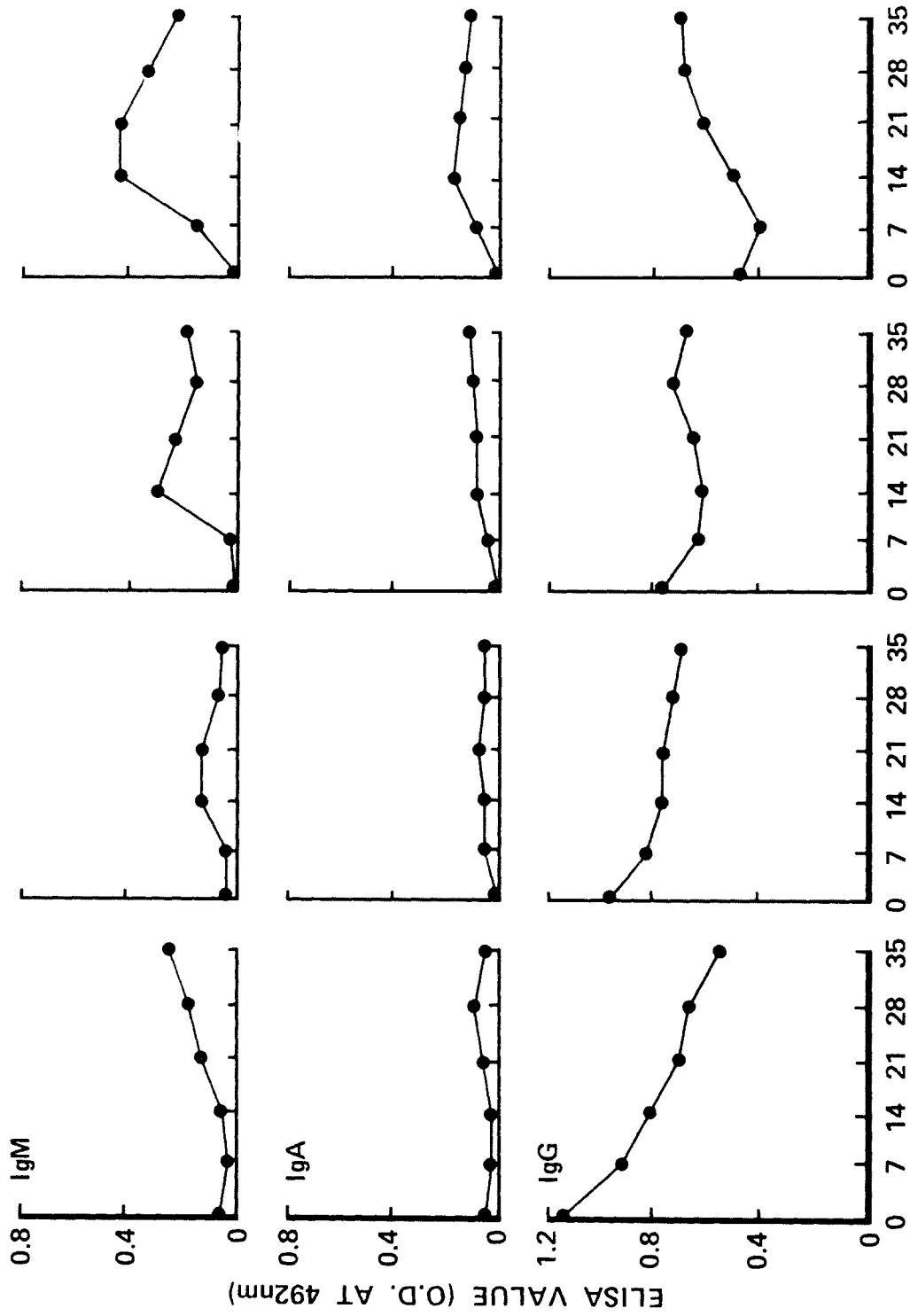


Table 7.19: Results of challenge tests on piglets from unvaccinated sows

Piglets vaccinated at 1 week old						Piglets vaccinated at 2 weeks old					
Piglet no.	Foot lesions				Result	Piglet no.	Foot lesions				Result
	LF	RF	LH	RH			LF	RF	LH	RH	
96/01	+	+	+	+	NP	96/03	+	+	+	+	NP
96/02	-	-	+	-	P	96/04	+	-	-	-	P
97/01	+	+	+	+	NP	97/03	-	-	-	-	P
97/12	-	-	-	-	P	97/04	+	+	+	+	NP
98/01	+	+	+	+	NP	98/03	+	-	-	-	P
98/02	+	-	+	+	NP	98/04	+	+	+	+	NP

Piglets vaccinated at 4 weeks old						Piglets vaccinated at 8 weeks old					
Piglet no.	Foot lesions				Result	Piglet no.	Foot lesions				Result
	LF	RF	LH	RH			LF	RF	LH	RH	
95/07	-	-	-	-	P	95/09	-	-	-	-	P
96/05	-	-	-	-	P	96/08	+	+	+	+	NP
96/06	+	+	-	-	P	96/09	-	-	-	-	P
96/07	+	-	-	-	P	96/10	-	-	-	-	P
97/05	+	-	-	-	P	97/07	-	-	-	-	P
97/06	-	-	+	-	P	97/08	-	-	-	-	P
97/11	+	+	+	+	NP	97/10	-	-	-	-	P
98/05	+	-	-	-	P						

P = Protected
NP = Not Protected
\+ = FMD lesions
\- = No FMD lesions

N.B. All 4 control pigs were +ve, on all 4 feet, and therefore not protected.

Table 7.20: Results of challenge tests on piglets from FMD vaccinated sows

Piglets vaccinated at 1 week old						Piglets vaccinated at 2 weeks old					
Piglet no.	Foot lesions				Result	Piglet no.	Foot lesions				Result
	LF	RF	LH	RH			LF	RF	LH	RH	
92/01	+	+	+	+	NP	91/03	+	+	+	+	NP
92/09	+	+	+	+	NP	91/04	+	+	+	+	NP
93/01	+	+	+	+	NP	92/03	+	+	+	+	NP
93/02	+	+	+	+	NP	92/04	+	+	+	+	NP
94/01	+	+	+	+	NP	93/03	+	+	+	+	NP
94/09	+	+	+	+	NP	93/04	+	+	+	+	NP
						94/03	+	+	+	+	NP
						94/04	+	+	+	+	NP

Piglets vaccinated at 4 weeks old						Piglets vaccinated at 8 weeks old					
Piglet no.	Foot lesions				Result	Piglet no.	Foot lesions				Result
	LF	RF	LH	RH			LF	RF	LH	RH	
91/05	+	+	+	+	NP	91/07	+	+	+	+	NP
91/06	+	+	+	+	NP	91/08	+	+	+	+	NP
92/05	+	+	+	+	NP	92/07	+	+	+	+	NP
92/06	+	+	+	+	NP	92/08	+	+	+	+	NP
93/05	+	+	+	+	NP	93/07	+	+	+	+	NP
93/06	+	+	+	+	NP	93/08	+	+	+	+	NP
94/06	+	+	+	+	NP						

P = Protected
NP = Not Protected
\+ = FMD lesions
\- = No FMD lesions

N.B. All 4 control pigs were +ve, on all 4 feet, and therefore not protected.

Table 7.21: Summary of challenge test results on 6-7 month old pigs

Piglets' age at vaccination	Source of piglets	
	Unvaccinated Sows	Vaccinated Sows
1 week old	2/6* (33.3%)**	0/6 (0%)
2 weeks old	3/6 (50%)	0/8 (0%)
4 weeks old	7/8 (87.5%)	0/7 (0%)
8 weeks old	7/8 (87.5%)	0/6 (0%)

* No. of piglets protected out of the total no. challenged.

** Percentage of piglets protected.

DISCUSSION

Before considering the prophylactic potential of FMD oil-emulsion vaccine in young pigs it was important to establish whether the vaccination had any harmful effect on the animals. From a practical point of view the most important aspect was thought to be the growth rate as this is of greatest economic importance to the farmer and also generally reflects the health of the animals. One group of workers (Chappuis et al, 1976) has reported that FMD oil vaccination at 14 days old retarded the growth rate of piglets while another group from the same institute later showed (Mougeot et al, 1980) that vaccination of 28 day old piglets did not retard growth or reduce the market value of the carcasses, since the oil vaccine was assimilated within 3 weeks. The present study however showed that piglets could be vaccinated as early as 7 days old with no harmful effects and no significant reduction in growth rate. Chappuis et al (1976) did not perform statistical analyses on their results, but if the differences demonstrated were significant then this could have been due to the fact that a different oil formulation was used to that described in this study.

The results of neutralizing antibody assays showed that piglets, born to unvaccinated sows, were capable of responding to FMD vaccination when only 1 week old. Although studies with FMD oil vaccines have not previously been carried out in piglets, born to unvaccinated sows, younger than 1 month old (Giraud et al, 1969; Morgan and McKercher, 1978), such an early ability to respond to antigenic stimulation has been observed previously in pigs injected with other viral (Niles and Reitz, 1920; Precausta, Kato and Brun, 1983) and non-viral antigens (Young, Hinz and Underdahl, 1955; Murdoch and Jungk, 1957).

However, examination of the nature and development of the anti-FMD activity in piglets from unvaccinated sows indicates that there are differences between the responses of piglets vaccinated when 1 or 2 weeks old and those vaccinated at 4 or 8 weeks old.

The response of the 4 or 8-week-old piglets was similar to that of adult sows (see Chapter 4). The neutralizing antibodies reached initial peak titres 2 to 4 weeks after vaccination, subsided slightly and then recovered and persisted until the piglets were challenged at 6 to 7 months old, at which time they were 87.5% protected. Furthermore, the antibody class response in both groups was similar, with IgM and IgA antibodies reaching peak activity at 7 to 14dpv and persisting for approximately 28 days while the IgG antibody activity did not reach a peak until 21 to 28dpv and then persisted at similar or increasing levels.

The initial peak titres observed in piglets vaccinated at 1 or 2 weeks old were higher than those observed in the piglets vaccinated at an older age (4 and 8 weeks old). These titres however declined steadily over the course of the experiment so that animals were only 33.3 to 50% protected from challenge at 6 to 7 months old. This result may simply have been due to the fact that in these animals there was a greater time interval (2 to 3 weeks extra) between vaccination and challenge; however the antibody class analysis did indicate certain deficiencies in their immune response. For instance, the IgM response of 1-week-old piglets was reduced and persisted for less than 21dpv in the 2-week-old group. Furthermore, the IgA response was poor in both 1 and 2-week-old piglets. It is unlikely that these results were due to normal variation between the groups since the piglets within each group

were littermates. These results suggest, therefore, that although the 1 and 2-week-old piglets were capable of mounting a significant immune response against FMD, following oil emulsion vaccination, their responses were not fully mature.

The results obtained in the piglets from FMD vaccinated sows were markedly different to those from unvaccinated sows in that the vaccinations carried out at 1, 2 and 4 weeks old appeared to have little or no effect on the levels of neutralizing antibody and the decay rates of the first 69 days ($t_{\frac{1}{2}}$=19.7 to 23.4 days) were similar to those of maternally derived antibodies in unvaccinated pigs ($t_{\frac{1}{2}}$=21 days; see chapter 6). It is also interesting to note that the antibody half-life from 3 to 226 days, in these animals, was between 44.3 and 48.7 days, which confirms the observation in Chapter 6 that the decay rate is related to the time over which measurements are taken. Furthermore vaccination at 8 weeks old of piglets from vaccinated mothers produced only a small increase in the neutralizing activity. From these results it is clear that the passive anti-FMD antibodies in the piglets, obtained by suckling from their immunized mothers, suppressed the piglets' active response to the oil vaccination, a point that is well illustrated in Figures 7.5a to 7.5d. This suppressive effect was confirmed by the results of challenge tests carried out when the piglets were 6 to 7 months old. In contrast to the results obtained with piglets from unvaccinated sows (33.3 to 87.5% protection), none of the piglets from vaccinated sows were immune to experimental infection with FMD virus in spite of the fact that they themselves had been vaccinated.

