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

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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 (Leece & 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 × 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

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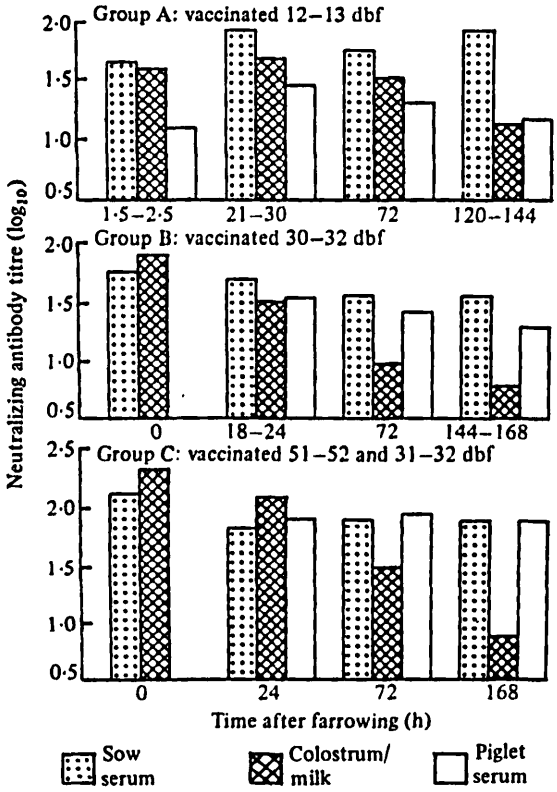

Fig. 1. The mean FMD neutralizing antibody titres of sows serum, colostrum/milk and piglets serum during the first week after farrowing.

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

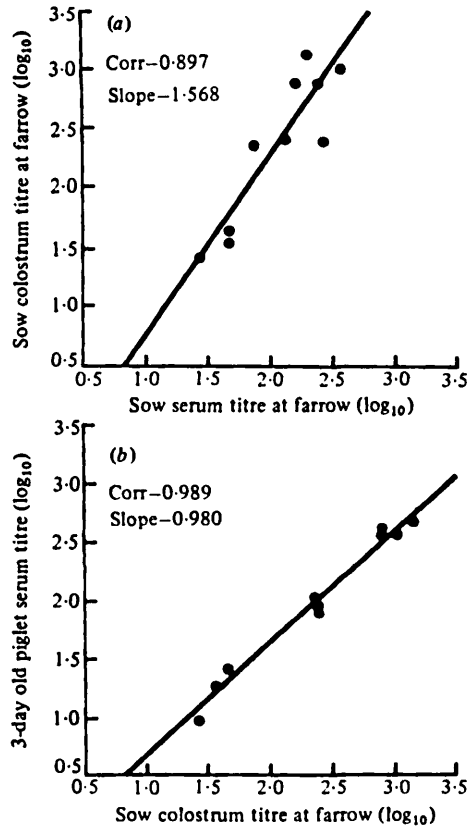

Fig. 2. Regression analysis of relationship between sows serum, colostrum and 3-day-old piglet serum titres.

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

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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.

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REFERENCES

Anderson, E. C., Masters, R. C. & Mowat, G. N. (1971). Immune response of pigs to inactivated foot and mouth disease vaccines – Response to emulsion vaccines. *Research in Veterinary Science* **12**, 342–350.

Bourne, F. J. (1971). Porcine Immunoglobulins. *Veterinary Annual* **12**, 74–85.

Bourne, F. J., Curtis, J., Johnson, R. H. & Collings, D. F. (1974). Antibody formation in porcine fetuses. *Research in Veterinary Science* **16**, 223–227.

Brambell, F. W. R. (1958). Pre-natal transference of antibodies. *Veterinary Record* **70**, 1060–1063.

Brown, P. (1976). *The Immune System of the Young Pig.* Agricultural research seminar on porcine immunology (EVR 5450), p. 60–64.

Curtis, J. & Bourne, F. J. (1971). Immunoglobulin quantitation in sow serum, colostrum and milk and the serum of young pigs. *Biochimica et Biophysica acta* **236**, 319–332.

Curtis, J. & Bourne, F. J. (1973). Half lives of immunoglobulins IgG, IgA and IgM in the serum of new born pigs. *Immunology* **24**, 147–155.

Dujin, T. (1971). Contribution to the knowledge of immunobiological protection of piglets against foot and mouth disease. *Acta Veterinaria Beograd* **21**, 111–119.

Francis, M. J. & Black, L. (1983). Antibody response in pig nasal fluid and serum following foot and mouth disease infection or vaccination. *Journal of Hygiene* **91**, 329–334.

Francis, M. J. & Black, L. (1984). The effect of sows vaccination regimen on the decay rate of maternally derived foot and mouth disease antibodies in piglets. *Research in Veterinary Science* **37** (in the press.)

Francis, M. J., Ouldridge, E. J. & Black, L. (1983). Antibody response in bovine pharyngeal fluid following foot and mouth disease vaccination and, or exposure to live virus. *Research in Veterinary Science* **35**, 206–210.

Kim, Y. B., Bradley, S. G. & Watson, D. W. (1966). Ontogeny of the Immune Response. I. Development of immunoglobulin in germfree and conventional colostrum-deprived piglets. *Journal of Immunology* **97**, 52–63.

Lecce, J. G. & Morgan, D. O. (1962). Effect of dietary regimen on cessation of intestinal absorption of large molecules (closure) in the neo-natal pig and lamb. *Journal of Nutrition* **78**, 263–268.

McKercher, P. D. & Giordano, A. R. (1967). Foot and mouth disease in swine. II. Some physical-chemical characteristics of antibodies produced by chemically treated and non-treated foot and mouth disease virus. *Archiv fur die Gesamte Virusforschung* **20**, 54–70.

OULDRIDGE, E. J., FRANCIS, M. J. & BLACK, L. (1982). Antibody response of pigs to foot and mouth disease oil emulsion vaccine: the antibody classes involved. *Research in Veterinary Science* **32**, 327–331.

PERRY, G. C. & WATSON, J. H. (1967). Variation in the absorption of colostrally secreted marker antibody in piglets. *Animal Production* **9**, 385–391.

PORTER, P. (1969). Transfer of immunoglobulins IgG, IgA and IgM to lacteal secretions in the parturient sow and their absorption by the neonatal piglet. *Biochimica et Biophysica Acta* **181**, 381–392.

PORTER, P. & HILL, I. R. (1970). Serological changes in immunoglobulins IgG, IgA and IgM and Escherichia coli antibodies in the young pig. *Immunology* **18**, 565–573.

YABIKI, T., KASHIWAZAKI, M. & NAMIOKA, S. (1974). Quantitative analysis of three classes of immunoglobulins in serum of newborn pigs and milk of sows. *American Journal of Veterinary Research* **35**, 1483–1489.