

**Longevity of the antibody response in pigs and sheep following a single administration of high potency emergency FMD vaccines****S. J Cox and P. V. Barnett**Institute for Animal Health, Pirbright Laboratory,  
Ash Road, Pirbright, Surrey GU24 0NF, U.K.**Summary**

Ideal vaccines should be capable of stimulating a potent and long-lasting immunity, after a single vaccination. Conventional FMD vaccines, however, require frequent re-vaccinations to maintain antibody at protective levels. Consequently the search for more effective FMD vaccines continues to occupy research teams world-wide. Studies using high potency emergency FMD vaccines, particularly those incorporating the 'ready-to-formulate' oil adjuvant Montanide ISA 206, have shown not only rapid sero-conversion but maintenance of a protective antibody response for at least 6 months in sheep and 4.7 months in pigs, after a single vaccination. Such long-lasting immunity, which is likely to overcome the need to revaccinate, would be invaluable in the control of an FMD outbreak. These high potency vaccines are worthy of further investigation, to monitor exactly how long protective immunity can be maintained following a single application, as this may provide not only benefits for the control procedures used in FMD-free countries but also a more efficient and cost effective means of maintaining herd immunity in endemic areas.

**Introduction**

Much research and effort has been directed towards the improvement of vaccines to FMD and to the understanding of host immune responses to natural FMD infection in order to develop vaccines which promote potent long lasting immunity ( as reviewed by Barteling and Vreeswijk, 1991, Doel, 1991 , 1996). However, depending on type and quality, the widely used conventional, low potency FMD vaccine formulations are often unable to provide an immunity that lasts longer than 6 months. Consequently livestock need to be re-vaccinated, depending upon the epidemiological situation, between one and three times a year for protection to be maintained. Ruminants require a primary vaccination followed by a booster vaccination at 3-4 weeks. Pigs generally only require a single dose of oil adjuvanted vaccine, which can result in a long duration of immunity (Anderson et al., 1971), but more often they require boosting at approximately 6 monthly intervals.

The main requirements of emergency vaccines are to rapidly induce protective immunity and reduce local virus replication thus limiting virus spread and transmission during an outbreak of FMD. In recent years, studies at the International Vaccine Bank (IVB), located at IAH Pirbright laboratory, using cattle (Doel et al., 1994, Salt et al., 1995), pigs (Salt et al., 1997) and sheep (Cox et al., 1999), have provided much data confirming the ability of such vaccines to prevent disease and transmission of FMDV. These studies, however, were interested in early immune events and were frequently terminated after 28 days so that the duration of the response remained unknown. As the need for a vaccine for both ruminants and pigs that confers long lasting immunity, ideally after a single vaccination remains, several recent additional studies have been performed using the same emergency vaccine

formulations to investigate the longevity of the antibody response in sheep and pigs. In sheep, both oil and aqueous vaccine formulations were investigated whereas the pig study evaluated the total neutralising antibody and some of the different classes of antibody present following vaccination with oil adjuvanted vaccine.

## Materials and methods

Vaccine formulations incorporating FMDV O<sub>1</sub> Lausanne or C<sub>1</sub> Oberbayern inactivated antigen as a water-in-oil-in-water (W/O/W) emulsion with Montanide ISA 206 (Seppic, Paris) were used in pigs. Studies in sheep used formulations incorporating FMDV O<sub>1</sub> Manisa and A<sub>22</sub> Iraq inactivated antigen as W/O/W or oil-in-water (O/W) emulsions with either Montanide ISA 206 or ISA 25 (Seppic, Paris) respectively or an aqueous (Al(OH)<sub>3</sub>)/saponin formulation. All the vaccines were prepared from antigen concentrates held currently by the IVB over liquid nitrogen and are highly potent with PD<sub>50</sub> values of 41, ≥112, ≥112 and 75 for O<sub>1</sub> Lausanne, C<sub>1</sub> Oberbayern, O<sub>1</sub> Manisa and A<sub>22</sub> Iraq respectively when tested as an Al(OH)<sub>3</sub>/saponin formulation. All vaccines used in sheep were administered as a 1.0ml volume (equivalent to half of a bovine dose), by the intramuscular route (W/O/W and O/W vaccines) into the right quadriceps mass or subcutaneously (aqueous vaccine) over the left shoulder. Pigs received a 2.0ml volume (equivalent to 1 bovine dose) by the intramuscular route immediately caudal to the ear.

Groups of three Large White crossbred Landrace pigs weighing 20-30 kg or three polled Dorset Horn sheep aged between 6 and 12 months were immunised with vaccines as detailed above. All animals were monitored daily for temperature and well-being for 10 days following vaccination and bled regularly for serology up to 141 days (pigs) and 168 days (sheep) post vaccination. Neutralising antibody titres against FMDV in serum were measured by a microneutralisation assay, essentially as detailed by Golding et al. (1976). End-point titres were calculated as the reciprocal of the last serum dilution to neutralise 100 TCID<sub>50</sub> of homologous FMDV in 50% of the wells. An antibody isotype analysis of the pig sera was performed using an ELISA (IDAS) as described by Salt et al. (1996). Anti porcine isotype reagents (anti IgM, IgG1, IgG2 and IgA) were supplied by Serotec, UK.

## Results

Temperatures remained normal and no side effects were recorded following vaccination with either the aqueous or oil formulations in the relevant target species. Neutralising antibody responses for sheep vaccinated with A<sub>22</sub> Iraq and O<sub>1</sub> Manisa over 168 days are shown in [figure 1](#). All animals had seroconverted by seven days post vaccination, regardless of adjuvant, and in most animals titres had peaked within 28 days. The sheep vaccinated with Montanide ISA 206 oil formulation ([fig 1, c and f](#)) maintained high titres of antibody for the duration of the trial, particularly for the A<sub>22</sub> Iraq vaccine. However, responses for the vaccines formulated with either Al(OH)<sub>3</sub>/saponin ([fig 1, a and d](#)) or Montanide ISA 25 ([fig 1, b and e](#)) demonstrated a gradual decline from peak antibody titres which leveled off after 66 days for most individuals.