Although a similar suppressive effect has also been observed in young pigs with many other antigens, including an oil-formulated Aujeskys disease vaccine (Wittmann, 1981), it is interesting to note that previous work on the use of FMD oil emulsion vaccines in young pigs has failed to detect any suppression by maternally derived antibodies (Giraud et al, 1969; Morgan and McKercher, 1978). These results have even led one group of workers to conclude that colostrally acquired antibodies are not involved in suppression or inhibition of the young pigs response to FMD oil emulsion vaccines (Morgan and McKercher, 1980). However, in their studies no comparisons were made with matched groups of piglets from unvaccinated sows. Furthermore, the inhibitory activity observed is dependent on the concentration of passive antibody present at the time of vaccination (see Figure 7.6) and also on the potency of the vaccine employed. The use of a highly potent vaccine and/or waning maternally derived antibody titres may produce a situation where inhibition is not easily demonstrable. Indeed from this study it would appear that some increase in antibody titres 21 days after vaccination should be expected when passive antibody levels fall below 1.9 $\log_{10}SN_{50}$.

The antibody class analysis provides some insight into the mechanisms involved in maternal antibody suppression. In piglets with relatively high levels of maternally derived IgG at the time of vaccination (1 and 2 weeks old) suppression occurred in all the antibody classes studied while in the piglets that possessed lower levels of passive IgG at time of vaccination (4 and 8 weeks old at vaccination) the active IgM response appears to have escaped from the suppressive effect.

Two possible mechanisms of passive antibody suppression have been proposed (Uhr and Moller, 1968; Solomon, 1971). Firstly, the active production of antibody may be suppressed by a rapid elimination of antigen from the circulation, due to the presence of high levels of maternally derived antibodies, which would reduce the chances of immunocompetent cells coming into contact with antigen. This would result in the type of total suppression observed in the piglets vaccinated at 1 or 2 weeks old. Secondly, the passive antibody may be suppressing antibody formation by a feedback action on those cells responsible for producing specific immunoglobulins. This feedback may act either by preventing secretion of antibody or by inhibiting further proliferation of stimulated cells. Such a mechanism may account for the observation that IgM activity appeared to escape from suppression by the lower levels of maternally derived IgG present in piglets vaccinated at 4 or 8 weeks old. It seems possible that both mechanisms are acting to a greater or lesser extent in the piglets and that this is dependent on the titre of passive antibody present in the piglets at the time of vaccination.

CHAPTER 8

THE EFFECT OF INJECTED NEUTRALIZING ANTIBODIES ON THE RESPONSE OF YOUNG PIGS TO VACCINATION

INTRODUCTION

Chapter 7 has shown that maternally derived antibodies can have a suppressive effect on the response of young pigs to oil-emulsion vaccination and that the degree of suppression is dependent on the neutralizing antibody titre present at the time of vaccination.

The purpose of the work described in this chapter was to establish whether suppression is a property of colostral antibody only or if injected anti-FMD IgG is also capable of inhibiting the response of pigs to vaccination. The importance of the time of administration was also studied since some authors recommend vaccination immediately prior to colostrum intake (Lee et al, 1980).

EXPERIMENTAL DESIGN

Twelve, four-month-old Large White pigs weighing 82.89±7.84Kgs, equivalent to a blood volume of approximately 4501ml, were divided into four groups of three and each group was vaccinated with oil emulsion batch O-115 and/or injected intraperitoneally with anti-FMD IgG antibody (250ml of 3.26 $\log_{10}SN_{50}$ per piglet prepared as described in Chapter 2) in the following manner:

Group 1: Vaccinated only.

Group 2: Injected with antiserum only.

Group 3: Injected with antiserum two days before vaccination.

Group 4: Injected with antiserum two days after vaccination.

The volume of passive antibody injected was calculated to give the piglets neutralizing antibody titres of approximately 2 $\log_{10}$/ml in their serum.

Blood samples were collected at the time of vaccination or passive antibody injection, one and/or two days later and then weekly for the next four months.

RESULTS

The results of neutralization tests on individual pigs in each group are shown in Table 8.1. In groups 3 and 4, which received passive antibody either 2 days before or 2 days after vaccination, certain animals that did not show a significant increase in neutralizing activity in their sera within 24-48 hours of the passive transfer, (NW55 in group 3, and NW57 and 59 in group 4), were excluded before calculating the group mean titres, shown in Table 8.1 and Figure 8.1. This Figure shows that the neutralizing antibody titres of groups 3 and 4 are similar to those of group 2, the passive antibody control group, throughout the experiment. This is supported by the calculated half-lives of the neutralizing antibodies in groups 2, 3 and 4 of 35.6, 39.6 and 34.3 days respectively. It is possible that the titre in group 4 may have increased during the first 20 to 30 days post vaccination before declining to the levels observed in groups 2 and 3. However, this result remains inconclusive as the observation is based on only a single animal's response. In group 1, the vaccination control group, the neutralizing activity gradually increased for the first 30 to 40dpv and then remained fairly stable at 1.7 to 1.8 $\log_{10}SN_{50}$ for the duration of the experiment (up to 120dpv). All four groups had similar mean titres at 30 to 35 days; however at no point after this did the titres of groups 2, 3 or 4 reach the levels observed in group 1.

Table 8.1: The effect of anti-FMD IgG injected intraperitoneally on the neutralizing antibody response of 4 month old piglets to vaccination

DPV	Group Treatment							
	Group 1 Vaccination only				Group 2 Passive antibody only			
	NW* 48	NW* 49	NW* 50	Mean (n=3)	NW* 51	NW* 52	NW* 53	Mean (n=3)
-2	-	-	-	-	-	-	-	-
0	<0.52**	<0.52	<0.52	<0.52	<0.52	<0.52	<0.52	<0.52
1	<0.56	0.52	<0.46	<0.51	1.95	1.91	1.65	1.84
2	<0.52	0.58	<0.52	<0.54	2.01	1.85	1.65	1.84
3	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-
8	1.09	1.15	1.40	1.21	1.81	1.86	1.66	1.77
14	0.89	1.45	1.70	1.35	1.85	1.75	1.44	1.68
23	1.24	1.71	1.80	1.58	1.90	1.65	1.50	1.68
30	1.30	1.45	1.96	1.57	1.80	1.50	1.44	1.58
36	1.35	1.40	1.91	1.55	1.55	1.41	1.25	1.40
44	1.50	1.50	2.01	1.67	1.55	1.40	1.30	1.41
51	1.60	1.75	1.95	1.77	1.55	1.34	1.10	1.33
58	1.80	1.75	1.81	1.79	1.55	1.19	0.89	1.21
65	1.96	1.61	1.80	1.79	1.34	1.15	1.05	1.18
72	1.85	1.60	1.80	1.75	1.24	1.19	1.04	1.16
79	1.85	1.75	1.80	1.80	1.65	1.05	0.84	1.18
86	2.06	1.55	1.65	1.75	1.45	0.99	0.90	1.11
100	2.01	1.55	1.70	1.75	1.25	0.94	0.86	1.01
107	2.00	1.61	1.85	1.82	1.09	0.86	0.74	0.90
114	2.16	1.61	1.90	1.89	0.99	0.80	0.66	0.82
120	2.01	1.65	1.85	1.84	1.25	0.70	0.66	0.87

* Pigs included in calculation of group mean

** $\log_{10} SN_{50}$ mean result of 3 tests

\- No sample

DPV Days post vaccination

Table 8.1 Contd.: The effect of anti-FMD IgG injected intraperitoneally on the neutralizing antibody response of 4 month old piglets to vaccination

DPV	Group Treatment							
	Group 3 Antibody injected 2 days before vaccination				Group 4 Antibody injected 2 days after vaccination			
	NW* 54	NW 55	NW* 56	Mean (n=2)	NW 57	NW* 58	NW 59	Mean (n=1)
-2	<0.52**	<0.46	<0.52	<0.50	-	-	-	-
0	1.86	<0.46	1.65	1.76	<0.52	<0.40	<0.52	<0.40
1	2.06	<0.52	1.55	1.81	-	-	-	-
2	2.05	<0.56	1.65	1.85	<0.56	<0.52	<0.52	<0.52
3	-	-	-	-	0.79	2.06	<0.55	2.06
4	-	-	-	-	0.90	2.11	0.66	2.11
8	1.90	1.54	1.50	1.70	1.65	2.00	1.60	2.00
14	1.86	1.76	1.80	1.83	1.39	2.11	1.09	2.11
23	1.81	2.06	1.90	1.86	1.40	2.30	1.14	2.30
30	1.65	1.91	1.61	1.63	1.45	1.76	1.19	1.76
36	1.50	1.81	1.45	1.48	1.25	1.61	1.20	1.61
44	1.35	1.71	1.35	1.35	1.45	1.50	1.20	1.50
51	1.35	1.96	1.30	1.33	1.35	1.45	1.40	1.45
58	1.15	1.81	1.30	1.22	1.45	1.25	1.73	1.25
65	1.09	1.90	1.09	1.09	1.40	1.24	2.01	1.24
72	1.09	2.26	1.05	1.07	1.45	1.20	2.01	1.20
79	1.04	1.95	0.99	1.01	1.40	1.19	2.16	1.19
86	1.00	1.85	1.09	1.05	1.25	1.15	2.26	1.15
100	0.99	2.15	0.94	0.97	1.61	1.05	2.26	1.05
107	0.94	-	1.50	1.22	1.61	1.25	2.35	1.25
114	0.99	2.21	1.35	1.17	1.95	1.30	2.31	1.30
120	0.84	2.16	1.03	0.94	1.81	1.30	2.40	1.30

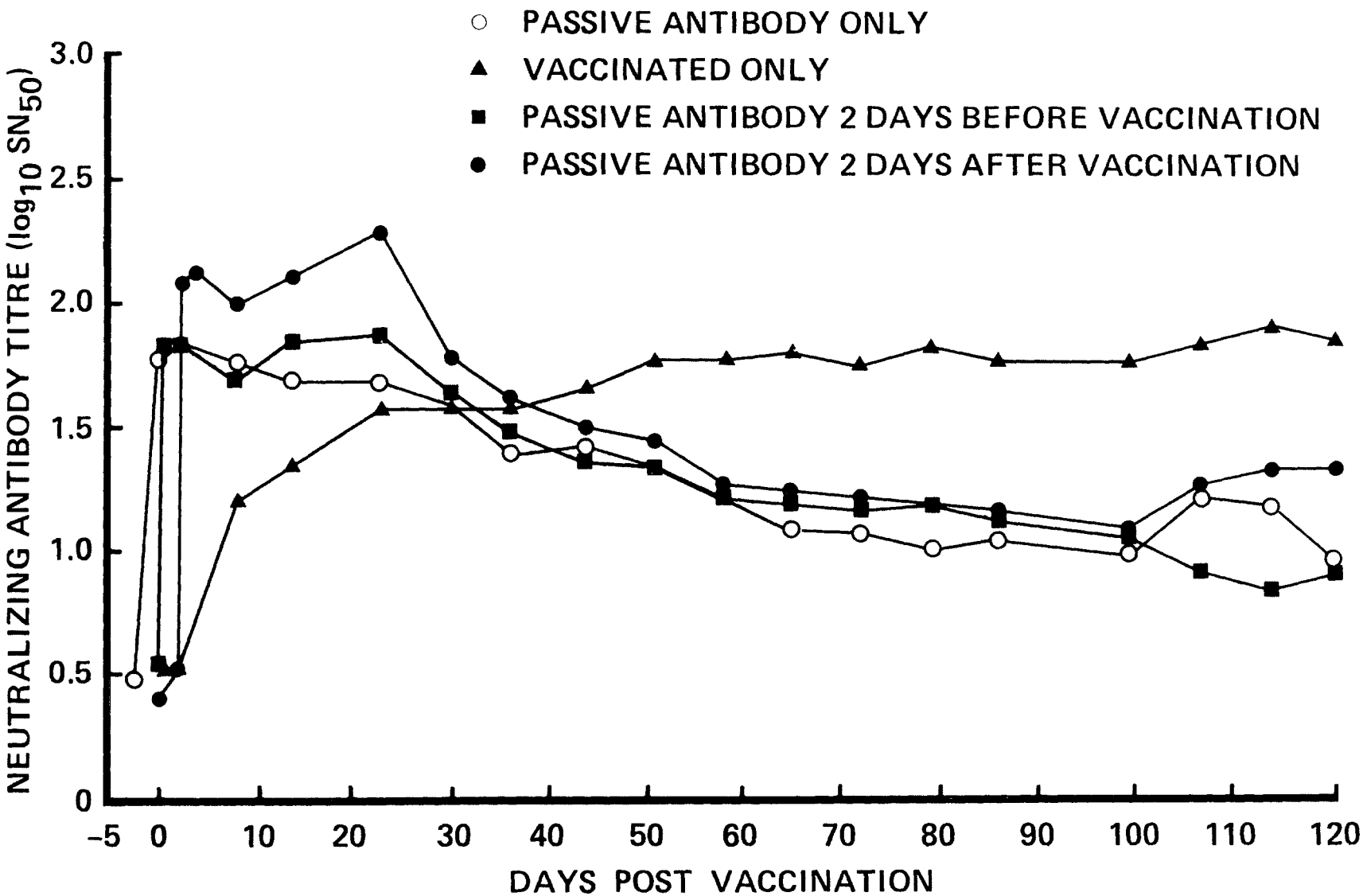
* Pigs included in calculation of group mean

