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# A review of emergency foot-and-mouth disease (FMD) vaccines

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**Abstract**

The primary objectives of this paper are to describe emergency foot-and-mouth disease (FMD) vaccines and review literature on emergency vaccine efficacy to protect animals against (1) clinical signs and (2) infection (local virus replication). The reviewed experiments suggest that in cattle, sheep and pigs, the vaccine could be effective in preventing disease within 4–5 days post-vaccination. These studies also suggest that the risk of spreading infection decreases as the interval between vaccine and challenge increases and that vaccination could reduce the amount of virus excreted compared to non-vaccinated animals. We suggest areas of future research to improve our knowledge of emergency vaccines. 

*Keywords:* Foot-and-mouth disease; Vaccine; Virus

## 1. Introduction

In the last two decades, many foot-and-mouth disease (FMD)-free countries have established strategic reserves of FMD vaccines for use in the event of an outbreak. Whilst these countries rely primarily on slaughter, movement restrictions and zoo-sanitary measures for control, such vaccines provide a supplement to disease control [1]. The most economical and popular method for holding reserves or banks of vaccine is by storing concentrated antigens indefinitely over liquid nitrogen for rapid formulation. Good examples include the International Vaccine Bank (IVB) in Pirbright, the North American Vaccine Bank and the European Union Vaccine Bank. Unlike conventional FMD vaccines, "emergency" vaccines are of higher potency (see later) (usually $\geq$6 protective dose 50 ($PD_{50}$)) which could indicate both rapid protective immunity and wider antigenic coverage within FMD serotypes. Such high potency is usually the result of increasing the antigen load per dose.

There is a large variability in serotypes and strains of FMD viruses that present various immunogenic properties [2]. Therefore, it is important that the "emergency" vaccine used produces cross-immunity with the field strain responsible for an outbreak. The antibody response developed following immunisation with $O_1$ Manisa vaccine produced by the IVB has been shown in vitro to neutralise the field Pan-Asia strain and to protect experimentally animals from the disease currently circulating in the UK (Barnett, unpublished results). Therefore, this paper will be particularly focused on the use of $O_1$ Manisa emergency vaccine.

Only a handful of East European countries are known to have used an emergency vaccine recently. These high potency FMD emergency vaccines were used in combination with movement restrictions, slaughter and ring-vaccination around the affected areas (Leforban, personal communication). Unfortunately, no specific surveillance programme to evaluate the effectiveness [1] of the emergency FMD vaccine against disease [2] or infection [3] in the vaccinated animals has been reported yet. In addition, the programmes implemented included destroying vaccinated animals after the spread of the disease had been under control. This complicates the possibility of assessing the long-term effectiveness of emergency FMD vaccines. Therefore, to date, most of the research has been experimental and primarily limited to the studies undertaken at the IVB. Research has been geared toward immediate use of emergency vaccines in a strategic emergency ring-vaccination where the perceived threat is from air-borne dispersal of virus. The most intensive area of research has been the development of protection against disease conferred by these "emergency"

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[1] Effectiveness: protection provided by the vaccine when used in the field.
[2] Disease: clinical signs or viraemia.
[3] Infection: local replication or carriage.

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FMD vaccines in the three main target species (cattle, sheep and pigs). The extent to which such vaccines could prevent the spread of disease (protection against infection) has also been assessed.

Given the infectious nature of the disease, housing required to accommodate the work, limits on space, the cost of large animals and ethical issues involving the infection of several target species, the experiments conducted to evaluate the potency and efficacy of emergency vaccine could only be conducted with a limited number of animals. Such limitations in turn reduce the possibility of finding statistically significant results. Where possible, research and surveillance of emergency vaccinated animals in the field would add substantially to knowledge on the effectiveness of high potency FMD vaccines. All the results in this paper are based on small numbers and should, therefore, be taken as indicators of the efficacy of emergency vaccine and not as broadly generalisable results. In contrast to human vaccines, where few controlled challenge studies have been undertaken, however, it is more feasible and acceptable to challenge a limited number of animals from the target host species.

The primary objectives of this paper are to describe the emergency FMD vaccines and the meaning of their potency value, review the available literature on the vaccine efficacy to protect animals against (1) FMD clinical signs and (2) local FMD virus replication experimentally and under potential field application. We also suggest areas of future research to improve our knowledge on the effectiveness of emergency vaccines. Throughout, we will distinguish between the ability of a vaccine to protect against the disease and its accompanying clinical signs as compared to total protection against infection (indicated by the absence of local replication of the virus), the so-called sterile immunity. This distinction is important because a vaccine protecting all animals against the clinical disease but not necessarily against the infection might not prevent the spread of the virus, thus failing to control the epidemic.

## 2. Potency of FMD emergency vaccines

### 2.1. Definition and estimation of potency

The potency is expressed as the number of 50% cattle protective doses ($PD_{50}$) contained in the dose stated on the vaccine label [3]. Potency is strictly meant as an indicator of the capacity of the vaccine to induce the type of immunity sought—for $PD_{50}$ analyses, this would be protective immunity. The FDA defines potency as "the specific ability or capacity of the product, as indicated by appropriate laboratory tests or by adequately controlled clinical data obtained through the administration of the product in the manner intended, to effect a given result" [5].