Neutralising antibody titres for pigs vaccinated with either O<sub>1</sub> Lausanne or C<sub>1</sub> Oberbayern are shown in [figure 2](#). All pigs had seroconverted by seven days post vaccination with peak titres generally being evident between 21 and 28 days post vaccination. Antibody isotype analyses of sera from pigs vaccinated with either of the two serotypes demonstrated typical

profiles and are shown in [figure 3](#). All animals responded to vaccination with a rapid IgM response which was prolonged in animals vaccinated with O<sub>1</sub> Lausanne. IgG<sub>1</sub> responses were generally earlier and reached higher titres than IgG<sub>2</sub>. One animal (TX 89) appeared unable to mount an IgG<sub>2</sub> response and no IgA was detected from any animal at any point after vaccination.

## Conclusions

Conventional aqueous vaccines in ruminants generally promote protective immunity within 8-10 days following primary vaccination and a secondary injection is required to maintain immunity at protective levels for about 6 months. Thereafter further re vaccinations at regular intervals, depending upon epidemiological situation, are required to maintain protective immunity. All the high potency vaccine preparations used in the sheep study generated a rapid antibody response (confirming previous observations, Cox et al., 1999) and in most sheep the antibody titres were maintained, at levels considered protective, for the duration of the study. The sheep vaccinated with A<sub>22</sub> Iraq serotype responded particularly well following vaccination with all three formulations although sheep vaccinated with the Montanide ISA 206 oil formulation were the only group to maintain peak titres for the duration of the trial. Peak antibody titres in the sheep vaccinated with O<sub>1</sub> Manisa vaccine formulations were less dramatic and more variable but again the Montanide ISA 206 oil formulated vaccine gave the best results. However, since the A<sub>22</sub> Iraq vaccine formulations gave a better response than the O<sub>1</sub> Manisa formulations but had originally shown lower potency as an aqueous formulation (75 and >112 respectively in cattle), maintenance of the response cannot relate entirely to this or indeed the payload of antigen per dose (1.246 and 2.394 for A<sub>22</sub> Iraq and O<sub>1</sub> Manisa respectively). Variation between serotypes which display differing levels of immunogenicity will inevitably contribute to the magnitude of the response (Doel.,1996). Since the sheep only received a single injection of vaccine these results suggest that higher potency vaccines may offer advantages over conventional aqueous vaccines particularly the oil adjuvanted formulations. Oil adjuvanted vaccines of various types have previously been shown to promote good protection in ruminants and pigs with immunity lasting for more than six months (reviewed Barteling & Vreeswijk, 1991).

Depending on the formulation used, conventional oil adjuvanted vaccines that are used in pigs normally only require a single injection to promote a protective immunity which develops within approximately 8 days and lasts for about 6 months. Our study with C<sub>1</sub> Oberbayern and O<sub>1</sub> Lausanne Montanide ISA 206 oil formulated vaccines show that high potency vaccines also promote neutralising antibody which is maintained at levels considered protective for at least 4.7 months and compare well with the level of protective immunity maintained in convalescent pigs following FMD infection (Doel., 1999). Highest titres were evident between 21 and 28 days which was later than had previously been observed (Barnett et al., 1996). Once peak titres had been achieved they were generally maintained although some minimal decline was evident in the antibody levels of those pigs vaccinated with the O<sub>1</sub> Lausanne formulation for the remainder of the trial period. Interestingly, Anderson et al (1971) observed similar antibody decline in pigs after vaccination with a single oil emulsion containing O<sub>1</sub> Swiss 1/66 but not with similar formulations containing an A or C serotype.

Individual isotype-specific antibody responses were measured in the pig sera. As previously shown by Ouldrige et al. (1982), and typical of primary responses, a rapid IgM response was prominent 3-5 days following vaccination which peaked within 7 – 14 days post immunisation. IgG responses were only generally detected 9 or more days post-vaccination.

This may have implications with regard to the role of the antibody response and the early protective immunity achieved within 4 days of vaccination (Salt et al., 1997). Although innate parameters are felt likely to be involved in such rapid protection (Cox et al., 1999), the role of the antibody response cannot be dismissed. However, if antibody does have a function at the early stages following vaccination, then the isotype profiles seen in this study suggest that IgM and not IgG plays an important role in early protection.

IgG<sub>1</sub> appears to be the first of the IgG isotypes detected which in most cases peaked around 28 days and was followed later by IgG<sub>2</sub> (apart from pig TX 90 which appeared unable to mount an IgG<sub>2</sub> response). Levels of IgG<sub>1</sub> tended to be higher than IgG<sub>2</sub> which is consistent with previous reports for cattle (Mulcahy et al., 1990) although the antibody isotypes of different species and their functions are not fully understood and therefore do not necessarily correlate with each other. Further studies are on going to monitor this primary response for 6-9 months following which the qualitative aspects of the response in terms of protection will be investigated.

In conclusion, these preliminary investigations in sheep and pigs have suggested that oil adjuvanted high potency 'emergency' vaccines provide not only a rapid immune response but also a long lasting immunity following a single application. Such attributes make them ideal for use in FMD-free countries where emergency ring vaccination may be necessary, as they are likely to overcome the need to re-vaccinate during an outbreak. Similar formulations would also be more efficient, and possibly more cost effective, for maintaining herd immunity in areas endemic for FMD by increasing the interval between re vaccinations.

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