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Emergency vaccination of sheep against foot-and-mouth disease: protection against disease and reduction in contact transmission

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Abstract

The ability of several emergency FMD vaccine formulations to elicit early protective immunity in sheep was examined. All vaccine formulations were shown to protect sheep against airborne challenge with homologous FMDV within 4 days of vaccination. Protection was associated in part with the induction of serum antibody responses but was also demonstrated in the absence of any detectable antibody response at the time of challenge. Aqueous Al(OH)₃/saponin vaccine formulations and oil emulsion vaccines based on Montanide ISA 206 adjuvant reduced virus replication and the numbers of animals subclinically infected up to 28 days post-challenge, when compared with non-vaccinated animals, consequently limiting transmission of the disease or infection to in-contact susceptible animals. © 1999 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Foot-and-mouth disease (FMD) is a highly contagious disease of ruminants and pigs. Epidemics in previously FMD-free countries can have serious economic consequences in terms of the direct cost of control measures and the loss of export trade [1]. Since 1991, the European Union (EU) has adopted a non-vaccination policy for the control of FMD, to allow greater movement of livestock and their products within the Single Market (Directive 90/423/EEC) and to allow improved trading with other FMD-free regions [2]. However, as a result of this strategy the European domestic livestock population is very susceptible to FMD should the virus be introduced.

Sheep and goats comprise the majority of the world's FMD-susceptible livestock [3]. All of the most recent outbreaks of FMD within and around the EU Member States [4–7] have involved sheep and in North Africa a definite predilection for sheep has been reported [5]. In Turkey, 18.5% of the total FMD cases reported in 1996 were associated with sheep and goats [7] and the most recent episode in Greece during 1996 involved 39 outbreaks, in which 5000 sheep and goats were destroyed [6]. In the event of an outbreak all EU member states employ a 'stamping out' strategy with associated zoo-sanitary measures such as the introduction of movement restrictions. However, if these control measures were unable to prevent the spread of FMD, a strategic emergency ring-vaccination would be used as an additional measure to limit the quantity of FMD virus circulating and reduce the number of new clinical cases. To support this contingency EU members and some other FMD-free states have access to FMD vaccine banks which store, at ultra-low temperature, concentrated, inactivated FMDV antigens that can be formulated rapidly into vaccine, as required [8]. The International FMD

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Vaccine Bank (IVB) at Pirbright is one such emergency FMD vaccine facility.

The most important properties of an emergency FMD vaccine are the early induction of protective immunity and a broad antigenic spectrum. The FMDV antigens held in the IVB can be formulated into highly potent vaccines for emergency use and have been shown previously to protect cattle against airborne challenge at 4 [9] and 2 days [10] post immunisation. More recently pigs have been shown to be protected against airborne challenge within 4 days of vaccination [11]. Previous studies have also shown that immunisation and subsequent challenge of pigs and cattle can reduce dramatically the titre and duration of FMDV excretion [11–13] thereby reducing contact transmission. Although these same emergency vaccine formulations may be utilised in sheep following an outbreak, no similar data is presently available for this species.

Pigs are considered particularly important in the dissemination of FMDV as they excrete large quantities of airborne virus [14] but sheep present problems of a different kind. Unlike pigs and cattle, FMD in sheep is frequently mild or inapparent [15, 16] so that infection and subsequent transmission can often go unobserved. The most common clinical sign is lameness, but this too is often absent. The airborne excretion of virus from sub-clinically infected sheep and from vaccinated and recovered animals [15, 16] further contributes to the problem of control. In addition, sheep that have had contact with FMDV may become carriers [17, 18]. An outbreak of FMD in sheep which remains undiagnosed until after the disease has spread, particularly where mixed animal husbandry is practised, could have devastating consequences. In Greece, for example, recent outbreaks in other species have been attributed to contact with infected sheep [6]. It is therefore of primary importance that an early protective immunity is attained in susceptible sheep flocks, reducing the likelihood of disease transmission.

Information relating to early protection following the vaccination of sheep is scarce. Gibson et al. [19] examined the outcome of airborne FMDV challenge of sheep vaccinated with either three or six times the standard dose of conventional monovalent type O FMD antigen, and showed that despite reducing virus replication the levels of protection attained were insufficient to prevent airborne infection of sheep vaccinated one week before challenge. Other more recent studies have either evaluated different FMD vaccine formulations [20–23] or assessed the immune response in pregnant ewes and their offspring [24, 25]. To date, no studies have examined the use of high potency

emergency FMD vaccines in sheep, particularly with regard to early protective immunity and the prevention of contact transmission. In this study we have examined the efficiency of several emergency FMD vaccines in eliciting an early protective immunity in sheep, their effect upon virus replication in the upper respiratory tract and their ability to limit FMDV transmission.

2. Materials and methods

2.1. Animals

52 Polled Dorset Horn sheep aged between 6 and 12 months were used for this study, which consisted of three separate trials. All infectivity studies were performed in a disease secure isolation compound at IAH, Pirbright.