To evaluate the potency of a specific strain of FMD virus vaccine, groups of usually five–eight cattle no less than 6 months old are vaccinated with reduced dose volumes or varying vaccine dilutions. The cattle are challenged with a vaccine-to-challenge interval of 21 days using an inoculation on the surface of the tongue of 10 000 $ID_{50}$ virulent bovine FMD virus of the same type and sub-type as the one contained in the vaccine. Two main methods have been used to dilute the vaccine. With the first method, used at the IVB, only the antigen component is diluted so that the same volume of vaccine is injected. The IVB uses dilutions of 1/2, 1/10 and 1/50. In the second method, used by the European Pharmacopoeia, the full vaccine formulation is diluted so that the vaccine is given with decreasing volumes. This method usually uses dilutions of 1/1, 1/4 and 1/16. Cattle are then closely monitored for 10 and 8 days post-challenge according to the IVB and the European Pharmacopoeia, respectively, for the appearance of FMD lesions on the feet and mouth. The proportion of animals vaccinated with all dilutions that do not generalise and develop lesions is used to calculate the potency of the vaccine usually by the Karber method [6]. The Karber method uses the formula $PD_{50} = 10^{\log(\text{dilution}^* \sum_G (\%\ \text{protected}_g) - 0.5)}$ where the dilution corresponds to the vaccine/antigen dilution, % $\text{protected}_g$ to the proportion of animals protected in dilution group *g*. It should be noted that the dilution at which the clinical signs are observed and the antigen level contained in the vaccine do not modify the value of the $PD_{50}$. At the IVB, this value is multiplied by two because the dilution series begins with 1/2 of the antigen payload and not the full dose of the vaccine. Fig. 1 illustrates the relationship between the proportion of animals protected and the $PD_{50}$, using both the IVB and the European Pharmacopoeia methods to estimate the values of the $PD_{50}$. It also shows the non-linear association between the percentage of cattle protected and the $PD_{50}$ and the different values of $PD_{50}$ obtained with the same percentage of cattle protected when using the two methodologies. Generally though, the larger the percentage of protection against clinical signs, the larger the $PD_{50}$ value.

### 2.2. Potency tests for $O_1$ Manisa emergency vaccines

Cox and Barnett reported the $O_1$ Manisa vaccine to have a $PD_{50}$ of at least 112 (Table 1). In other words, the antigen content in the vaccine formulation could be diluted 112 times or more and still protect 50% of cattle against challenge 21 days after vaccination.

### 2.3. Potency tests for other FMD emergency vaccines

Table 1 also indicates the $PD_{50}$ estimated for other antigen strains of FMD held in the IVB. Even within the same serotype, the $PD_{50}$ can vary widely. The range of values observed against the payload of antigen reflects the various immunogenicities of the different strains and additionally could be influenced by the virulence of the virus challenge itself.

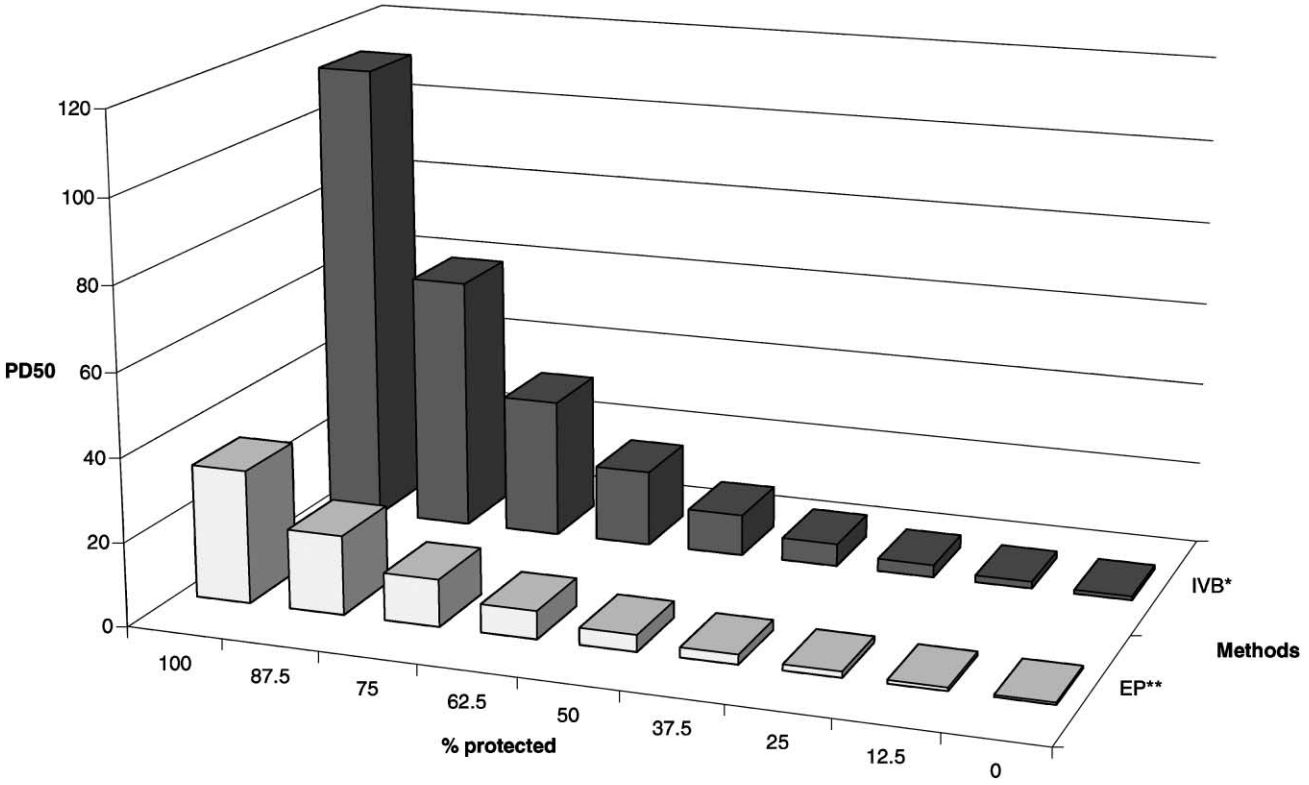


Fig. 1. Association between the method used (European Pharmacopoeia and IVB) to determine the potency of FMD emergency vaccine, the percentage of cattle protected against FMD challenge and the potency ($PD_{50}$). IVB*: IVB method to determine the potency of emergency FMD vaccine using antigen dilutions of 1/2, 1/10 and 1/50 (fully shaded bar). EP**: European Pharmacopoiea method to determine the potency of emergency FMD vaccine using reduced volume vaccine dilutions of 1/1, 1/4 and 1/16 (hatched bar).