** $\log_{10}SN_{50}$ mean result of 3 tests

\- No sample

DPV Days post vaccination

Figure 8.1: Effect of injected passive antibody on the vaccination response of 4 month old pigs



DISCUSSION

The results have confirmed and extended the conclusions reached in Chapter 7 by showing that antibodies injected 2 days before vaccination have a suppressive effect which is similar to that obtained with natural maternally derived antibodies. Thus passive antibodies, whether derived from the colostrum or injected intraperitoneally, can suppress the pigs response to FMD oil-emulsion vaccination. Furthermore a result was obtained suggesting that antibody may also suppress the vaccination response of piglets when administered 2 days after vaccination. In this case the small increase in the neutralizing activity over the first 30dpv may have been due to an IgM response while the response >30 days after vaccination, which is known to be mainly due to IgG (Ouldridge, Francis and Black, 1982) was suppressed. If this were true then passive antibody administered at different times after vaccination could be used to study the IgM to IgG switching mechanism in this species. However, since this observation was based on a single animal only, further data would be required before any firm conclusion could be drawn. The poor uptake by some of the animals of passive antibody may have been due to aggregation of the antibody molecules as a result of polyethylene glycol precipitation. Ammonium sulphate precipitation may be a preferable method for future studies although extensive dialysis would be required before animal inoculation in order to eliminate toxicity. Once again the injected antibody half-life in the pigs sera ($T_{\frac{1}{2}}$=34.3 to 39.6 days measured over 120 days) confirms the observations made in Chapter 6 relating the serum antibody decay rate to the time over which measurements are taken.

CHAPTER 9

GENERAL DISCUSSION

In conclusion, the results have shown that pregnant sows respond well to FMD vaccination and that initial double vaccination, with the doses given one 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 high risk areas a six month interval or revaccination at time of service would be advisable. Such a regimen would ensure that sows pass high titres of passive antibody to their litters.

The passage of antibodies from the sow to her piglets is a rapid and efficient process. The sow's colostral neutralizing antibody titre at the time of farrowing may be taken as a good indicator of the mean serum antibody titre that would be present in her three-day-old litter, regardless of antibody class involved. However, the interval between vaccination and farrowing can affect the concentration of neutralizing antibodies in the colostrum since an interval of 12-13 days resulted in colostral titres that were lower than the sow's serum titres at farrowing while an interval of 30-32 days generally resulted in colostral titres that were higher than serum titres. Therefore, sufficient time should be allowed for the transfer/concentration of antibodies from serum, into the colostrum, to take place and sows should not be vaccinated, or revaccinated, within 30 days of farrowing.

From the time piglets are three days old the class of antibody which they derive from the sow has a considerable influence on the persistence

of the antibodies in their sera because each antibody class has a different decay rate. The results obtained here demonstrated that neutralizing IgM antibodies have a more rapid decay rate than IgG antibodies, and it appears that during the first 70 days of a piglet's life the decay of neutralizing IgG antibody is mainly the result of dilution due to the expanding blood volume. Since the class of maternally derived antibodies passed onto piglets depends on that present in the sow at farrowing, it is desirable to vaccinate sows in such a way that IgG predominates in their sera at that time. This provides further support for the recommendation that sows should not be vaccinated or revaccinated within 30 days of farrowing. Regular revaccination of sows at time of service would be ideal.

In breeding stock which have been previously vaccinated on one or several occasions revaccination produces a rise in both IgG and IgM in the immediate post vaccination period. However, in primovaccinates IgM is primarily responsible for neutralizing activity in the early post vaccination period and it is often only in the third or fourth week after vaccination that IgG class antibodies contribute significantly to the neutralization titres. Hence, the design of the vaccination regimen is especially important when susceptible sows are being vaccinated for the first time, for example in a country that is introducing a pig vaccination campaign. A suitable regimen would be a double vaccination with the first dose being given at time of service and the second dose at least one month before farrowing. Furthermore while no adverse effect of FMD vaccination on pregnant sows has ever been demonstrated it seems wise to avoid vaccination during the first month of pregnancy when the foetus is most prone to damage.

Young pigs devoid of maternally derived antibodies are capable of responding to FMD vaccination at one week old, with no deleterious effects on their growth rate. In these circumstances, however, their immunity to experimental infection at six months old is not complete. Consequently, it would be preferable to delay vaccination of susceptible piglets until they are at least one month old or alternatively, in high risk areas, animals vaccinated at one week old should receive a booster dose at one to two months old to ensure adequate protection.

In piglets born to immunized sows the maternally derived antibodies have a suppressive effect on the early vaccination responses. In this study the suppression was complete in one, two and four-week-old piglets and partial in 8 week old piglets. However, it was clear that such suppression was affected by the titres of maternally derived antibody present in the piglets at time of vaccination and also by the potency of the vaccine used. The vaccine in this study for example, which contained 13.4μg of 146S viral antigen per dose, did not induce a positive neutralizing antibody response in piglets when the maternal antibody titre was greater than 1.9 $\log_{10}SN_{50}$ at time of vaccination. Thus, provided the potency of the vaccine is known, it should be possible for field personnel to estimate the time at which maternally derived antibodies subside to a level that would not interfere with vaccination and design a suitable vaccination regimen on this basis. This could be carried out simply by monitoring the antibody levels in the sow population, since these correlate well with maternally derived antibody titres in their litters, and by following the growth rate of the piglet litters, since this can be regarded as an indicator of the neutralizing IgG half-life in the serum until the piglets are at least 70 days old. In the present study, for example vaccination of the

litters born to vaccinated sows, would have been ineffective if carried out before the piglets were two months old. Thus, it would be advisable to delay vaccination of piglets, born to sows possessing a high titre of IgG antibodies, until they are two to three months old.

Regarding the possibility of future work in this field, it may be possible to overcome the suppressive effect of passive antibody by giving piglets repeat vaccinations. Such an approach has been proposed by Nathans (1965) on the basis that a first vaccination, with aqueous vaccine, primed the animals for an enhanced response to the second vaccination even though the response to the first vaccination was undetectable. It may also be possible to produce an especially potent vaccine for use in very young stock which would overcome the suppressive effect of maternal antibodies. However, both these approaches would require further investigations into the relationship between the passive antibody titre, the vaccine potency and the timing of the vaccination. Results of such a study could produce information that would enable vaccine manufacturers to recommend the most suitable vaccination regimen for vaccine batches of known potency.

Finally, the results obtained here emphasise the importance of designing vaccination programs on a rational basis since vaccines incorrectly administered may result in piglets with deficient titres of maternal antibody, piglets which suffer a break in immunity between subsidence of passive protection and active immunization, or piglets which respond poorly to vaccination as a result of the suppressive effects of maternally derived antibodies. It is hoped that this thesis will form the basis of a better understanding of how and when to administer FMD vaccines to pig stock in order to optimise their effects,

and of the immunological mechanisms involved in the humoral response of young pigs. Furthermore, fundamental information obtained here should be applicable not only to current vaccines but also to the new generation of synthetic or expressed peptide vaccines that may be produced in the future.

APPENDIX 1

MEDIA

(a) Virus Maintenance Medium (VMM)

Constituent	Volume (ml)
Eagles B.S.S. (x10 conc.)	80
Tryptose Phosphate Broth (2% w/v)	100
Sodium Bicarbonate (4.4% w/v)	100
Glucose (20% w/v)	18
Antibiotics -	
Penicillin (200,000 i.u./ml)	1.0
Neomycin (200,000 i.u./ml)	1.0
Polymyxin (200,000 i.u./ml)	1.0
Distilled water	Up to 1,000

N.B. Medium also contained 0.001% Phenol Red as a pH indicator.

(b) Microtest medium

Constituent	Volume (ml)
Eagles B.S.S. (x10 conc.)	80
Tryptose Phosphate Broth (2% w/v)	100
Glucose (20% w/v)	18
Sodium Bicarbonate (4.4% w/v)	20
Sterile Bovine Serum	20
Antibiotics -	
Penicillin (200,000 i.u./ml)	1.0
Neomycin (200,000 i.u./ml)	1.0
Polymyxin (200,000 i.u./ml)	1.0
Distilled water	Up to 1,000

N.B. Medium also contained 0.001% Phenol Red as a pH indicator

APPENDIX 2

METHODS OF ANALYSIS

a) Analysis of microneutralization test results - a worked example

The results from the example test plates shown in Figures A2.1(a) to A2.1(d) and A2.2(a) to A2.2(d) would be calculated as follows:

(i) Virus titre

$$\left(6.1 + \frac{0.5}{2}\right) - \left(\frac{75}{24} \times 0.5\right)$$

$= 6.35 - 1.56$

$= 4.79\ TCID_{50}$

(ii) Virus dose

$4.79 - (4.6-2) = 2.19\ \log_{10}$

(iii) Correction factor

Expected dose ($\log_{10}$)	Observed dose ($\log_{10}$)	Control serum titre ($\log_{10}$)
3	3.19	2.11
2	2.19	2.85
1	1.19	3.61

Slope = 7.332 Correlation = -0.9999

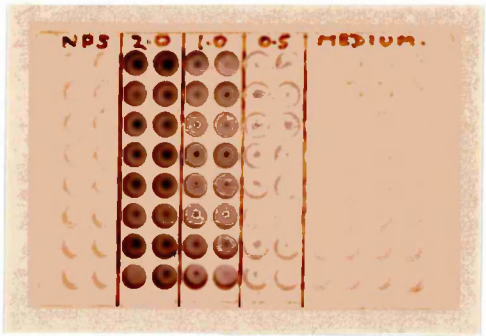
2.19 $\log_{10}$ value from slope = 2.86

2.00 $\log_{10}$ value from slope = 3.00

Correction factor = +0.14

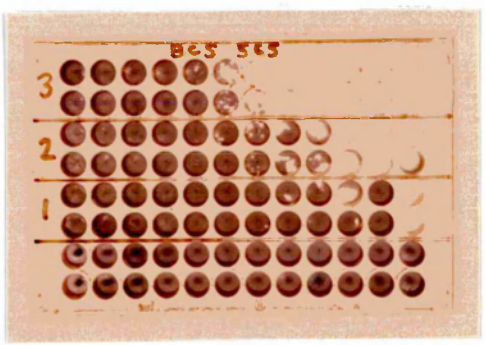
Figure A2.1 : Examples of Microneutralization Control Plates

(a) Medium, Cell and Negative Serum Controls



Normal Pig Serum Control | Cell Control | Medium Control

(b) Positive Serum Control



Virus Dose ($TCID_{50}$)