2.2. Preparation of vaccines

Vaccine formulations incorporating FMDV O₁ Lausanne inactivated antigen as either a water-in-oil-in-water (W/O/W) emulsion with Montanide ISA 206 (Seppic, Paris) [26], or as an aqueous Al(OH)₃/saponin formulation [9] were used in trial 1. Trial 2 examined a single vaccine formulation of FMDV Asia1 India 8/79 inactivated antigen in a W/O/W emulsion, again using Montanide ISA 206. In trial 3, a single aqueous Al(OH)₃/saponin formulation, using FMDV C₁ Oberbayern inactivated antigen, was used. All the vaccines were prepared from antigen concentrates held currently by the IVB over liquid nitrogen and were highly potent with PD₅₀ values of 41, 61 and ≥112 for O₁ Lausanne, Asia1 India and C₁ Oberbayern per bovine dose respectively, when tested in an Al(OH)₃/saponin formulation. The formulated vaccines contained the following amounts of 146S antigen per 1.0 ml sheep dose, 3.05 µg (O₁ Lausanne), 5.25 µg (Asia1 India) and 1.4 µg (C₁ Oberbayern).

2.3. Vaccination

All vaccines were administered as a 1.0 ml volume, equivalent to half of a bovine dose, by either the intramuscular route using the right quadriceps mass (W/O/W vaccine) or subcutaneously over the left shoulder (aqueous vaccine). Vaccinations were staggered to allow simultaneous challenge of all sheep in each trial.

2.4. Infection of donor pigs

Pigs were used as a source of airborne virus for the challenge of vaccinated sheep and unvaccinated controls. For each trial, three pigs were infected by intradermal injection of the heel bulbs of one hind foot [28] with 10^4 cattle ID_{50} of the appropriate FMD virus, homologous to the vaccine strain used to immunise the sheep. To maximise the release of airborne virus each of three pigs were infected either 72, 48 or 24 h (trial 3 only) prior to challenge and all were used as donors.

2.5. Challenge of animals

2.5.1. Trial 1

Groups of three sheep were immunised at 10, 6, 4 and 3 days, respectively, prior to challenge with either the W/O/W emulsion or aqueous vaccine formulation, in order to investigate the development of protection stimulated in outbred sheep by two 'emergency vaccines' and the effect on local virus replication in the respiratory tract. Airborne challenge was carried out by allowing the vaccinated and four non-vaccinated control sheep to circulate for 2 h around three pigs showing early clinical signs of FMD, which were housed in open-topped, side-ventilated crates. The FMDV donor pigs were destroyed immediately after the challenge period. The challenged sheep were then rehoused in their original immunisation groups. The four non-vaccinated control sheep were housed in a separate box. All the sheep were examined daily for clinical signs of FMD and rectal temperatures were recorded for up to 11 days post-challenge. Heparinised blood and oesophageal–pharyngeal fluid samples were collected at regular intervals for virus isolation. Blood samples were also collected weekly for serology. The trial was terminated four weeks after challenge.

2.5.2. Trial 2

To determine the effect of 'emergency vaccines' upon the transmission of FMDV to susceptible in-contact sheep as well as to investigate the development of protection and effect on local virus replication in the upper respiratory tract, groups of five sheep were housed together, three of which were to be vaccinated and two to act as the susceptible in-contact controls. Sheep were immunised at 10, 6, 4 and 3 days prior to challenge respectively, with a single W/O/W emulsion formulation. One group of three sheep remained unvaccinated. The airborne challenge method was as described above for trial 1 except that the duration of exposure was extended to four hours to increase the severity of the challenge. Following challenge all sheep

were rehoused in their original immunisation groups with the two susceptible in-contacts for 28 days to assess transmission of FMDV by direct contact. All of the sheep were examined daily for clinical signs of FMD and rectal temperatures were recorded. Blood and oesophageal–pharyngeal samples were collected on a regular basis and examined for the presence of virus and/or antibody. This trial was also terminated four weeks after challenge.

2.5.3. Trial 3

To further investigate the kinetics of protection stimulated in outbred sheep and the effect of 'emergency vaccination' upon the transmission of FMDV to susceptible in-contact sheep this trial was performed using an aqueous vaccine formulation. The protocol was essentially the same as trial 2 except that the donor challenge pigs failed to show clinical signs of FMD at the predetermined challenge date. Consequently, the challenge was delayed 24 h and the vaccination groups were re-designated as 11, 7, 5 and 4 days pre-challenge. In all other respects the trial was similar to trial 2.

2.6. Serology

Neutralising antibody titres to FMDV in serum samples were measured in a microneutralisation assay, essentially as detailed by Golding et al. [29]. End-point titres were calculated as the reciprocal of the last serum dilution to neutralise 100 TCID₅₀ of homologous FMDV in 50% of the wells.