Table 1
Formulations, concentration of the vaccine, potency value ($PD_{50}$) for the strains of emergency vaccines held at the IVB, Pirbright, UK

| Strain | Formulation | μg antigen/vaccine dose | $PD_{50}$ | Reference |
|---|---|---|---|---|
| $O_1$ Manisa | $Al(OH)_3$/saponin | 2.40 | ≥112 | Barnett, unpublished data |
| $C_1$ Oberbayern | $Al(OH)_3$/saponin | 1.40 | ≥112 | [1] |
| $A_{15}$ Thailand | $Al(OH)_3$/saponin | 8.94 | ≥112 | Barnett, unpublished data |
| $A_{22}$ Iraq | $Al(OH)_3$/saponin | 1.25 | 75 | Barnett, unpublished data |
| Asia 1 India | $Al(OH)_3$/saponin | 5.25 | 61 | [9] |
| $O_1$ Lausanne | $Al(OH)_3$/saponin | 3.05 | 41 | [1] |
| $A_{24}$ Cruzeiro (4219) | $Al(OH)_3$/saponin | 12.47 | 18.3 | Barnett, unpublished data |

### *2.4. Formulations of FMD emergency vaccines*

The effective formulation of FMD inactivated vaccines requires adjuvants and $Al(OH)_3$/saponin (for ruminants) and mineral oil-based formulations (for pigs and ruminants) have been widely employed in experimental studies. However, depending on the formulation, stability of the antigen and its "shelf life" can vary greatly. For example, the $Al(OH)_3$/saponin formulation currently used by the IVB has a very short "shelf life" compared to oil-adjuvanted formulations [7,8]. An intensive research programme by the IVB resulted in the adoption of Montanide ISA 206 oil adjuvant for its emergency vaccines. This is also used in the commercial sector and primarily gives the IVB the versatility to formulate a vaccine that is effective in pigs. The IVB is currently licensed to produce an aqueous formula, shown to be less effective in pigs, but is aiming toward the licensing of its oil-based vaccine.

## 3. Efficacy of FMD emergency vaccine to protect experimentally against clinical signs and viraemia

### *3.1. Definition and estimation of efficacy*

Last defines efficacy as "in clinical epidemiology, the extent to which a specific intervention, procedure, regimen or service produces a beneficial result under ideal conditions;

the benefit or utility to the individual or the population of the service, treatment regimen or intervention" [4]. The efficacy of a vaccine is usually assessed in clinical trials through three phases. In phase I, the safety of the product is tested on a limited number of animals. In phase II, a limited number of animals are vaccinated and then challenged with the micro-organism being evaluated. The results described in this paper roughly correspond to phase II clinical trials that have limited generalisability because of the small number of animals tested. In phase III trials, a large number of animals in the field are vaccinated to estimate both the efficacy and the effectiveness against the disease and the infection. Only in phase III, it is possible to obtain more generalisable conclusions. There has never been, to our knowledge, a phase III trial for emergency FMD vaccines.

### 3.2. $O_1$ Manisa emergency vaccines

To our knowledge, there is no published data on early protection against disease for $O_1$ Manisa using challenged animals.

### 3.3. Other emergency vaccines

Experiments have been conducted at the IVB to evaluate early protection against disease using infected pigs to simulate indirect contact (air-borne). The general challenge involves infection of three pigs as the source of air-borne transmission, generally 72, 48 and 24 h prior to challenge, with $10^4$ cattle $ID_{50}$ of the appropriate FMD virus, homologous to the strain used in the vaccine under investigation. Vaccinated and non-vaccinated control animals are allowed to mingle during 1–4 h, around the three infected pigs physically separated to restrict transmission to air-borne route only. After the challenge period has been completed, the infected pigs used for challenge are removed. Vaccinated animals are then re-housed to their original immunisation groups and non-vaccinated control animals kept isolated from one another and from the vaccinated animals to avoid over-challenge. Vaccinated and non-vaccinated control animals are then monitored for various periods of time to evaluate the development of clinical signs of the disease. The protocol used for the indirect contact challenge has evolved through time with the improvement to the experimental approach and the containment facilities used. Early experiments were performed in high containment facilities with isolation boxes not independently supplied with air and restricted capacity that limited physical separation between vaccinated and non-vaccinated control animals. This led to over-challenge of both vaccinated and non-vaccinated controls in these early experiments [1] (Section 3.3.3).

#### 3.3.1. Cattle

Initial studies have demonstrated that both oil and $Al(OH)_3$/saponin-adjuvanted monovalent vaccines containing either $O_1$ Lausanne or $C_1$ Oberbayern were capable of protecting cattle against disease with vaccine-to-challenge interval as short as 4 days [1]. None of the 28 cattle vaccinated with vaccine-to-challenge intervals of 4–21 days with oil or Al(OH)3/saponin vaccines showed any clinical signs 10 days post-challenge, whereas, all four control cattle showed clinical signs. The early protection in the group with a vaccine-to-challenge interval of 4 days was achieved in the absence of significant quantities of neutralising or virus-specific antibodies. It should, however, be noted that the same cattle were used to test the protection of, first $O_1$ Lausanne vaccine against the disease and second, $C_1$ Oberbayern vaccine "approximately" 4 months after the start to the initial experiment. Thirteen out of 28 animals still had detectable neutralising serum antibodies when vaccinated with $C_1$ Oberbayern. Therefore, there could have been some residual cross-immunity modifying these results [1]. In fact, cross-immunity against serotypes $A_{22}$ and C has been reported in calves and lambs vaccinated against $O_1$ serotypes [11] and would have some age and genetic components [12].