1,000 — 2.11

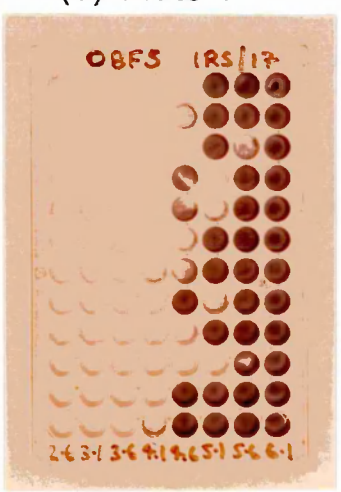
100 — 2.85

10 — 3.61

Serum Titre (Log_{10} SN_{50})

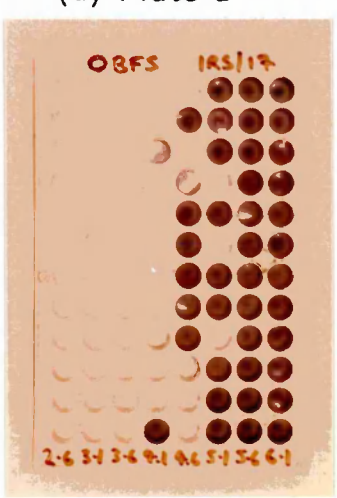
Virus Titration Plates

(c) Plate 1



35 Intact Cell Sheets

(d) Plate 2

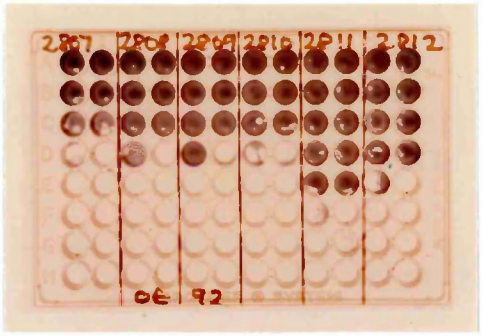


40 Intact Cell Sheets

Figure A2.2 : Examples of Microneutralization Test Plates

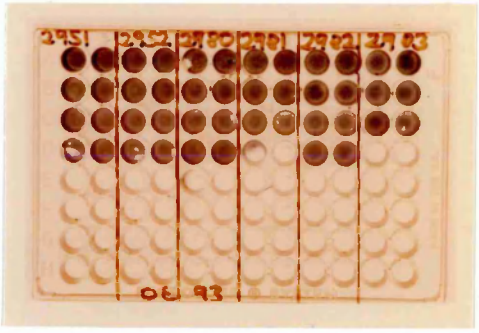
6 Samples per Plate

(a)



1.34 1.51 1.51 1.34 1.95 1.65
TITRES (log_{10})

(b)

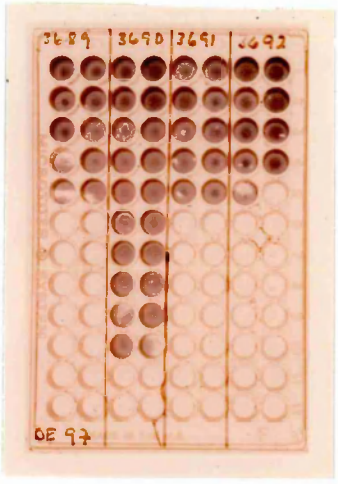


1.65 1.65 1.65 1.34 1.65 1.34
TITRES (log_{10})

Sample Dilution

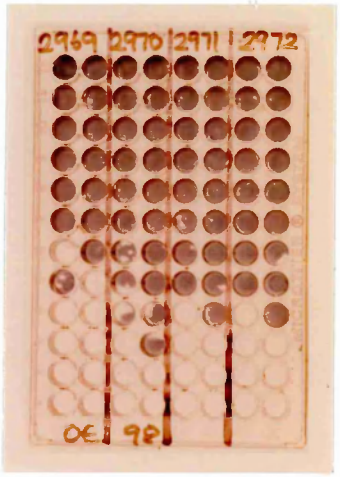
4 Samples per Plate

(c)



1.65 3.31 1.95 1.65
TITRES (log_{10})

(d)



2.56 3.16 3.01 3.01
TITRES (log_{10})

Sample Dilution

iv) Test sample results

Fig.	Tag No.	Sample no.	Observed titre (log_{10})	Corrected titre (log_{10})
2a	OE92	2807	1.34	1.48
		2808	1.51	1.65
		2809	1.51	1.65
		2810	1.34	1.48
		2811	1.95	2.09
		2812	1.65	1.79
2b	OE93	2951	1.65	1.79
		2952	1.65	1.79
		2980	1.65	1.79
		2981	1.34	1.48
		2982	1.65	1.79
		2983	1.34	1.48
2c	OE97	3689	1.65	1.79
		3690	3.31	3.45
		3691	1.95	2.09
		3692	1.65	1.79
2d	OE98	2969	2.56	2.70
		2970	3.16	3.30
		2971	3.01	3.15
		2972	3.01	3.15

b) Methods for analysing ELISA data

(i) The absorbance method - This method is most suitable for testing large numbers of samples and was used in the antibody class analysis of sows' and piglets' serum, as well as of sows' colostrum/milk. Test samples were assayed for pig anti-FMD IgG, IgM or IgA at a single dilution (1 in 50) and the OD value at 492nm for each sample was recorded. Mean OD values from two tests were calculated and corrected by substracting negative control values which were either -

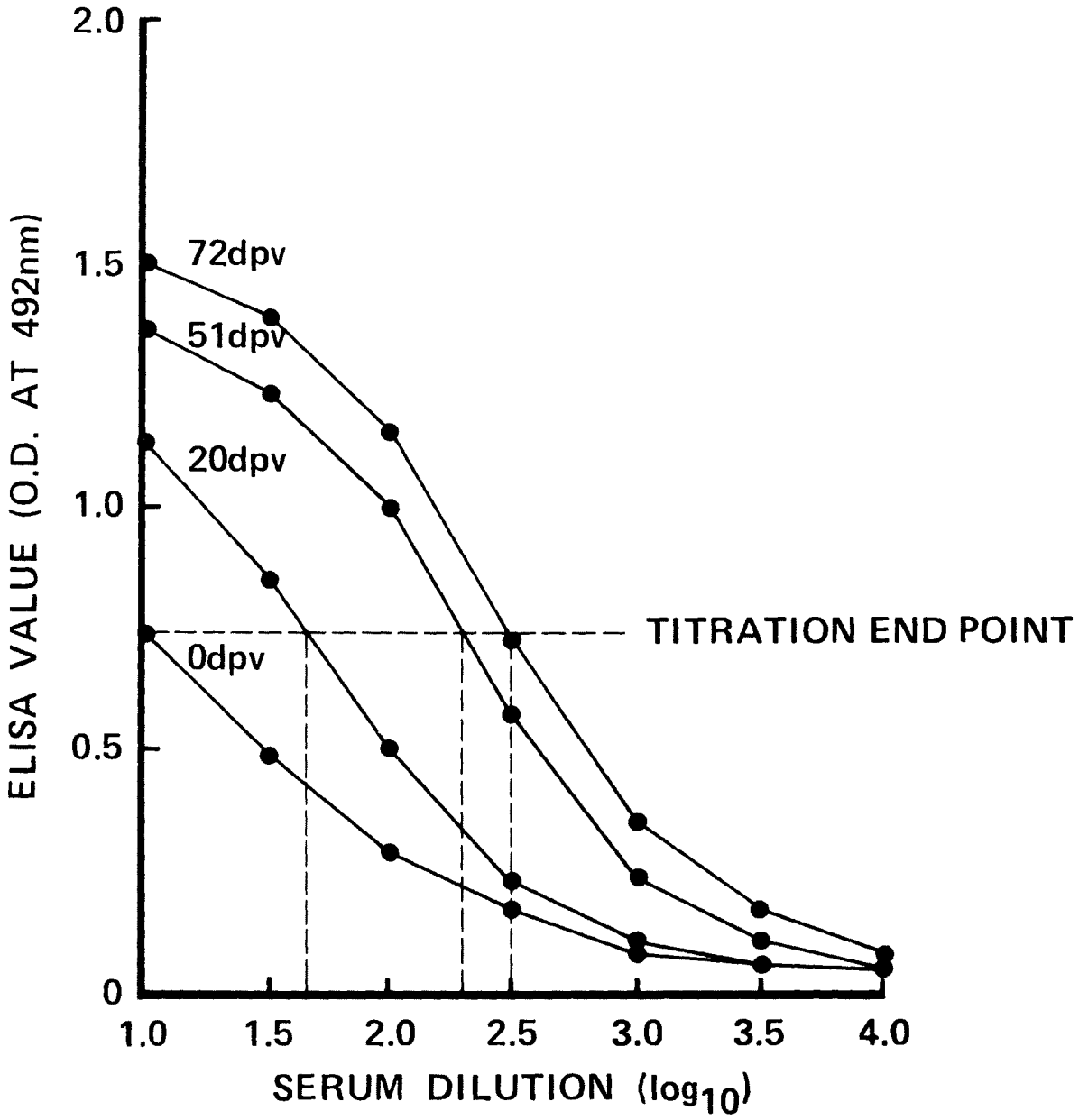
a) Pre-vaccination serum from adult sows diluted 1:50.

b) Colostrum or milk from non-vaccinated sows diluted 1:50.

c) Pre-vaccination serum from piglets born to non-vaccinated sows diluted 1:50.

(ii) The titration method - The antiserum under test was serially diluted in 0.5 $\log_{10}$ steps from 1.0 to 4.0 $\log_{10}$. Mean values from duplicate tests were calculated and plotted graphically as OD at 492nm against the $\log_{10}$ reciprocal antiserum dilution. A line was then drawn using the OD value given by a negative pre-vaccination serum sample, taken from the same animal, at a 1.0 $\log_{10}$ dilution and the dilution at which that line intersected the graphs of post vaccination samples was taken as the $\log_{10}$ antibody titre. An example is shown in Figure A2.3 for sera collected from a sow at 0, 20, 51 and 72 days post vaccination (dpv).

Figure A 2.3: The titration method for ELISA analysis of pig sera on various days post vaccination (dpv)



(iii) The logistic saturation model - The logistic model used a computer programme (Hingley and Ouldridge, 1985) to fit a sigmoid shaped curve to OD values obtained in the ELISA using a fixed serum dilution (1.0 $\log_{10}$) and variable antigen concentrations (0-8µg/ml 146S). The parameterisation of the model used was -

$$OD_i = OD_{max} \times \frac{(\bar{K} X_i)^{\underline{a}}}{1 + (\bar{K} X_i)^{\underline{a}}} + \varepsilon_i$$

where

OD_i = Optical density corrected for no antigen (blank) for the ith observation.
OD_{max} = Plateau value of optical density.
$\bar{K}$ = Average intrinsic association constant.
$\underline{a}$ = Heterogeneity index.
X_i = Concentration of antigen for ith observation.
ε_i = Normally distributed error (mean 0, variance σ^2) to ith observation.

Values obtained were used to study the functional nature of the antibody population being measured. $\bar{K}$ and $\underline{a}$ values were corrected by subtracting the value obtained with -ve serum (Odpv) from values obtained with +ve sera (>Odpv) from the same animal.

c) Calculation of sedimentation coefficients for purified pig immunoglobulins

IgG (see Figure A2.4(a))

Centrifuge Temp. (°C)	=	20
Centrifuge speed (RPM)	=	40,000
R1-R2 (mm)	=	228
Balance cell distance (mm)	=	16
Scan interval (mins)	=	10
No. of scans	=	9
R1-P (mm)	=	70, 76, 82, 88, 94, 100, 105, 112, 120.