2.7. Virus isolation

Heparinised blood and oesophageal–pharyngeal samples were examined for the presence of virus by inoculation of monolayers of primary calf thyroid (BTY) cells, as described by Ferris and Dawson [29]. Four BTY tubes were used for each sample, 250 μ l of sample/tube. Inoculated tubes were incubated at 37°C on roller drums and examined at 24, 48 and 72 h for cytopathic effect (cpe). The virus specificity of any cpe was confirmed by ELISA [30].

3. Results

3.1. Trial 1

In the non-vaccinated control group, three out of four sheep became viraemic and two out of four pyrexia ($\geq 40^\circ\text{C}$) within 4 days of challenge. Clinical signs

of FMD (i.e. vesicular lesions) were not recorded in this group. All four sheep had FMDV positive oesophageal–pharyngeal samples from 4 days post-challenge and remained infected at 28 days post-challenge (Table 1). All four sheep seroconverted to FMDV, including animal SU27 which was not shown to be either viraemic or pyrexia (Fig. 1).

Neither pyrexia nor viraemia was detected in any of the vaccinated sheep. However, FMDV was detected in the oesophageal–pharyngeal samples from some of the vaccinated sheep (Table 1). Both formulations induced detectable serum antibody responses from 4 days post immunisation as measured by microneutralisation assay (Fig. 1).

3.2. Trial 2

Neither vaccinated nor non-vaccinated sheep showed any signs of clinical disease following the four hour airborne challenge, however viraemia were evident in the three non-vaccinated controls, two of which were also pyrexia (Table 2). None of the vaccinated sheep or the in-contact sheep housed with the non-vaccinates were viraemic within the 8 day examination period post-challenge, but some of the sheep in-contact with vaccinated sheep did become viraemic at 3 or 6 days after rehousing with the challenged vaccinates (Table 2). Measurement of rectal temperatures up to 13 days post-challenge identified pyrexia ($\geq 40^{\circ}\text{C}$) in

Table 1

Outcome of challenge of sheep vaccinated with either aqueous or Montanide ISA 206 W/O/W formulated FMD type O1 Lausanne vaccine prior to challenge with homologous FMDV (trial 1)

Vaccine	Animal	Pyrexia ^a	Viraemia	O-P ^b virus isolation (days post-challenge)					
				2	4	7	9	28	
Aqueous	10 ^c	SU47	N ^d	N	– ^e	–	–	–	–
		SU48	N	N	–	–	–	–	–
		SU49	N	N	–	–	–	–	–
	6	SU41	N	N	+ ^f	+	+	+	+
		SU42	N	N	–	–	–	–	–
		SU43	N	N	–	–	–	–	–
	4	SU35	N	N	–	–	–	–	–
		SU36	N	N	–	–	–	–	–
		SU37	N	N	–	–	–	–	–
	3	SU29	N	N	–	–	–	–	–
		SU30	N	N	+	+	+	+	+
SU31		N	N	+	+	–	–	–	
Oil	10	SU50	N	N	–	–	–	–	–
		SU51	N	N	–	–	–	–	–
		SU52	N	N	–	–	–	–	–
	6	SU44	N	N	–	–	–	–	–
		SU45	N	N	+	+	+	+	–
		SU46	N	N	+	+	–	–	–
	4	SU38	N	N	–	+	+	–	+
		SU39	N	N	+	+	+	+	–
		SU40	N	N	–	+	+	+	–
	3	SU32	N	N	+	–	–	–	–
		SU33	N	N	–	–	–	–	–
SU34		N	N	+	–	–	–	–	
None ^g	SU25	N	Y ^h	–	+	+	–	+	
	SU26	Y	Y	–	+	+	+	+	
	SU27	N	N	+	+	+	+	+	
	SU28	Y	Y	–	+	+	+	+	

^a $\geq 40^{\circ}\text{C}$. ^b Oesophageal–pharyngeal fluid sample. ^c Days vaccinated prior to challenge. ^d N. ^e No virus detected. ^f Virus detected. ^g Non-vaccinated control group. ^h Yes.