Another group of cattle were also protected against disease when vaccinated against Asia 1 India 8/79 with a vaccine-to-challenge interval of only 2 days when using an ISA 206 oil formulation and with a vaccine-to-challenge interval of only 3 days when using an $Al(OH)_3$ formulation, suggesting that ISA 206 "outperformed" the aqueous vaccine [13]. The cattle with a vaccine-to-challenge interval of only 3 days did not show any detectable specific neutralising serum antibody prior to challenge [13].

#### 3.3.2. Sheep

Sheep are naturally more resistant to clinical FMD signs than other species [2,15]. Therefore, the presence of viraemia in both the vaccinated and control groups is thought to be a better indicator of active "disease" in that species. All vaccinated sheep showed protection against clinical disease and viraemia after an air-borne challenge with a vaccine-to-challenge interval as short as 3 days for $O_1$ Lausanne and a vaccine-to-challenge interval as short as 4 days for $C_1$ Oberbayern and Asia 1 India [9]. For each FMD virus strain, 24 sheep had been vaccinated with vaccine-to-challenge intervals of 3, 4, 6 and 10 days with $Al(OH)_3$ or ISA 206-adjuvanted vaccine formulations. In the control group for the $O_1$ Lausanne and the Asia 1 India strains, as expected, none of the seven control animals showed clinical signs of the disease but six developed viraemia. For the $C_1$ Oberbayern strain, two control sheep showed clinical signs of FMD, developed pyrexia and viraemia, whereas, the third one remained free of signs. All control sheep had seroconverted 7 days post-challenge.

#### 3.3.3. Pigs

Emergency vaccination of 28 pigs with $C_1$ Oberbayern using ISA 206 and 25 oil-adjuvanted formulations given with vaccine-to-challenge intervals of 4–21 days conferred protection against disease, whereas, all three control animals developed clinical signs 4 days post-challenge [14].

However, also in that study, another group of animals vaccinated with vaccine-to-challenge intervals of 2 and 5 days did develop clinical signs of FMD. Therefore, clinical signs can occur in animals vaccinated with vaccine-to-challenge intervals of up to 5 days [14]. This result contrasts with what was found using the same adjuvants and challenge methods using $O_1$ Lausanne [1]. In that experiment, only the 12 pigs vaccinated with vaccine-to-challenge intervals of at least 21 days were free of clinical signs. Nineteen of the 24 pigs vaccinated with vaccine-to-challenge intervals of $\leq$16 days developed clinical signs [1]. However, it should be noted that the facilities that were used to perform these early experiments were not ideal. Animals could not be housed in their individual vaccination groups and more importantly could not be separated from the unvaccinated controls. Therefore, these animals would have been subjected to re-challenge by the more severe direct contact route, from time to time, which makes interpretation of the results more difficult. These results underline the importance of experimental design and the holding accommodation.

### *3.4. General comments on the ability of emergency vaccines to protect experimentally against clinical signs*

The experiments described above suggest that, in all species tested, the vaccine could be effective in preventing disease with a vaccine-to-challenge interval as short as 4–5 days under an indirect aerosol challenge. This does not mean, however, that we can claim with certainty that all animals would be protected against the disease 4–5 days after vaccination given that variation could be introduced by the vaccine strain, the animal species, the breed and the virulence of the challenge strain. In addition, the experiments described above do not inform about the effect of direct contact between animals, which would be likely to occur in the field situation. In field conditions, it would, however, be more important for the vaccine to protect at an early stage against virus replication in the oro-pharynx (see later).

### *3.5. Duration of protection against clinical signs and of antibodies levels*

#### *3.5.1. Cattle*

There has been little study on the long-term antibody response and protection against disease for emergency vaccines in cattle. FMD virus specific antibody responses were monitored for 92 days following a single intramuscular vaccination involving the use of Montanide ISA 206 oil adjuvant with an old IVB antigen (A24 Cruzeiro lot 625 $PD_{50} = 8$) [7]. Given that this vaccine was of low potency in comparison to all the other current IVB antigens, the antibody response by 92 days was at a level similar to that measured 7 days post-vaccination. In an another study, where the cattle were vaccinated with $O_1$ Lausanne and subsequently ("approximately" 4 months later), vaccinated with $C_1$ Oberbayern, antibodies against $O_1$ Lausanne were still present in cattle initially vaccinated with an $Al(OH)_3$ or oil formulation [1]. No challenge against that strain was done at this latter time point, so it is difficult to say if the cattle would have been protected against disease or not.

#### *3.5.2. Sheep*

No study has looked at the long-term protection of emergency vaccine against disease. However, the antibody levels against $O_1$ Manisa and $A_{22}$ Iraq could remain high up to 168 days post-vaccination [10]. The levels remained higher when an oil-based formulation was used. In one sheep, the neutralising antibodies against $O_1$ Manisa became undetectable after 66 days when the aqueous formulation was used [10]. The antibody levels reached higher peaks and remained higher with $A_{22}$ Iraq vaccine, which has a potency of 75 compared to >112 for $O_1$ Manisa.