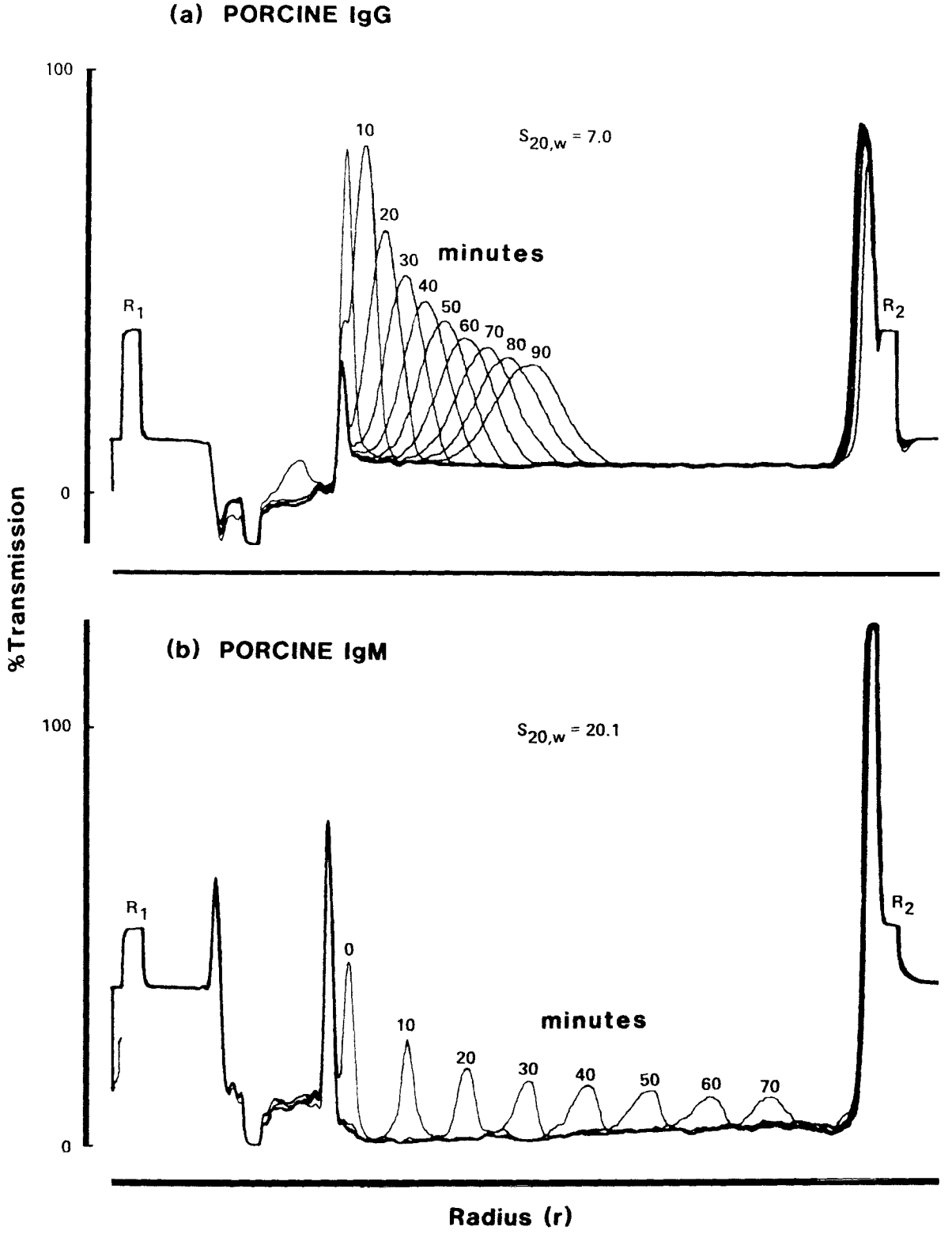
S value = 6.412 $S_{20}W$ = 6.412 x 1.0936 = 7.012

IgM (see Figure A2.4(b))

Centrifuge Temp. (°C)	=	20
Centrifuge speed (RPM)	=	40,000
R1-R2 (mm)	=	228
Balance cell distance (mm)	=	16
Scan interval (mins)	=	10
No. of scans	=	7
R1-P (mm)	=	82, 100, 118.5, 137, 155, 173.5, 192.5

S value = 18.384 $S_{20}W$ = 18.384 x 1.0936 = 20.105

Figure A2.4: Schlieren traces of porcine immunoglobuli sedimentation



APPENDIX 3

ELISA OPTICAL DENSITY VALUES

Table A3.1: Immunoglobulin class activity in singly vaccinated sows

NUO2				NUO5			
DPV	Conjugate specificity			DPV	Conjugate specificity		
	IgM	IgA	IgG		IgM	IgA	IgG
► 0	0*	0	0	► 0	0	0	0
7	0.13	0.04	0	7	0.17	0.07	0
[12]	0.17	0.14	0.03	[13]	0.12	0.07	0.21
13	0.21	0.10	0.12	14	0.07	0.16	0.38
15	0.22	0.12	0.19	16	0.07	0.48	0.57
18	0.23	0.10	0.18	18	0.04	0.46	0.60
25	0.16	0.08	0.28	25	0.03	0.44	0.59
32	0.16	0.07	0.45	32	0.05	0.33	0.58
39	0.10	0.09	0.46	39	0.05	0.35	0.52
46	0.11	0.07	0.49	46	0.04	0.30	0.43
53	0.10	0.04	0.49	53	0.02	0.34	0.38
60	0.06	0.08	0.43	60	0	0.41	0.36
67	0	0.02	0.31	67	0	0.46	0.37
74	0.01	0.04	0.31	74	0	0.33	0.25
80	0.01	0.07	0.37	80	0	0.40	0.33

NUO1				NUO4			
DPV	Conjugate specificity			DPV	Conjugate specificity		
	IgM	IgA	IgG		IgM	IgA	IgG
► 0	0	0	0	► 0	0	0	0
6	0.14	0	0.03	6	0.10	0.03	0
13	0.13	0.05	0.14	13	0.17	0.05	0.02
20	0.13	0.09	0.53	20	0.16	0.08	0.27
27	0.14	0.18	0.75	27	0.16	0.11	0.39
[32]	0.06	0.10	0.70	[30]	0.13	0.12	0.29
33	0.04	0	0.67	31	0.13	0.13	0.34
35	0	0.01	0.75	33	0.10	0.13	0.42
38	0	0.01	0.86	37	0.09	0.13	0.37
45	0	0	0.92	44	0.06	0.07	0.16
52	0	0.06	1.05	51	0.05	0.03	0.10
59	0	0.20	1.13	58	0.02	0.07	0.07
66	0	0.27	1.08	65	0	0.11	0.07
73	0	0.31	1.05	72	0.01	0.11	0.07
80	0	0.18	1.11	79	0.01	0.14	0.13

► = Vaccination [ ] = Farrow *Mean OD at 492nm DPV = Days post vaccination

Table A3.2: Immunoglobulin class activity in sows revaccinated 20 days post initial vaccination

NU00				NU03			
DPPV	Conjugate specificity			DPPV	Conjugate specificity		
	IgM	IgA	IgG		IgM	IgA	IgG
► 0	0*	0	0	► 0	0	0	0
8	0.09	0.03	0.12	8	0.07	0	0.01
14	0.08	0.01	0.28	14	0.25	0.03	0.05
►20	0.05	0.01	0.41	►20	0.23	0.20	0.50
27	0.14	0.13	1.01	27	0.40	0.40	1.25
34	0.15	0.10	1.04	34	0.31	0.34	1.39
41	0.11	0.13	1.06	41	0.22	0.40	1.25
48	0.04	0.04	0.89	48	0.20	0.39	1.07
51	0.01	0.10	0.92	52	0.07	0.39	0.99
52	0.01	0.06	0.93	53	0.07	0.30	0.92
54	0.03	0.10	0.85	55	0.03	0.45	0.99
58	0	0.07	0.92	59	0	0.44	1.07
65	0	0.03	0.98	66	0	0.48	1.08
72	0	0.07	1.07	73	0	0.59	1.29
79	0	0.05	1.04	80	0	0.52	1.39

► = Vaccination

□ = Farrow

*Mean OD at 492nm

DPPV = Days post primary vaccination

Table A3.3: Immunoglobulin class activity in sows revaccinated 57 days post initial vaccination

DPPV	OE91			OE92			OE93			OE94		
	Conjugate specificity			Conjugate specificity			Conjugate specificity			Conjugate specificity		
	IgM	IgA	IgG	IgM	IgA	IgG	IgM	IgA	IgG	IgM	IgA	IgG
► 0	0*	0	0	0	0	0	0	0	0	0	0	0
3	0	0.14	0.09	0	0.02	0.04	0	0.01	0.05	0.01	0.07	0.04
10	0.16	0.19	0.20	0.14	0.09	0.06	0.11	0.03	0.10	0.14	0.09	0.14
17	0.14	0.09	0.25	0.18	0.22	0.41	0.15	0.06	0.19	0.18	0.10	0.58
24	0.09	0.02	0.38	0.16	0.11	0.78	0.14	0.03	0.22	0.17	0.11	0.92
31	0.02	0.01	0.39	0.16	0.09	0.78	0.14	0.02	0.37	0.15	0.12	0.97
38	0.04	0.03	0.51	0.16	0.07	0.72	0.13	0.04	0.36	0.07	0.09	0.89
45	0.01	0	0.46	0.13	0.08	0.62	0.05	0.01	0.34	0.05	0.07	0.79
► 57	0.05	0.04	0.53	0.09	0.04	0.54	0	0.03	0.34	0.01	0.08	0.77
60	0	0	0.56	0.18	0.07	0.61	0.05	0.03	0.37	0.10	0.07	0.81
66	0.10	0.13	1.28	0.13	0.25	1.18	0.11	0.19	1.00	0.15	0.49	1.51
77	0.04	0.08	1.18	0.08	0.16	1.12	0.05	0.12	0.94	0.05	0.37	1.48
87-89	0.03	0.08	0.95	0.03	0.08	0.79	0.03	0.06	0.63	0	0.12	1.23
94-96	0.03	0	0.94	0.05	0.08	0.81	0	0.05	0.60	0	0.26	1.22

► = Vaccination ☐ = Farrow *Mean OD at 492nm DPPV = Days post primary vaccination

Table A3.4: Immunoglobulin class involved in the vaccination response of piglets born to unvaccinated sows

Age at vaccination (weeks)	Antibody class	Days post vaccination					
		0	7	14	21	28	35
1	IgM	0	0.47*	0.45	0.38	0.29	0.25
	IgA	0	0.09	0.10	0.11	0.09	0.10
	IgG	0	0.01	0.37	0.98	1.18	1.22
2	IgM	0	0.74	0.61	0.19	0.14	0.16
	IgA	0	0.21	0.18	0.08	0.10	0.13
	IgG	0	0.05	0.54	1.09	1.27	1.26
4	IgM	0	0.90	0.79	0.34	0.21	0.19
	IgA	0	0.50	0.33	0.13	0.11	0.15
	IgG	0	0.12	0.80	1.28	1.34	1.34
8	IgM	0	0.68	0.58	0.37	0.06	0.04
	IgA	0	0.39	0.28	0.11	0.03	0.03
	IgG	0	0.11	0.52	1.01	1.13	1.19

*Corrected ELISA OD values at 492nm for samples diluted 1:50

Table A3.5: Immunoglobulin class involved in the vaccination response of piglets born to FMD vaccinated sows

Age at vaccination (weeks)	Antibody class	Days post vaccination					
		0	7	14	21	28	35
1	IgM	0.05*	0.03	0.05	0.12	0.17	0.23
	IgA	0.04	0.01	0.02	0.05	0.08	0.05
	IgG	1.15	0.93	0.85	0.70	0.69	0.57
2	IgM	0.03	0.03	0.12	0.12	0.05	0.04
	IgA	0.01	0.04	0.04	0.08	0.05	0.05
	IgG	0.99	0.84	0.76	0.77	0.73	0.70
4	IgM	0	0.02	0.29	0.22	0.14	0.18
	IgA	0	0.03	0.09	0.08	0.09	0.11
	IgG	0.77	0.65	0.62	0.65	0.72	0.68
8	IgM	0	0.14	0.42	0.43	0.31	0.20
	IgA	0	0.08	0.17	0.16	0.13	0.10
	IgG	0.49	0.41	0.51	0.63	0.69	0.71

*Corrected ELISA OD value at 492nm for a sample diluted 1:50

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J. Hyg., Camb. (1984), **93**, 123–131
Printed in Great Britain

The effect of vaccination regimen on the transfer of foot and mouth disease antibodies from the sow to her piglets

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(*Received* 10 *October* 1983; *accepted* 22 *February* 1984)

SUMMARY

Four groups of pregnant sows were inoculated with type O_1 foot and mouth disease (FMD) oil emulsion vaccine at various times before farrowing and samples of the sow's serum, colostrum and milk, and piglet's serum, collected during the first week after farrowing, were analysed for FMD virus neutralizing activity.