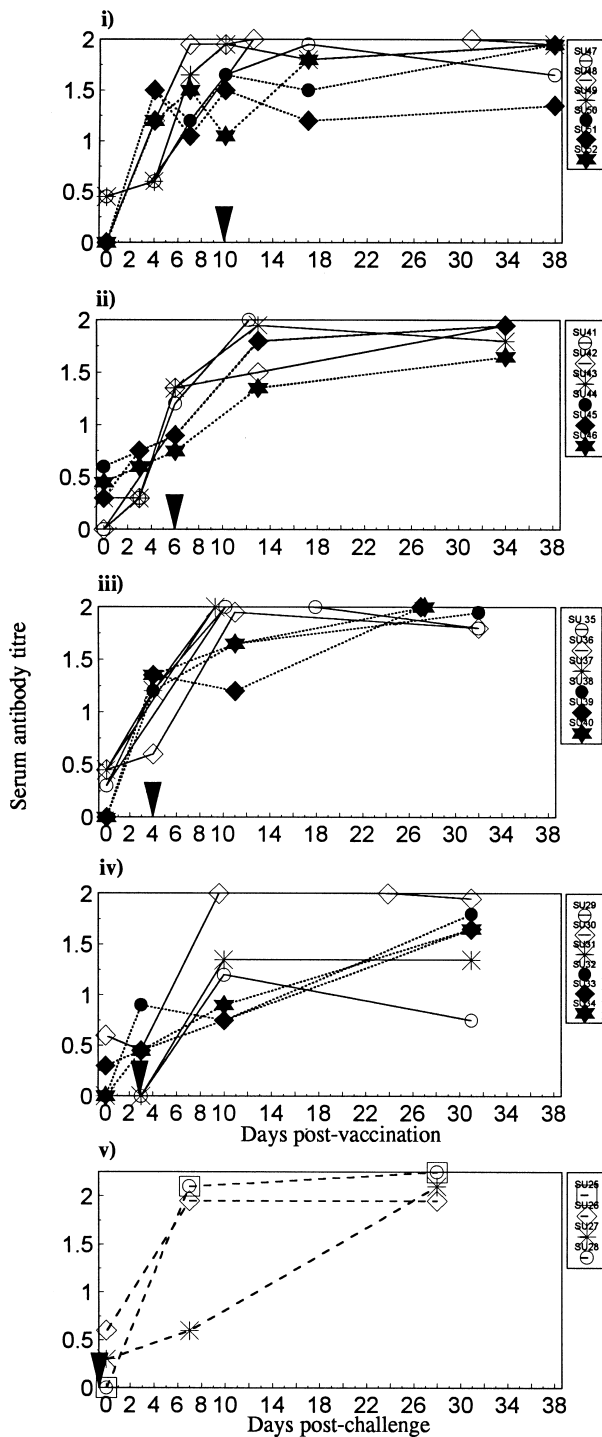


Fig. 1. Serum antibody responses in sheep following vaccination and challenge with FMDV type 01 Lausanne as measured by microneutralisation assay. Sheep were vaccinated at (i) 10, (ii) 6, (iii) 4 and (iv) 3 days prior to challenge or (v) remained unvaccinated prior to challenge. Arrows mark the day of challenge. Solid lines represent sheep vaccinated with the aqueous formulation; dotted lines the sheep vaccinated with Montanide ISA 206 W/O/W formulation and dashed lines the non-vaccinated sheep.

some vaccinates and their in-contacts (Table 2). SV56, an animal in-contact with the group vaccinated 3 days prior to challenge was also inappetent. The in-contact animal SV46, housed with the group vaccinated 6 days prior to challenge, showed lameness in the right hind leg and in-contact SV41, housed with the 10 day group, also became lame in three feet and was subsequently withdrawn from the trial.

FMDV was isolated from oesophageal–pharyngeal samples from several animals, as shown in Table 2. Positive virus isolations were detected in the vaccinated, non-vaccinated and in-contact sheep.

The antibody responses of all the sheep were monitored over a 28 day period following challenge (Fig. 2). The non-vaccinated sheep showed rapid antibody responses 4 days following challenge but sero-conversion did not occur in either in-contact susceptible sheep housed with the non-vaccinates, despite the isolation of FMDV from their oesophageal–pharyngeal samples. All vaccinated sheep had detectable antibody titres by the day of challenge. In-contacts associated with the 10 and 6 day vaccinated groups had sero-converted by 10 or more days post-challenge. Neither of the in-contact sheep housed with the 4 day vaccinated group sero-converted, but one out of the two in-contacts associated with the 3 day group had a detectable antibody response more than 7 days post-challenge.

3.3. Trial 3

Following a 4 h airborne challenge by indirect contact with the infected donor pigs, two of the challenged sheep in the non-vaccinated group (TG30 and TG31) exhibited clinical signs of FMD. At 6 days post-challenge TG30 had unruptured vesicles on the heel bulb of the left foreleg and by the 7th day lesions were apparent on all four feet. TG31 had foot lesions on both hind feet at 11 days post-challenge. Pyrexia and viraemia were also recorded post-challenge in TG30 and TG31 (Table 3). The two control sheep (TG33 and TG35) in contact with this group also developed lesions on both hind feet at 7 and 11 days, respectively, after rehousing with the challenged non-vaccinates. These two in-contact sheep developed pyrexia and viraemia within 13 days of challenge. FMDV was detected in oesophageal–pharyngeal fluid samples from TG30 up to 6 days post-challenge and TG31 up to 27 days post-challenge and from in-contacts TG33 and TG35 at 13 days after rehousing with the challenged animals (Table 3). All three non-vaccinated challenged sheep had sero-converted by 7 days post-challenge (Fig. 3), including TG32 which showed no clinical signs of disease or pyrexia and from which FMDV

Table 2

Development of pyrexia, viraemia and isolation of FMDV in oesophageal–pharyngeal fluid following challenge of sheep vaccinated with Montanide ISA 206 W/O/W formulated FMD type Asia 1 India vaccine prior to challenge and subsequent transmission to in-contact sheep (trial 2)