#### *3.5.3. Pigs*

A study was carried out to examine the longevity of the humoral response following "emergency" vaccination in pigs. A single formulation of either $O_1$ Lausanne or $C_1$ Oberbayern inactivated antigen in a double oil-emulsion (ISA 206) was used and the antibody response monitored for 141 days. Results indicated that the antibody levels were maintained for the duration of the trial, with peak antibody levels between 21 and 28 days post-vaccination [10]. A repeat trial was performed in which 7 months (218 days) after vaccination all pigs were challenged by indirect contact for 2 h with clinically diseased pigs infected with $C_1$ Oberbayern. Pigs that had received a homologous challenge showed no signs of clinical disease (Barnett, unpublished results) suggesting that protective immunity against disease could be maintained for at least this period of time in this species using the oil adjuvant formulation.

### *3.6. Comments on the duration of protection against clinical signs and of antibody levels*

The cellular and humoral immunological response to infection and to the vaccine remains poorly described [2,16,17], but cellular immunity has been reported to play a role [18,19]. The studies described above have used humoral immunity response as indicators of the overall immunity to the vaccine. More studies on the cellular and other types of immunological responses are needed. The duration of protective immunity following emergency high potency vaccination of cattle should be examined.

## 4. Efficacy of FMD emergency vaccines to protect experimentally against local replication of the virus

### *4.1. $O_1$ Manisa emergency FMD vaccine*

To our knowledge, there has been no study assessing the early protection of emergency vaccinated animals in terms

of local replication of the virus or of becoming carriers after challenge by $O_1$ Manisa.

### 4.2. Other emergency vaccines

This was examined through the same experiments used to assess early protection against disease. Heparinised blood and oesophageal–pharyngeal fluid samples (probang samples) were collected at regular intervals for viral isolation 1–3 months post-challenge. Detailed data for cattle and sheep are summarised in Tables 2 and 3.

#### 4.2.1. Cattle

The level of virus persistence in vaccinated and non-vaccinated cattle after homologous virus challenge has been studied [1]. The results using $Al(OH)_3$/saponin formulation are summarised in Table 2. When the animals were first vaccinated against and then challenged with $O_1$ Lausanne, with vaccine-to-challenge intervals ranging from 4–21 days, all 27 cattle showed cytopathic effect on at least one probang test tube on at least one occasion during the post-challenge period of 10 weeks. Since, there were fewer tubes with cytopathic effects when the vaccine-to-challenge interval was 16 or 21 days, the authors concluded that animals with shorter vaccine-to-challenge intervals were more liable to become "carriers". The authors also observed that virus excretion was reduced more effectively by "emergency" vaccines, particularly $Al(OH)_3$/saponin-based formulations, administered with a longer vaccine-to-challenge interval [1]. In a later early protection experiment, Salt et al. [13]

Table 2
Studies on the isolation of FMD virus in the oesophageal–pharyngeal fluids in cattle (predominantly Friesian and Friesian/Hereford crosses aged 6–9 months) after vaccination and challenge using indirect contact with infected pigs during 1 h

| Vaccine strain | Adjuvant formulation | IVC (days)[a] | Virus isolation in oesophageal–pharyngeal fluids (# positive/# animals tested)[b] | | | | | | | | | | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1[c] | 2[c] | 3[c] | 4[c] | 5[c] | 6[c] | 7[c] | 8[c] | 9[c] | 10[c] | |
| $O_1$ Lausanne | $Al(OH)_3$ | 21 | 0/2 | 1/2 | 2/2 | 0/2 | 0/2 | 0/2 | 0/2 | 0/2 | 0/2 | 1/2 | [1] |
| | ISA 206 | 21 | 3/3 | 3/3 | 3/3 | 1/3 | 1/3 | 0/3 | 3/3 | 1/2 | 3/3 | 2/3 | [1] |
| | $Al(OH)_3$ | 16 | 2/3 | 3/3 | 3/3 | 2/3 | 3/3 | 1/2 | 1/3 | 1/3 | 2/3 | 1/3 | [1] |
| | ISA 206 | 16 | 0/2 | 3/3 | 1/3 | 1/3 | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 | [1] |
| | $Al(OH)_3$ | 12 | 1/3 | 3/3 | 3/3 | 1/3 | 1/3 | 1/3 | 3/3 | 1/3 | 1/3 | 1/3 | [1] |
| | ISA 206 | 12 | 3/3 | 2/2 | 3/3 | 2/3 | 2/3 | 1/2 | 1/3 | 1/3 | 2/3 | 2/3 | [1] |
| | $Al(OH)_3$ | 8 | 2/3 | 3/3 | 3/3 | 2/3 | 2/3 | 2/3 | 1/2 | 2/3 | 2/3 | 2/3 | [1] |
| | ISA 206 | 8 | 2/3 | 3/3 | 3/3 | 1/3 | 3/3 | 1/3 | 2/3 | 3/3 | 3/3 | 3/3 | [1] |
| | $Al(OH)_3$ | 4 | 1/2 | 1/1 | 2/2 | 1/2 | 1/2 | 1/2 | 1/2 | 0/0 | 1/2 | 1/1 | [1] |
| | ISA 206 | 4 | 2/2 | 2/2 | 2/2 | 2/2 | 2/2 | 2/2 | 2/2 | 0/2 | 2/2 | 2/2 | [1] |
| | NV[d] | – | 1/1 | 2/2 | 2/2 | 2/2 | 2/2 | 2/2 | 2/2 | 2/2 | 2/2 | 1/1 | [1] |
| $C_1$ Oberbayern | $Al(OH)_3$ | 21 | 1/3 | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 | 0/1 | 0/3 | 0/3 | [1] |
| | ISA 206 | 21 | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 | 1/3 | 0/3 | 0/3 | [1] |
| | $Al(OH)_3$ | 16 | 0/3 | 0/2 | 0/2 | 0/2 | 0/2 | 0/2 | 0/2 | 0/2 | 0/2 | 0/2 | [1] |
| | ISA 206 | 16 | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 | [1] |
| | $Al(OH)_3$ | 12 | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 | [1] |
| | ISA 206 | 12 | 1/3 | 1/3 | 3/3 | 1/3 | 0/3 | 0/3 | 0/2 | 0/2 | 0/2 | 0/2 | [1] |
| | $Al(OH)_3$ | 8 | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 | [1] |
| | ISA 206 | 8 | 1/3 | 0/3 | 1/3 | 0/3 | 0/3 | 0/3 | 1/3 | 1/3 | 0/3 | 0/3 | [1] |
| | $Al(OH)_3$ | 4 | 0/2 | 0/2 | 1/2 | 1/2 | 1/2 | 0/2 | 0/2 | 0/2 | 0/2 | 0/2 | [1] |
| | ISA 206 | 4 | 0/2 | 0/2 | 1/2 | 0/2 | 0/2 | 0/2 | 0/2 | 0/2 | 0/1 | 0/2 | [1] |
| | NV | – | 1/2 | 2/2 | 2/2 | 2/2 | 2/2 | 0/2 | 1/2 | 0/2 | 1/2 | 0/2 | [1] |
| Asia 1 India 8/79 | $Al(OH)_3$ | 12 | 0/2 | | | 0/2 | | | | | | | [13] |
| | ISA 206 | 12 | NA[e] | | | 1/2 | | | | | | | [13] |
| | $Al(OH)_3$ | 8 | NA | | | 1/3 | | | | | | | [13] |
| | ISA 206 | 8 | 3/3 | | | 2/3 | | | | | | | [13] |
| | $Al(OH)_3$ | 4 | 3/3 | | | 3/3 | | | | | | | [13] |
| | ISA 206 | 4 | 2/2 | | | 2/2 | | | | | | | [13] |
| | $Al(OH)_3$ | 3 | NA | | | 2/3 | | | | | | | [13] |
| | ISA 206 | 3 | NA | | | 1/3 | | | | | | | [13] |
| | $Al(OH)_3$ | 2 | NA | | | 3/3 | | | | | | | [13] |
| | ISA 206 | 2 | 3/3 | | | 3/3 | | | | | | | [13] |
| | NV | – | 2/2 | | | ND[f] | | | | | | | [13] |