No FMD neutralizing antibodies were detectable in the piglets serum at birth but they were present 1·5 h after suckling and peak titres were reached 1–3 days later. There was no significant difference between the antibody titres of colostrum samples collected from different teats at farrowing. However, similar samples collected 3 days later showed significant ($P < 0{\cdot}005$) fore to hind variation. The principal FMD virus neutralizing antibody class present in the sow's serum at farrowing and in their 3-day-old piglets was governed by the inoculation schedule employed. When the last vaccinations were given $\simeq$ 30 days before farrowing (dbf) the predominant FMD virus neutralizing class was IgG. However, when the sows were vaccinated only $\simeq$ 12 dbf the predominant class was IgM. A significant correlation was observed between the sow's serum titres and colostrum titres at farrowing ($r = 0{\cdot}90$), and also between sows colostrum titres at farrowing and their 3-day-old piglets serum titres ($r = 0{\cdot}99$).

INTRODUCTION

Pigs develop within an impermeable epitheliochorial placenta and there is little or no placental transmission of immunoglobulins from the mother to the pig foetus (Brambell, 1958). Consequently, piglets are largely devoid of immunoglobulins at birth (Kim, Bradley & Watson, 1966; Porter, 1969; Dujin, 1971; Bourne *et al.*, 1974) and colostral antibodies are of major importance for their survival.

When designing a vaccination regimen for breeding sows it is important to understand the factors affecting the transfer of immunity to the young but to date this subject has received little attention as far as foot and mouth disease (FMD) oil emulsion vaccination is concerned. The fact that most or all absorption of colostral antibodies through the intestinal wall of the newborn piglet occurs during the first 24–48 h after birth (Lecce & Morgan, 1962; Bourne, 1971; Dujin, 1971; Yabiki, Kashiwazaki & Namioka, 1974) led us to consider whether the vaccination regimen of the sow, and hence the proportion of the various antibody classes in

the sows serum during the critical few days around farrowing, might influence the passive transfer of maternally derived antibodies to the piglets. The predominant antibody class in the piglets is of special importance since IgG (half life of 6·5–22·5 days), IgM (half life of 1·3–7·8 days) and IgA (half life of 2–3·5 days) vary considerably in their decay rates (Porter & Hill, 1970; Curtis & Bourne, 1971; 1973; Francis & Black, 1984). Hence inappropriate vaccination schedules administered to breeding sows may result in piglets which are not only deficient in their initial antibody levels but also become susceptible to FMD at an earlier stage due to the rapid decay rate of the passive antibodies.

The purpose of the present study was to provide detailed information about the influence of the sow's FMD vaccination schedule on the class and titre of neutralizing antibodies in her serum and colostrum at farrowing, and on the efficiency of transfer of these antibodies to her progeny.

MATERIALS AND METHODS

Vaccination schedule

Ten pregnant large white sows, never previously vaccinated against or in contact with FMD, were divided into three groups of two and one group of four animals and inoculated intramuscularly with a 2 ml dose of O_1BFS 1860/67 FMD single oil emulsion vaccine containing 5·23 μg 140S antigen according to the following schedules: group A, sows 1 and 2, vaccinated once at 12–13 days before farrowing (dbf); group B, sows 3 and 4, vaccinated once at 30–32 dbf; group C, sows 5 and 6, vaccinated twice at 51–52 and 31–32 dbf and group D, sows 7, 8, 9 and 10, vaccinated twice at 87–89 and 30–32 dbf.

Sampling

Blood was taken from sows 1 to 6 at the time of farrowing and at 1, 3 and 5–7 days later. Their piglets were bled just before suckling (Groups B and C), or 1·5–2·5 h after (Group A) and again 1, 3 and 5–7 days later. Blood samples were also collected from sows 7 to 10 at the time of farrowing and from their piglets at 3 days after farrowing. The blood was kept at room temperature for 24 h and the serum was separated by centrifugation. The serum was then stored at −20 °C and inactivated at 56 °C for 30 min prior to testing.

Colostrum/milk was collected from sows 1 to 6 at the time of farrowing and at 1, 3 and 5–7 days later. Samples taken on each occasion from two fore, (one left and one right), two central and two hind teats were pooled. Colostrum/milk was also collected from sows 7 to 10 at the time of farrowing and at 3 and 7 days later but here the teat samples were analysed separately. Each sample was centrifuged at $50000 \times \boldsymbol{g}$ for 30 min at 4 °C and the whey separated, stored at −20 °C and inactivated at 56 °C for 30 min prior to testing.

Neutralizing antibody assessment

The FMD virus neutralizing activity of the serum and whey was demonstrated using a micro neutralization test described previously by 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.

Analysis of anti-FMD virus antibody class activity

Neutralizing activity. Samples of serum from each sow at the time of farrowing, and pooled samples from each litter at 3 days old, were fractionated by gel filtration. A 2·5 ml sample was applied to a 26 mm × 950 mm glass column (LKB Instruments Ltd) containing Bio-Gel A.5M gel filtration medium (Bio-Rad Laboratories) and was eluted with 0·1 M tris buffer (pH 7·2) containing 0·3 M-NaCl. A total of 180 × 2·7 ml fractions was collected and analysed for optical density at 280 nm, neutralizing activity and antibody class (using a combination of ELISA, immunoelectrophoresis and sedimentation coefficient analysis). The reciprocals of the 50% neutralization dilution endpoints for IgM, IgA and IgG rich column fractions were added together in order to estimate the level of neutralizing activity attributable to each antibody class.

Enzyme linked immunosorbent assay (ELISA). Serum samples collected from two sows (number 1 which had been vaccinated once 12 dbf and number 5 which had been vaccinated twice 52 and 32 dbf) at farrowing, and from their litters 3 days later, were selected for anti-FMD virus immunoglobulin class analysis using an indirect ELISA method (Francis, Ouldridge & Black, 1983). Briefly, microplates were coated overnight at room temperature with FMD virus 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) 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 (O.D.) at 492 nm was measured in an LKB Multiskan.

RESULTS

Fig. 1 shows the mean group FMD virus neutralizing activity of the serum and colostrum of sows within groups A, B and C, and of their litters between 0 and 168 h after farrowing. At farrowing the mean serum titre of the sows vaccinated once at 12–13 dbf (group A) was 1·67 $\log_{10}$ while that of the animals vaccinated 30–32 dbf (group B) was 1·78 $\log_{10}$. The sows vaccinated twice (group C) had mean titres of 2·13 $\log_{10}$ at farrowing. The serum titres were closely correlated with the colostral titres ($r = 0{\cdot}897$) as illustrated in Fig. 2*a*. The interrelationship between the serum and colostrum titres was reflected by the slope of this regression line ($M = 1{\cdot}568$) which was in turn influenced by colostral titres which were lower than the serum titres in group A but higher than them in both groups B and C.

The serum titres of sows in groups B and C all vaccinated at least a month before farrowing declined during the first 24 h after farrowing and then remained fairly constant for the next 5–6 days while the serum titres of group A sows (vaccinated 12 dbf) increased during the first 21–30 h after farrowing before showing signs of declining. This result was also reflected in the colostral titres which increased during the first 30 h after farrowing in group A but declined steadily in groups B and C. The FMD virus neutralizing antibody activity in the colostrum/milk taken from the various teats of sows 7–10 during the first week after farrowing is shown in Table 1. There was no significant variation between samples collected from sows at the time of farrowing. The maximum deviation between titres from different

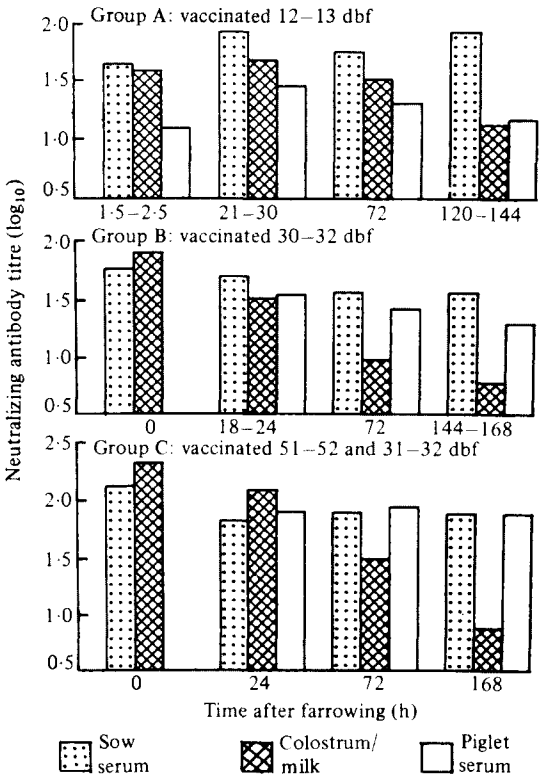

Fig. 1. The mean FMD neutralizing antibody titres of sows serum, colostrum/milk and piglets serum during the first week after farrowing.

teats occurred 3 days after farrowing and at this time there was a significant ($P > 0{\cdot}005$) fore to hind variation as measured by a two way analysis of variance. The colostral titres at farrowing were also correlated ($r = 0{\cdot}989$) with the group mean 3-day-old piglet serum titres as illustrated in Fig. 2*b* and in this case a direct relationship was suggested by the slope of the regression ($M = 0{\cdot}980$).

Sera collected from group B and C piglets prior to suckling had no detectable FMD neutralizing activity whereas the mean titre of serum samples collected from group A piglets 1·5–2·5 h after farrowing was 1·10 log_{10}. Furthermore, individual piglets in these litters had titres as high as 1·36 and 1·72 log_{10}. Peak antibody titres were observed in piglet groups A and B at 18–30 hours after farrowing and in group C at 72 h after farrowing.

Table 2 shows that the class of antibodies present in the serum of the 3-day-old piglets was related to that predominating in the serum of their dams at time of farrowing and this in turn was dictated by the vaccination regimen employed. In sow number 1 which was vaccinated only 12 days previously the neutralizing activity in the serum at farrowing was confined to the IgM class and so too was the antibody transferred to the serum of the piglets when 3 days old. On the other hand sow number 5 which had been vaccinated twice at 52 and 32 dbf the neutralising activity in her serum was confined to the IgG class. Here there was no FMD neutralizing IgM antibody in the serum of the 3-day-old piglets and the

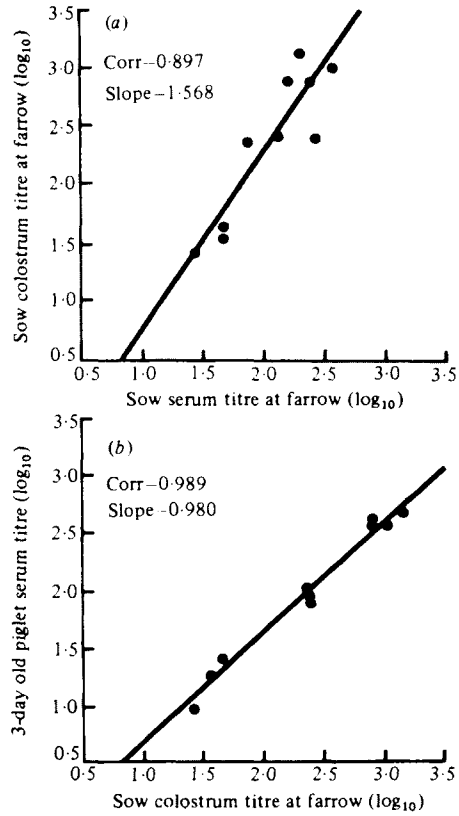

Fig. 2. Regression analysis of relationship between sows serum, colostrum and 3-day-old piglet serum titres.