Vaccinated	Animal	Pyrexia ^a	Viraemia	O-P ^b virus isolation (days post-challenge)				
				2	4	7	9	28
10 ^c	SV17	N ^d	N	+ ^e	+	+	+	+
10	SV18	N	N	- ^f	-	-	-	-
10	SV19	N	N	+	+	+	+	-
Contact	SV20	N	N	-	-	-	-	ND ^g
Contact	SV41	Y ^h	Y	-	-	+	+	ND
6	SV42	N	N	+	+	-	+	+
6	SV43	N	N	+	-	-	-	-
6	SV44	N	N	-	-	-	-	-
Contact	SV45	N	Y	-	+	+	+	ND
Contact	SV46	Y	Y	-	-	-	-	ND
4	SV47	Y	N	-	-	-	-	-
4	SV48	Y	N	-	-	-	-	-
4	SV49	Y	N	-	-	-	-	-
Contact	SV50	N	N	-	-	-	-	ND
Contact	SV51	Y	N	-	-	-	-	ND
3	SV52	N	N	-	-	-	-	-
3	SV53	N	N	-	-	-	-	-
3	SV54	Y	N	-	-	-	-	-
Contact	SV55	Y	N	-	-	-	-	ND
Contact	SV56	Y	N	-	-	+	+	ND
NV ⁱ	SV57	Y	Y	+	+	+	+	-
NV	SV58	N	Y	-	+	+	-	-
NV	SV59	Y	Y	+	+	+	+	+
Contact	SV60	N	N	+	+	-	-	ND
Contact	SV61	N	N	+	+	-	-	ND

^a $\geq 40^{\circ}\text{C}$. ^b Oesophageal–pharyngeal. ^c Days vaccinated prior to challenge. ^d No. ^e Virus detected. ^f No virus detected. ^g Not done. ^h Yes. ⁱ Non-vaccinated control group.

was neither isolated from blood or oesophageal–pharyngeal samples. Both sheep in-contact with this group had sero-converted by 13 (TG33) and 20 (TG35) days post-challenge (Fig. 3).

All of the vaccinated sheep were protected from clinical FMD and failed to transmit disease or infection to the susceptible in-contacts housed with them after challenge. These in-contacts remained clinically normal, sero-negative (with the exception of TG37) and non-viraemic throughout the trial although occasional transient rectal temperatures of $\geq 40^{\circ}\text{C}$ were measured in some animals (Table 3). This latter observation was also true for several sheep in the vaccinated groups post-challenge (Table 3). The oesophageal–pharyngeal fluid samples from only one vaccinated animal (TG25), vaccinated 5 days pre-challenge, were positive for FMDV (Table 3).

Animals vaccinated at 11 and 7 days prior to challenge had developed detectable antibody titres by day of challenge whereas the antibody titres of animals vaccinated at 5 and 4 days prior to challenge, except for TG25, were still undetectable on the day of challenge (Fig. 3).

4. Discussion

The early protective immunity induced by emergency FMD vaccines in cattle and pigs [9–11] would also be useful in sheep. This study was undertaken to provide data, previously absent, to support the use of high potency emergency FMD vaccines in sheep. The vaccines used in this study were formulated from antigens held in the International FMD Vaccine Bank (IVB) at