[a] IVC: interval vaccine-to-challenge.
[b] Oesophageal–pharyngeal samples (probang samples). Cytopathogenic effect was measured and the virus specificity of any cytopathogenic effect was confirmed by ELISA.
[c] No. of weeks post-challenge.
[d] NV: not vaccinated.
[e] NA: not available.
[f] ND: not done.

Table 3
Studies on the isolation of FMD virus in the oesophageal–pharyngeal fluids in sheep (Polled Dorset Horn aged 6–12 months) after vaccination and challenge using indirect contact with infected pigs during 2 h ($O_1$ Lausanne) or 4 h ($C_1$ Oberbayern and Asia 1 India)

| Vaccine strain | Adjuvant formulation | IVC (days)[a] | Virus isolation in oesophageal–pharyngeal fluids (# positive/# animals)[b] | | | | | | | | | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 2[c] | 4[c] | 6[c] | 7[c] | 9[c] | 13[c] | 20[c] | 27[c] | 28[c] | |
| $O_1$ Lausanne | $Al(OH)_3$ | 10 | 0/3 | 0/3 | | 0/3 | 0/3 | | | | 0/3 | [9,20] |
| | ISA 206 | 10 | 0/3 | 0/3 | | 0/3 | 0/3 | | | | 0/3 | [9,20] |
| | $Al(OH)_3$ | 6 | 1/3 | 1/3 | | 1/3 | 1/3 | | | | 1/3 | [9,20] |
| | ISA 206 | 6 | 2/3 | 2/3 | | 1/3 | 1/3 | | | | 0/3 | [9,20] |
| | $Al(OH)_3$ | 4 | 0/3 | 0/3 | | 0/3 | 0/3 | | | | 0/3 | [9,20] |
| | ISA 206 | 4 | 1/3 | 3/3 | | 3/3 | 2/3 | | | | 1/3 | [9,20] |
| | $Al(OH)_3$ | 3 | 2/3 | 2/3 | | 1/3 | 1/3 | | | | 1/3 | [9,20] |
| | ISA 206 | 3 | 2/3 | 0/3 | | 0/3 | 0/3 | | | | 0/3 | [9,20] |
| | NV[d] | – | 1/4 | 4/4 | | 4/4 | 3/4 | | | | 4/4 | [9,20] |
| Asia 1 India | ISA 206 | 10 | 2/3 | 2/3 | | 2/3 | 2/3 | | | | 1/3 | [9] |
| | Contact[e] | 10 | 0/2 | 0/2 | | 1/2 | 1/2 | | | | ND[f] | [9] |
| | ISA 206 | 6 | 2/3 | 1/3 | | 0/3 | 1/3 | | | | 1/3 | [9] |
| | Contact | 6 | 0/2 | 1/2 | | 1/2 | 1/2 | | | | ND | [9] |
| | ISA 206 | 4 | 0/3 | 0/3 | | 0/3 | 0/3 | | | | 0/3 | [9] |
| | Contact | 4 | 0/2 | 0/2 | | 0/2 | 0/2 | | | | ND | [9] |
| | ISA 206 | 3 | 0/3 | 0/3 | | 0/3 | 0/3 | | | | 0/3 | [9] |
| | Contact | 3 | 0/2 | 0/2 | | 1/2 | 1/2 | | | | ND | [9] |
| | NV | – | 2/3 | 3/3 | | 3/3 | 2/3 | | | | 1/3 | [9] |
| | Contact | – | 2/2 | 2/2 | | 0/2 | 0/2 | | | | ND | [9] |
| $C_1$ Oberbayern | $Al(OH)_3$ | 11 | 0/3 | | 0/3 | | | 0/3 | 0/3 | 0/3 | | [9,20] |
| | Contact | 11 | 0/2 | | 0/2 | | | 0/2 | 0/2 | 0/2 | | [9,20] |
| | $Al(OH)_3$ | 7 | 0/3 | | 0/3 | | | 0/3 | 0/3 | 0/3 | | [9,20] |
| | Contact | 7 | 0/2 | | 0/2 | | | 0/2 | 0/2 | 0/2 | | [9,20] |
| | $Al(OH)_3$ | 5 | 1/3 | | 1/3 | | | 1/3 | 1/3 | 1/3 | | [9,20] |
| | Contact | 5 | 0/2 | | 0/2 | | | 0/2 | 0/2 | 0/2 | | [9,20] |
| | $Al(OH)_3$ | 4 | 0/3 | | 0/3 | | | 0/3 | 0/3 | 0/3 | | [9,20] |
| | Contact | 4 | 0/2 | | 0/2 | | | 0/2 | 0/2 | 0/2 | | [9,20] |
| | NV | – | 2/3 | | 1/3 | | | 1/3 | 1/3 | 1/3 | | [9,20] |
| | Contact | – | 0/2 | | 0/2 | | | 2/2 | 0/2 | 0/2 | | [9,20] |