Table 1. *FMD neutralizing activity in colostrum/milk collected from six different teats of sows for 1 week after farrowing*

Sow tag no.	Days after farrowing	Sample teat: Left fore	Left central	Left hind	Right fore	Right central	Right hind	Mean titre (Log_{10})	S.D.
7	0	2·90	2·99	3·14	2·94	2·94	3·19	3·02	0·12
	3	1·89	1·74	1·89	1·65	1·79	2·45	1·90	0·28
	7	1·43	1·54	1·59	1·49	1·54	1·74	1·56	0·11
8	0	2·83	2·98	3·03	2·88	2·73	2·93	2·90	0·11
	3	1·72	2·13	2·28	1·68	1·72	2·48	2·00	0·34
	7	1·58	1·12	1·68	0·95	0·99	1·37	1·28	0·31
9	0	2·55	2·80	3·00	2·90	3·10	3·05	2·90	0·20
	3	1·20	1·19	2·10	1·25	2·15	2·90	1·80	0·70
	7	0·66	0·62	0·69	0·80	1·55	1·13	0·91	0·36
10	0	2·96	3·36	3·06	3·11	3·11	3·31	3·15	0·15
	3	1·86	2·50	2·86	1·80	1·90	2·36	2·21	0·43
	7	1·45	2·26	1·90	1·45	1·45	2·06	1·76	0·36

Table 2. *Effect of vaccination regimen on anti-FMD immunoglobulin class activity in sow and piglet serum*

(*a*) Sow serum at farrow

Test	Sow 1: Group A (vaccinated 12 dbf)			Sow 5: Group C (vaccinated 52 and 32 dbf)		
	IgM	IgA	IgG	IgM	IgA	IgG
ELISA (O.D.)	0·17	0·14	0·03	0·01	0·10	0·92
Neutralizing antibody titre (Log_{10})	2·18	< 0·75	< 0·75	< 0·90	< 0·90	2·62

(*b*) Piglet serum 3 days old

Test	Litter 1: Group A			Litter 5: Group C		
	IgM	IgA	IgG	IgM	IgA	IgG
ELISA (O.D.)	0·38	0·34	0·19	0·15	0·20	0·90
Neutralizing antibody titre (Log_{10})	1·75	< 0·50	< 0·50	< 0·54	1·96	2·77

main neutralizing activity was due to IgG antibodies. However, in this case some neutralizing IgA activity was also evident in the piglets serum. The principal immunoglobulin class responsible for the anti-FMD activity detected in the indirect ELISA was the same as that responsible for neutralization. However, some activity was also detectable in the other classes possibly due to the increased sensitivity of the test system.

DISCUSSION

The vaccination regimen employed in this study, for the breeding sows, appeared to have the desired effect of providing sows with different levels of anti-FMD antibody class activity at farrowing. Vaccination $\simeq$ 12 dbf produced serum FMD neutralizing antibodies which were mainly, or exclusively, of the IgM class at farrowing while vaccination a month or more before farrowing resulted in predominantly IgG class neutralizing antibodies in the serum of the farrowing sows. This confirms previous observations relating to the development of various neutralizing antibody classes in pigs after FMD oil emulsion vaccination (McKercher & Giordano, 1967; Anderson, Masters & Mowat, 1971; Ouldridge, Francis & Black, 1982). Furthermore, the 3-day-old piglets from sows vaccinated 12 dbf had only neutralizing IgM antibodies in their serum while those from sows vaccinated a month or more before farrowing had predominantly IgG. Some neutralizing IgA was also observed in 3-day-old piglets however, since none was detectable in the sows serum at farrowing, its source is not clear.

The results of the IgG and IgM analysis demonstrated that in general the class of FMD virus neutralizing antibody transferred to the piglets was dependent on the antibody class predominating in the sows serum at time of farrowing and stresses the importance of adopting a rational approach to vaccination since FMD virus neutralizing antibodies of different classes have markedly different decay rates in piglets. In litters from sows vaccinated only 12–13 dbf, which receive

predominantly anti-FMD IgM, the half-life of FMD virus neutralizing activity is 4–8 days while in litters from sows vaccinated or revaccinated 30–32 dbf, which receive predominantly anti-FMD IgG, the half-life of FMD virus neutralizing activity is 7–21 days (Francis & Black, 1984).

No FMD virus neutralizing antibodies were detectable in any piglet sampled at birth and prior to suckling which agrees with previous findings relating to the impermeability of the sow's epitheliochorial placenta (Brambell, 1958; Kim, Bradley & Watson, 1966; Porter, 1969; Dujin, 1971; Bourne *et al.* 1974). Nevertheless, group 1 piglets attained antibody levels as high as 1·36 $\log_{10}$ within 1·5 h and 1·72 $\log_{10}$ within 2·5 h of birth demonstrating the piglets remarkable capacity to absorb protective antibodies from their mothers colostrum. The peak of passive antibody activity was observed in the 18–30 h samples collected from litters derived from singly vaccinated sows which supports published findings that antibody absorption ceases within 24–48 h of suckling (Leece & Morgan 1962; Bourne, 1971; Dunin, 1971; Yabiki, Kashiwazaki & Namioka, 1974). However, the maximal level of passive antibody in piglets from revaccinated sows occurred 72 h after farrowing. It is therefore possible that the vaccination regimen of the sow, and therefore the class and titre of FMD virus neutralizing antibodies in the colostrum at farrowing, may have influenced the rate at which the antibodies were absorbed by the piglets.

There was no significant teat to teat variation in colostral antibody titres at the time of farrowing contrary to published data on the subject (Perry & Watson, 1967). Therefore, any variations observed between the serum titres of individuals within a litter is not due to variation in the colostrum titres from different teats of the sow. It seems likely that the greater degree of variation which occurred 3 days later was due to selective suckling by the young piglets. If this hypothesis is correct then the piglets displayed a preference for suckling from the fore teats on the sow as demonstrated by the significantly lower titres in samples collected from these teats 3 days after suckling.

Despite the differences in neutralizing antibody class and rate of absorption which have been discussed it was possible to establish a relationship between FMD virus neutralizing antibody levels in the sow and her young. There was a significant correlation ($r = 0{\cdot}99$) between the sow's colostral titres at farrowing and 3-day-old piglet serum titres and since the regression slope was very close to 1·00 ($m = 0{\cdot}98$) a direct relationship between the two is indicated. This observation would imply that the piglet gut absorbs FMD virus neutralizing antibodies of different immunoglobulin classes non-selectively which would support published data on the subject (Bourne, 1971; Brown, 1976). There was also a good correlation ($r = 0{\cdot}90$) between sows serum and colostral titres at farrowing. However, the slope of the regression between the two ($m = 1{\cdot}57$) suggest that the mechanism of concentrating antibodies from the serum into the colostrum is either selective for immunoglobulin class or influenced in some other way by the sow's vaccination regimen.

It is important for herd immunity that young pigs are protected against FMD from as soon as possible after birth until they are actively immunized by vaccination, generally at 1–2 months old. This study has demonstrated the role of maternally derived antibodies in providing the young pigs with such protection and has emphasised the importance of maintaining high titres of FMD virus

neutralizing antibodies in breeding sows, particularly around the time of farrowing. Therefore, it would be advisable to revaccinate sows during pregnancy. However, since it has been shown that the interval between vaccination and farrowing affects the concentration of FMD virus neutralizing antibodies in the colostrum, this should not be carried out within 1 month of farrowing. Furthermore, a 1 month time interval would reduce the proportion of FMD neutralizing IgM antibodies, which are also provoked by revaccination (Ouldridge, Francis & Black, 1982) and have a rapid decay rate (Francis & Black, 1984), passed onto the young. In a situation where fully susceptible breeding sows are being vaccinated against FMD, for example in a country introducing a pig vaccination campaign for the first time, regimens that produce anti-FMD IgM at farrowing should be avoided. Therefore, susceptible pregnant sows should receive a double vaccination with the booster dose being given 1 month before farrowing.

We are grateful to Professor F. J. Bourne and Dr T. J. Newby of the Department of Animal Husbandry, Bristol University for supplying the immunoglobulin specific conjugates used in the ELISA.

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Research in Veterinary Science 1984, 37, 72-76

Effect of the sow vaccination regimen on the decay rate of maternally derived foot-and-mouth disease antibodies in piglets

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Three pairs of sows were vaccinated against foot-and-mouth disease (FMD) at various intervals before farrowing and samples of blood were collected from their piglets periodically for 70 days after birth. When the sows were vaccinated 12 to 13 days before farrowing the predominating FMD neutralising antibody at time of parturition was IgM and the observed half lives of the maternally derived antibodies in the piglets were short (four to eight days). However, when sows were last vaccinated 30 to 32 days before farrowing, the maternally derived FMD neutralising antibodies in the piglets were predominantly IgG and the observed half lives were seven to 21 days. These observed half lives for IgG were shown to be closely related to the period over which the maternally derived antibodies could be demonstrated and to the rate of increase of the piglet's blood volume over the same period. If corrections were made for increase in blood volume the decay rate of IgM antibodies in piglets was seven to 18 days while the decay rate for IgG was greater than 408 days. This result suggested that there was little or no IgG catabolism or excretion during the first 70 days of the piglet's life.

SINCE piglets develop within an epitheliochorial placenta (Brambell 1958) they are largely devoid of immunoglobulins at birth (Kim et al 1966, Porter 1969, Dujin 1971, Bourne et al 1974) and, consequently, colostral antibodies make an important contribution to protection from disease during their first weeks of life (Renshaw 1975, Brandenbury and Wilson 1979, Wittmann and Jakubik 1979, Bridger and Brown 1981).

To date, relatively little attention has been directed towards the foot-and-mouth disease (FMD) vaccination regimen of the sow and its effect on the class of antibody in the serum and colostrum at the time of farrowing. Since the antibody class is likely to influence the persistence of maternally derived antibodies in piglets (Porter and Hill 1970, Curtis and Bourne 1971) this experiment has been designed to determine the effect of various sow vaccination regimens on the decay rate of the FMD neutralising antibodies passed to their piglets and to investigate the influence of piglet growth rates on these results. The information so obtained should facilitate predictions about the duration of colostral protection in piglets born to FMD vaccinated sows and enable one to design optimal vaccination regimens for breeding stock on this basis.

Materials and methods

Vaccination schedule

Six pregnant Large White sows were divided into three groups of two and vaccinated intramuscularly behind the ear using a 2 ml dose of FMD virus strain O_1BFS 1860 oil emulsion vaccine (Batch 0-115, Wellcome) which contained 5·2 μg of intact 146S FMD immunising antigen (Brown and Newman 1963) per dose. Sows 1 and 2 were vaccinated 30 to 32 days before farrowing, sows 3 and 4 were vaccinated 51 to 52 days before farrowing and then revaccinated 31 to 32 days before farrowing and sows 5 and 6 were vaccinated 12 to 13 days before farrowing.

Serum sampling

Blood samples were collected from each piglet at three days, one week and then weekly after farrowing for 10 weeks. This blood was kept at room temperature for 24 hours and the serum was separated by centrifugation. The serum was then stored at $-20°C$ and inactivated at 56°C for 30 minutes prior to testing.

Bodyweight and blood volume

Immediately before each blood sampling the weight of the piglets was recorded and converted to blood volume using the formulae of von Engelhardt (1966) as shown: Up to 25 kg bodyweight, $BV = 9{\cdot}5 \times W^{-0{\cdot}068}$, and above 25 kg bodyweight, $BV = 17{\cdot}9 \times W^{-0{\cdot}27}$, where BV is blood volume (ml [100 g bodyweight]$^{-1}$) and W is bodyweight (kg).