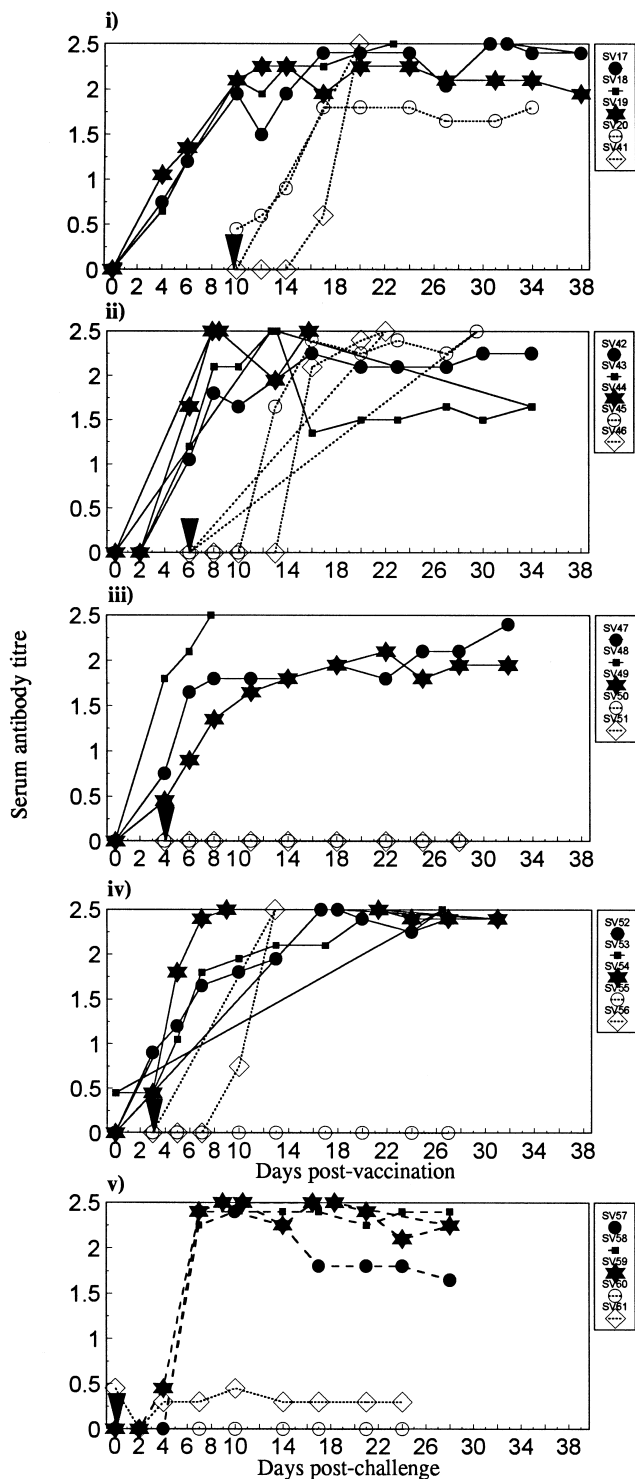


Fig. 2. Serum antibody responses in sheep following vaccination and challenge with FMDV type Asia 1 India as measured by microneutralisation assay. Sheep were vaccinated at (i) 10, (ii) 6, (iii) 4 and (iv) 3 days prior to challenge or (v) remained unvaccinated prior to challenge. Arrows mark the day of challenge. Solid lines represent vaccinated sheep, dashed lines the non-vaccinated sheep and dotted lines represent susceptible in-contacts housed with the challenged vaccinated or non-vaccinated sheep.

Pirbright which comprises a strategic reserve of concentrated inactivated FMD antigens. In addition, the IVB is unique amongst similar banks in having an approved manufacturing facility to formulate emergency vaccines, as either an aqueous $\text{Al}(\text{OH})_3$ /saponin or as an oil adjuvanted product [26] at very short notice.

Previous studies in pigs have concluded that aqueous $\text{Al}(\text{OH})_3$ /saponin FMD vaccines are unsuitable for this species [33, 34] and that stronger immunity is generated using oil adjuvanted formulations [33–35]. In sheep, studies on the longer term generation of immunity have given conflicting results as to which adjuvant formulation would be the most suitable. A significant difference in the antibody response to Asia1 FMD vaccines formulated with either aluminium hydroxide or oil adjuvants was observed by Nair and Sen [20], who showed higher titre antibody responses to oil vaccines which persisted longer than those of the aqueous formulations. In a later study, however, utilising types Asia1 and O FMD antigens, no differences were observed between the antibody responses to PEG-concentrated aluminium hydroxide gel vaccines and oil adjuvanted vaccines over a 2 month period [21] despite the antigen payload being greater in the aqueous formulation. A recent study by the present authors to determine the kinetics of induction and duration of serum antibody levels following a single FMD vaccination of sheep support (in part) some of the previous observations. Sheep vaccinated with A₂₂ Iraq antigen, formulated as oil emulsion (Montanide ISA 25 or 206, Seppic, France) or as $\text{Al}(\text{OH})_3$ /saponin vaccines, and monitored over a 6 month period, developed similar rapid antibody responses regardless of adjuvant, which peaked between 7–21 days post-vaccination (unpublished results). However, only the sheep vaccinated with the ISA 206 oil formulation maintained their serum antibody titres for the duration of the trial.

In trial 1 of the present study, both aqueous and oil based FMD vaccine formulations induced neutralising antibody responses within 4 days and protected all sheep against viraemia following an airborne challenge with homologous FMDV as early as 3 days post-immunisation. Under the same challenge conditions non-vaccinated sheep developed viraemia and pyrexia in the absence of any clinical signs. The Asia1 oil-based formulation used in the second trial also induced serum antibody responses within 4 days in all the vaccination groups and protected sheep from clinical disease and viraemia. However, pyrexia was still apparent in the 3 and 4 day vaccination groups. The aqueous C₁ Oberbayern vaccine formulation used in trial 3 was less efficient in the induction of neutralising serum

Table 3

Development of pyrexia, viraemia and isolation of FMDV in oesophageal–pharyngeal fluid following challenge of sheep vaccinated with aqueous FMDV type C1 Oberbayern vaccine prior to challenge and subsequent transmission to in-contact sheep (trial 3)