[a] IVC: interval vaccine-to-challenge.
[b] Oesophageal–pharyngeal samples (probang samples). Cytopathogenic effect was measured and the virus specificity of any cytopathogenic effect was confirmed by ELISA.
[c] No. of days post-challenge.
[d] NV: not vaccinated.
[e] Contact: each contact animals group was housed for 28 days with a specific vaccinated/challenged or non-vaccinated group.
[f] ND: not done.

suggested that this may be the result of the presence in the upper respiratory tract of secretions of humoral antibody. Re-vaccination of the same animals with another antigen ($C_1$ Oberbayern) approximately 4 months later, resulted in far fewer animals locally replicating the virus (eight cattle out of 27 presented some cytopathic effect on at least one tube at least once) [1]. In addition to the two controls, only two cattle showed local virus replication 4 weeks and none were carriers 10 weeks post-challenge, including the control animals. Although, this may have been related to the ability of the individual strains to establish a persistent state, it was conceivably the result of pre-existing immunity to the previous vaccine. Local mucosal immunity was not examined.

Another potent vaccine (Asia 1 India, $PD_{50} = 61$) was administered to cattle that were followed 30 days post-challenge for virus excretion [13]. Animals had vaccine-to-challenge intervals ranging from 2 to 12 days. At least one cattle in all vaccinated groups, except for two cattle with vaccine-to-challenge interval of 12 days, were found to be locally replicating virus 9 days post-challenge. This contrasts with the results obtained with $C_1$ Oberbayern and calls for further experiments using another highly potent vaccine in cattle to assess its effectiveness in reducing the number of animals being persistently infected and possibly becoming carriers after challenge.

### *4.2.2. Sheep*

Both oil and $Al(OH)_3$/saponin-adjuvanted vaccine formulations for $O_1$ Lausanne and $C_1$ Oberbayern were found to reduce the frequency of virus replication and the number of animals infected, when monitored up to 28 days post-challenge [9,20]. Table 3 summarises the results. All sheep with vaccine-to-challenge intervals of at least 7 days remained free of virus in the oesophageal–pharyngeal tract,

throughout the trial. However, with Asia 1 India strain, two of the three sheep with vaccine-to-challenge interval of 10 days excreted the virus for at least 9 days post-challenge. For all viral strains, a smaller proportion of vaccinated animals were excreting virus than the control animals but generally for a similar period of time [9,20]. Comparison of $O_1$ Lausanne or $C_1$ Oberbayern, using both oil and $Al(OH)_3$/saponin formulations [20], partly supported the previous observations in cattle where vaccines administered with longer vaccine-to-challenge intervals were most effective at reducing virus excretion. However, there was no tendency toward one particular adjuvant being more efficient at reducing the level of virus excretion as had previously been suggested using cattle. There was also suggesting evidence of a relationship between potency and the incidence of virus replication in the oro-pharynx [20]. Inclusion of susceptible in-contact sheep following challenge of Asia 1 India vaccinated sheep [9], demonstrated evidence of transmission, even from animals with vaccine-to-challenge interval of 10 days (one contact sheep developed viraemia in that group). With $C_1$ Oberbayern, none of the contact animals developed clinical signs or viraemia when housed with sheep with vaccine-to-challenge intervals from 3 to 10 days. This was observed even if one sheep with a vaccine-to-challenge interval of 5 days was shown to excrete virus constantly from 2 to 27 days post-challenge suggesting that the level of virus excreted was insufficient to cause clinical disease or viraemia.

#### *4.2.3. Pigs*

Virus excretion from pigs vaccinated against $C_1$ Oberbayern demonstrated that immunisation, with a vaccine-to-challenge interval of 7 days, reduced the air-borne excretion of virus to prevent contact transmission from vaccinated ($n = 3$) to in-contact pigs ($n = 2$) [14]. However, the in-contacts of pigs vaccinated with vaccine-to-challenge intervals of 5 days or less all developed clinical signs of FMD. The virus was isolated in the air of the isolation rooms from all vaccine-to-challenge interval groups. However, in the vaccine-to-challenge interval group of 7 days, the virus was isolated in the air only once, 2 days post-challenge. Therefore, vaccine-to-challenge intervals of $<5$ days did not protect pigs from spreading the infection and the disease. These results suggest that transmission could be reduced with longer vaccine-to-challenge intervals.