Neutralising antibody assessment

The neutralising activity of the piglet serum samples was demonstrated using a microneutralisation test (Francis et al 1983). Each test was done in triplicate and the results were recorded as the mean $\log_{10}$ reciprocal of the serum dilution which gave confluent cell sheets in 50 per cent of the microplate wells.

Analysis of anti-FMD antibody class activity

Pooled samples of serum from each litter at three days old were fractionated by gel filtration. A 2·5 ml sample was applied to a 26 mm × 950 mm glass column (LKB Instruments) containing Bio-Gel A-5 M gel filtration medium (Bio-Rad Laboratories) and was eluted with 0·1 M Tris buffer containing 0·3 M sodium chloride. A total of 180×2·7 ml fractions were collected and analysed for optical density, neutralising activity and antibody class (using a combination of enzyme-linked immunosorbent assay, immunoelectrophoresis and sedimentation coefficient analysis). The neutralising titres observed within IgM, IgA and IgG-rich regions of the column were added together and the percentage FMD neutralising activity for each class in the three-day-old litters was calculated.

Calculation of observed half lives

The observed half lives of maternally derived antibodies for each litter were calculated from the decrease in the mean $\log_{10}$ neutralising antibody titres for that litter over time using a linear regression and the following two conditions. First, only piglets with $\log_{10}$ antibody titres greater than 1·0 at three days old were taken into account and second, in any litter where the antibody levels of individual piglets had fallen to their least detectable value (as indicated by subsequent bleedings) the mean half life of the litter was determined up to that point. Results were based on seven to eight piglets per litter except in litter 2 where, due to the sow's poor vaccination response, only four piglets met the first condition.

Calculation of the catabolism and, or, excretion rate

The antibody titres observed were corrected for increases in blood volume according to the following formula:

$$\frac{\text{Blood volume at sample point (a)}}{\text{Blood volume at start of the experiment (three days after birth) (b)}} \times \text{FMD neutralising antibody titre at sample point (c)} = \text{Corrected titre}$$

In practice, the $\log_{10}$ values for the blood volumes were subtracted from each other (a-b) and the result was added to the $\log_{10}$ observed antibody titre (c) to give the corrected $\log_{10}$ titre. These corrected titres were used to calculate the half lives of neutralising antibodies due to catabolism/excretion using a linear regression as before.

Results

The results of the antibody class analysis (Table 1) showed that in piglets born to sows vaccinated or revaccinated 30 to 32 days before farrowing, 60 to 91 per cent of the FMD neutralising activity resided in the IgG fraction, whereas in those piglets born to sows vaccinated 12 to 13 days before farrowing 100 per cent of the FMD neutralising activity was in the IgM fraction.

The mean neutralisation titres ($\log_{10}$) for each litter, calculated from the mean of three titrations on each serum sample, are shown in Figs 1a to 1f together with the linear regression slopes. The fit of the linear regression slopes to individual piglet data was highly significant ($r>0·9$, $P<0·001$) for all litters. The observed half lives of maternally derived antibodies (mean per litter) were 18·4 (n = 8) and 7·4 (n = 4) days in the piglets born to sows 1 and 2 which were vaccinated 32 and 30 days before farrowing respectively; 20·9 (n = 8) and 20·6 (n = 8) days in piglets born to sows 3 and 4 vaccinated at 51 and 31, and 52 and 32 days before farrowing respectively; and 4·2 (n = 8) and 7·8 (n = 7) days in piglets born to sows vaccinated 12 and 13 days before farrowing respectively.

TABLE 1: FMD neutralising antibody class activity in three-day-old piglets serum and the maternal antibody decay rates

Litter	Sow vaccination (days before farrowing)	Percentage of neutralising activity in each antibody class IgM	IgA	IgG	Observed half life of neutralising antibody (days)	Half life corrected for blood volume increase (days)
1	32	2	7	91	18·4	>408
2	30	20	20	60	7·4	Infinity
3	51 and 31	0	13	87	20·9	Infinity
4	52 and 32	6	6	88	20·6	Infinity
5	12	100	0	0	4·2	6·9
6	13	100	0	0	7·8	18·4

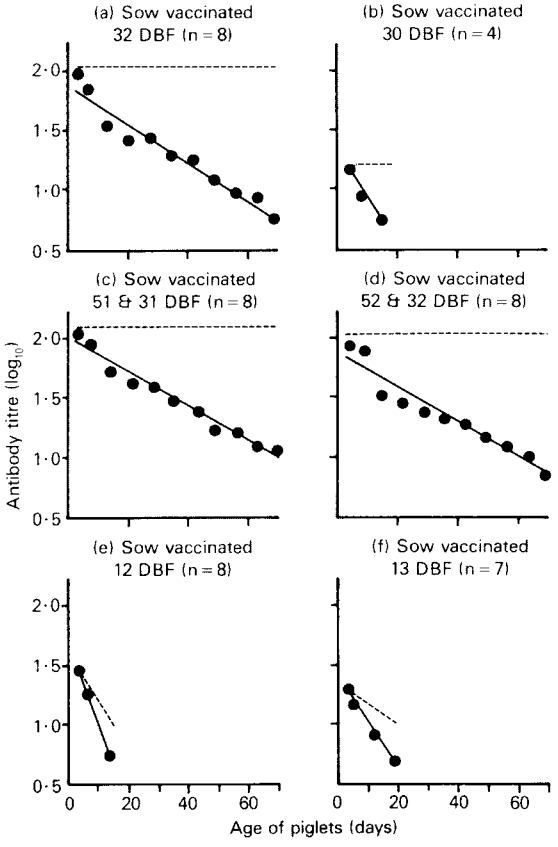

FIG 1: Observed (———) and corrected (- - -) decay rates of maternally derived FMD antibodies in piglets. DBF Days before farrowing

The mean increase in bodyweight (kg) and blood volume (ml) for the 43 piglets is given in Table 2. This table also shows rate of increase in the mean blood volumes calculated from a linear regression analysis of the mean $\log_{10}$ values.

TABLE 2: Mean bodyweight, blood volume and blood volume doubling over the first 70 days of life

Age of piglets (days)	Mean bodyweight (kg)	Mean blood volume (ml)	Blood volume doubling time from day 3 (days)
3	1·75	160·04	—
6-7	2·50	223·15	7·02
13-14	3·97	343·40	8·68
20-21	5·77	486·56	10·81
27-28	7·92	653·64	12·19
34-35	10·52	851·61	13·39
41-43	13·64	1084·85	14·49
48-49	16·60	1302·76	15·58
55-56	18·67	1453·55	16·92
62-63	20·79	1606·81	18·31
69-70	22·91	1759·00	19·80

The half lives of antibodies in piglets from sows 5 and 6 vaccinated 12 and 13 days before farrowing, corrected for blood volume increase, were 6·9 and 18·4 days respectively (Figs 1e and 1f) while the corrected half lives in piglets from sows 1 to 4 vaccinated or revaccinated 30 to 32 days before farrowing ranged from greater than 408 days to infinity (Figs 1a to 1d).

Discussion

The vaccination schedules used in this experiment were based on previously published observations (Ouldridge et al 1982) and resulted in litters with principally either IgG or IgM FMD neutralising antibodies in their sera three days after sucking. The litters which received principally neutralising IgM antibodies had observed mean half lives of 4·2 and 7·8 days. These results, based on FMD virus neutralising activity, were somewhat higher than values published elsewhere of 1·3 to 4·5 days which were based on the decay of normal serum IgM (Porter and Hill 1970, Curtis and Bourne 1971, Curtis and Bourne 1973, Klobasa et al 1981). However, although only neutralising IgM activity could be detected in the three-day-old piglet serum pools of these litters, it seems possible that low levels of neutralising IgG were present at farrowing which were not detectable by the technique employed, but nonetheless lengthened the observed half lives of maternally derived antibodies in the litters. The observed mean half lives in piglet litters 1 to 4 which received principally IgG antibodies were generally longer (7·4, 18·4, 20·6 and 20·9 days). A similar range of passive IgG half live values (7·5 to 19·7 days) appears in the literature (Porter and Hill 1970, Curtis and Bourne 1971, Curtis and Bourne 1973, Launais et al 1978, Frenyo et al 1980/81, Klobasa et al 1981, Paul et al 1982).

In seeking an explanation for the wide variation in the passive antibody half lives it was attempted to identify the true half lives due to catabolism/excretion of the neutralising passive antibodies by taking into account the influence of dilution produced by increased blood volume with size. Although previous authors have drawn attention to the effect that the rapid growth rate of young pigs might have on their observed antibody half life values (Porter and Hill 1970, Curtis and Bourne 1971, Curtis and Bourne 1973) and used this to explain litter to litter variation in results obtained (Curtis and Bourne 1971, Curtis and Bourne 1973), few have actually measured and corrected for bodyweight increase. The corrected half lives of passive IgM antibodies in the litters due to catabolism were calculated to be between 6·9 and 18·4 days. With regard to the IgG antibodies the results suggested

that there was little or no IgG catabolism/excretion during the first 10 weeks of the pigs' life and that all, or almost all, of the fall off in the piglet's neutralising IgG titres was due to their increased size and vascular compartment volume.

In an earlier study on porcine parvovirus antibody the authors corrected for bodyweight increase in 16-to 24-week-old piglets and obtained a mean correct half life value of 29 days (Paul et al 1982). Therefore the low catabolic rate observed in this report may be due to the age of the piglets studied. Since young pigs do not attain adult levels of IgG until they are 12 to 16 weeks old (Curtis and Bourne 1971, Frenyo et al 1980/81) it is possible that in piglets less than 10 weeks old, as used in this study, catabolism is controlled by the level of total serum IgG. The control of IgG catabolism by the total serum IgG concentration has been reported in other species (Fahey and Robinson 1963, Sell 1964, Waldmann and Strober 1969). Furthermore, IgM and IgA catabolism has been shown to be independent of serum concentration (Waldmann and Stober 1969) which would explain why catabolism was observed in litters 5 and 6.

We were also able to demonstrate that the doubling time of the vascular volume depended largely on the age and period over which the observations were made. For example, when measured in three- to seven-day-old piglets the doubling time for the blood volume was approximately seven days but when measured in the same piglets over three to 70 days the blood volume doubling time was almost 20 days. Thus, although a linear regression provides a good fit to the data ($r>0{\cdot}95$ in all six litters), the observed half life is not strictly linear and is influenced by the rapid blood volume doubling time in the first few weeks of the piglet's life. The influence of these findings on the half life of the maternally derived IgG antibodies is apparent in the case of litter 2 where the relatively short half life of 7·4 days can be ascribed to the fact that the titres of maternally derived antibodies were low and could only be measured for 13 days after farrowing. During this period the doubling time of the blood volume was correspondingly short (8·7 days). This variation in observed half life values due to piglet age and time over which observations were made has been reported previously for porcine parvovirus antibody (Paul et al 1982).

Using this hypothesis differences in previously published IgG half life values could be accounted for to a large extent by relating them to the observation period used in each instance. For example, an IgG half life of 12 to 14 days (Curtis and Bourne 1971) was calculated over the first four weeks of life during which time the blood volume doubles, on average, every 12·2 days, while a half life of 17 days (Launais et al 1978) was calculated over the first 10 weeks of life during which time the mean blood volume doubles every 19·8 days (Table 2).

For practical purposes, a linear regression drawn through the observed neutralising titres of all the piglets in a litter is useful in that it can be used to predict the time at which the titres drop below protective levels (Black et al 1984) and also when the vaccination inhibitory activity subsides to below acceptable limits.

To provide maximum passive protection for the piglets against FMD it would seem advisable to give previously vaccinated sows a booster vaccination during pregnancy. Unvaccinated sows should be inoculated twice during this period. In order to ensure that antibodies predominantly of IgG class and virus neutralising are passed to the piglets, an interval of at least 30 days is required between final vaccination and time of farrowing. Finally, although no deleterious effect of FMD vaccination in pigs has ever been demonstrated it seems wise to avoid vaccinating during the first month of pregnancy when the fetus is most prone to damage.

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Received for publication October 3, 1983