Vaccinated	Animal	Pyrexia ^a	Viraemia	O-P ^b virus isolation (days post-challenge)				
				2	6	13	20	27
11 ^c	TG18	N ^d	N	– ^e	–	–	–	–
11	TG19	N	N	–	–	–	–	–
11	TG20	N	N	–	–	–	–	–
Contact	TG40	Y ^f	N	–	–	–	–	–
Contact	TG41	Y	N	–	–	–	–	–
7	TG21	Y	N	–	–	–	–	–
7	TG22	N	N	–	–	–	–	–
7	TG23	N	N	–	–	–	–	–
Contact	TG34	N	N	–	–	–	–	–
Contact	TG39	Y	N	–	–	–	–	–
5	TG24	Y	N	–	–	–	–	–
5	TG25	Y	N	+ ^g	+	+	+	+
5	TG26	Y	N	–	–	–	–	–
Contact	TG37	N	N	–	–	–	–	–
Contact	TG42	Y	N	–	–	–	–	–
4	TG27	N	N	–	–	–	–	–
4	TG28	N	N	–	–	–	–	–
4	TG29	N	N	–	–	–	–	–
Contact	TG36	N	N	–	–	–	–	–
Contact	TG38	N	N	–	–	–	–	–
NV ^h	TG30	Y	Y	+	+	–	–	–
NV	TG31	Y	Y	+	–	+	+	+
NV	TG32	N	N	–	–	–	–	–
Contact	TG33	Y	Y	–	–	+	–	–
Contact	TG35	Y	Y	–	–	+	–	–

^a $\geq 40^{\circ}\text{C}$. ^b Oesophageal–pharyngeal. ^c Days vaccinated prior to challenge. ^d No. ^e No virus detected. ^f Yes. ^g Virus detected. ^h Non-vaccinated control group.

antibody responses when compared to the other formulations and in some cases antibody was not detected before 6 days post-immunisation. Nevertheless, all sheep were protected from clinical disease and viraemia, although some sheep in the 5 and 7 day vaccinated groups developed pyrexia. This rapid development of protection from clinical FMD following the use of IVB-derived antigens is similar to that shown in cattle [9, 10] and pigs [11].

It is well established that immunity to FMD correlates with neutralising antibody titre directed against the structural proteins of the virus capsid, both in cattle [37] and pigs [17, 36]. In addition, protection has also been demonstrated through passive transfer of convalescent serum in pigs [37, 38]. However, the immune mechanisms which afford early protection following vaccination are unclear. The aqueous O₁ Lausanne FMD vaccine formulation used in trial 1

gave a more rapid antibody response than the similarly formulated C₁ Oberbayern antigen used in trial 3. Such difference in antibody response may in part be due to the antigen payload and/or serotype. Nevertheless, despite the absence of antibody at the early time points following vaccination, the C₁ Oberbayern aqueous formulation was still efficient at inducing rapid and early immunity to infection. Examples of animals with serum antibody titres that were either undetectable or at levels that conventionally would not be considered protective, but were nonetheless immune to FMDV challenge have previously been reported [4, 11, 29]. Several factors may be important in generating such protection including the dynamic state of the immune system following vaccination [19], the lag period after challenge for primary viral replication and subsequent replication at secondary sites [38] and the stimulation and interaction

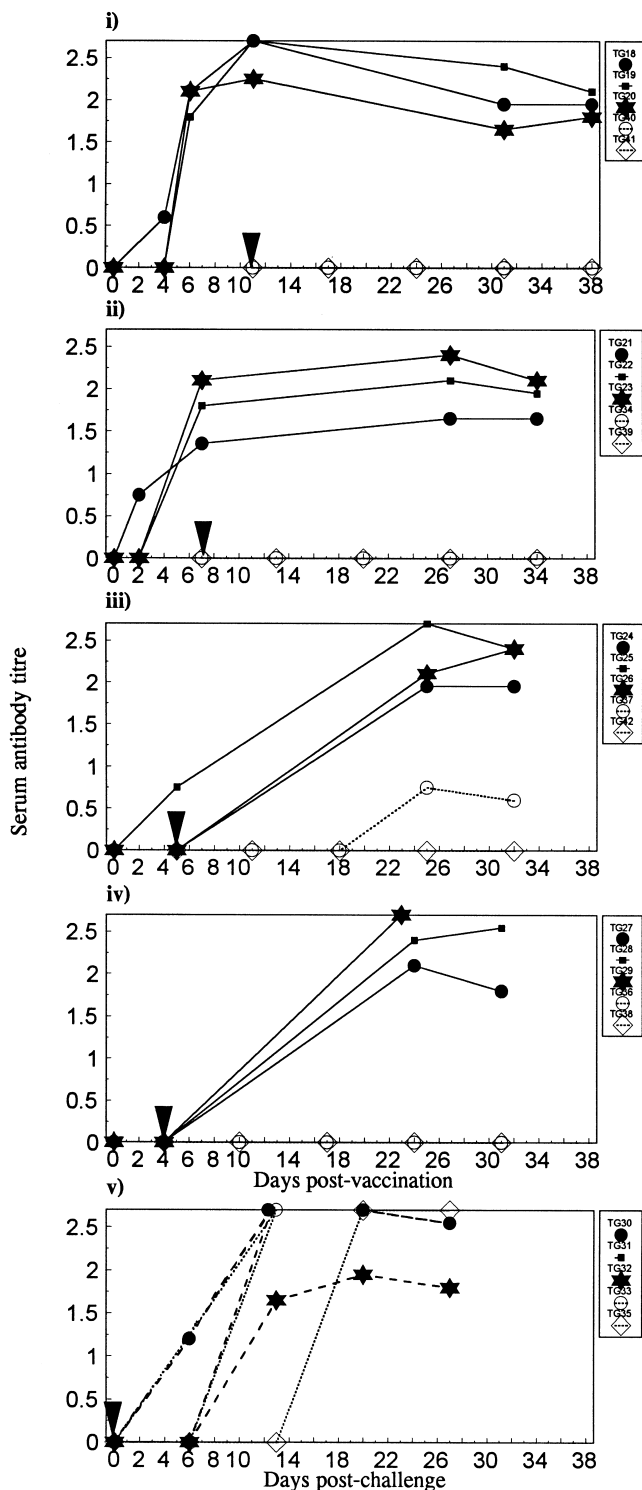


Fig. 3. Serum antibody responses in sheep following vaccination and challenge with FMDV type C1 Oberbayern as measured by microneutralisation assay. Sheep were vaccinated at (i) 11, (ii) 7, (iii) 5 and (iv) 4 days prior to challenge or (v) remained unvaccinated prior to challenge. Arrows mark the day of challenge. Solid lines represent vaccinated sheep, dashed lines the non-vaccinated sheep and dotted lines represent susceptible in-contacts housed with the challenged vaccinated and non-vaccinated sheep.

of adaptive and innate immunity, either systemically or in the mucosa of the upper respiratory tract. Indeed, the nature and importance of non-antibody-mediated immunity may be paramount to the development of future vaccines designed to generate early protection. Further work needs to be done to elucidate early immune mechanisms including non-specific elements of the immune system.

The effect of emergency vaccination on local virus replication and excretion was also examined in this study. Inhalation of airborne FMDV, leading to replication in the respiratory tract is considered to be the most common route by which livestock become infected. Hence, ideally an emergency vaccine should protect the respiratory tract against infection, or if not, it should produce a rapid and marked reduction in local virus replication and subsequent excretion to be effective in preventing spread to susceptible livestock. FMD vaccines can protect sheep from clinical disease and can also result in the reduction of virus replication within the respiratory tract and duration of FMDV excretion [12,19]. Both vaccine formulations used in trial 1 reduced the incidence of virus replication and the number of animals infected up to 28 days when compared with the non-vaccinated animals. With the exception of the sheep immunised at 4 days prior to challenge with oil adjuvanted vaccine there were examples in all the groups of animals remaining completely free of virus in the oesophageal–pharyngeal tract for the duration of the trial. The importance of these observations was examined further in trials 2 and 3 to assess what effect such impairment on the replication of FMDV in the oropharynx had on transmission to susceptible in-contact animals. The results from trial 2 showed that transmission occurred in sheep in contact with groups vaccinated at 10, 6 and 3 days before challenge as shown by the development of viraemia and/or pyrexia, sero-conversion and virus isolated from oesophageal–pharyngeal fluid samples. Transmission from sheep vaccinated at 10 and 6 days prior to challenge was logical considering the repeated isolation of virus from their oesophageal–pharyngeal samples. However, no virus isolations were observed in the oesophageal–pharyngeal samples from the 3 day vaccinees, despite evidence of transmission to the in-contact animal SV56. Animals immunised 4 days prior to challenge were non-viraemic and free of virus in the respiratory tract and no viral transmission was evident. Interestingly, the two in-contacts housed with the non-vaccinates were also non-viraemic, non-pyrexial and did not sero-convert, however virus was isolated on two occasions, from both animals, indicating that viral transmission had still occurred. Only two vaccinees

(SV17 and SV42) and one non-vaccinate (SV59) were FMDV positive at 28 days.

The third trial was more conclusive. Only one vaccinated animal (TG25), in the group vaccinated 5 days before challenge, yielded virus from oesophageal–pharyngeal samples up to 27 days post-challenge, but no virus was isolated from the in-contacts housed with this sheep. Assuming that the limited virus isolations were not due to the inadequacies of traditional techniques alluded to by De Clercq et al. [40], these results suggest that this particular aqueous formulation was efficient in inhibiting local virus replication in the oropharynx of vaccinated animals following challenge, and thereby reduced contact transmission. Further confirmation that virus transmission was inhibited was the absence of any sero-conversion in all of the in-contact sheep.

Overall, these results suggest that both oil and aqueous emergency vaccines previously shown to be efficacious in eliciting early protective immunity in cattle and pigs provide a rapid and protective immunity in sheep as early as three days following vaccination. Moreover, these highly potent emergency vaccines can reduce virus replication in the oropharynx, consequently decreasing virus excretion, and thereby limiting the transmission of the disease to susceptible non-vaccinated sheep.

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