### *4.3. General comments on virus replication and carrier state of animals after vaccination against FMD and indirect air-borne challenge*

The studies described above suggest that the risk for animals to spread infection (or become carriers for ruminants) decreases as the interval between vaccine and challenge increases and that vaccination could reduce the amount of virus excreted compared to non-vaccinated animals. In field conditions, the interval between vaccination and challenge is impossible to know so the speed with which immunity is acquired after vaccination is of vital importance. Also, there may not always be a close antigenic relationship between the field and vaccine strains. This supports the importance of maintaining a movement restriction policy in the affected areas during and after the period of vaccination. Also, it suggests that in a situation where the slaughtering of animals in an area cannot be achieved within 48 h, vaccination could be used as a tool to reduce in part further spreading of the infection. The studies described above do show a variation according to the strain, and protection against infection and local virus replication could be better for vaccines with higher $PD_{50}$ values. In pigs, $C_1$ Oberbayern was the only strain reliably tested for effect on local virus replication and transmission and the results were encouraging as long as pigs were challenged at least 7 days after vaccination.

### *4.4. Maximum duration of protection against infection after single dose of emergency vaccine*

The use of emergency high potency vaccines as an additional control measure is normally perceived to be followed by culling vaccinated animals. However, there may be circumstances in which the slaughter of such animals is not desired. In such a situation, if there were a resurgence of disease some months later that threatened previously vaccinated animals, it would be of immense value to know the maximum duration of protective immunity following a single immunisation of these high potency vaccines.

### *4.5. Differentiation between infected and vaccinated animals*

It has been well established that FMD virus capsid protein is immunogenic and will initiate the production of neutralising antibodies in animals. The presence of structural antibodies against protein is an indication of contact with the FMD virus, through infection or vaccination [2]. Recent work has shown that non-structural proteins (NSPs) of the FMD virus could be used to differentiate between vaccinated and infected animals on a herd but not on an individual animal basis [16,21–23]. The NSP polyprotein 3ABC has been shown sensitive and specific to differentiate between infected and vaccinated animals at a herd level [16,19,24]. For conventional vaccines used repeatedly, the test is not as reliable at the individual level as FMD vaccine contains traces of NSP that can induce specific antibody to the 3ABC NSP [25,26]. In cattle, it has been observed that some vaccinated animals exposed to infection can become asymptomatic carriers without seroconverting to 3ABC NSP, especially in animals with mild viral replication in the oro-pharynx [27]. Antibody against NSP is also slower to develop and of shorter duration compared to antibody against FMDV structural proteins. Research is currently going on to develop RT−PCR tests to detect FMDV specific viral RNA in clinical samples, but these are not readily available yet for large-scale field surveys [16].

There has been one specific study in which the detection of carrier state in 60 sheep vaccinated with two strains of emergency vaccines and 15 controls was examined [28]. The high potency emergency vaccines did not stimulate NSP-3ABC responses to a sufficient level for detection in all 59 animals negative to the probang tests. In addition, in 15 out of 16 cases where virus replication and/or viraemia was observed, the 3ABC was a reliable indicator for the presence of FMD virus.

In conclusion, it would be impossible to identify individual vaccinated animals as carrier using this test after the implementation of a vaccination programme. The virus excretion by carriers is intermittent, therefore, even testing the whole vaccinated population for virus—which is not feasible—would not solve the problem. Nevertheless, the data indicate that NSP serology would be useful as a herd test to detect active infection that had not been detected by clinical inspection.

## 5. Conclusions and considerations for future research

High potency FMD emergency vaccines have been shown experimentally to prevent animals from developing signs of FMD or viraemia when vaccinated at least 3–5 days before challenge. To date, high potency FMD emergency vaccines have been used to help animal health authorities to gain time when stamping out infected and dangerous contact premises. Therefore, the long-term effectiveness of emergency vaccines in the field remains unknown.

Clearly from the question that have been raised over the past few months on the use of emergency FMD vaccines for the current UK FMD outbreak and the lessons that have been learnt in the field, there is an essential need to instigate additional avenues of research. The following summarises areas which certainly merit attention.

1. Experiments on emergency vaccines should be conducted over a range of different animal breeds, species (e.g. goats) and age groups.
2. All the early protection studies with emergency FMD vaccines published so far have involved indirect aerosol challenge, depicting a situation in which infected premises could infect a neighbouring farm through windborne spread. Recent events in UK, following the incursion of the Pan-Asian O serotype strain of FMD, has shown how easily FMD virus can spread unnoticed in sheep flocks, leading to wide geographical spread of the disease from infected to susceptible animals almost certainly as a result of direct contact at livestock distribution centres and markets. The benefits of using emergency vaccines within an infected area have, in recent years, also been considered. There is, therefore, significant merit in extending previous early protection studies to see how rapid and effective emergency vaccines are at protecting susceptible livestock from direct contact virus challenge. This should include the use of $O_1$ Manisa vaccine and a Pan-Asian virus challenge.
3. The highly potent emergency vaccines have been shown experimentally to reduce virus replication in the oro-pharynx, consequently decreasing virus excretion and thereby limiting the transmission of the disease to susceptible stock. Local virus replication and the subsequent carrier status has been a key issue recently in the consideration to use vaccine in the current UK outbreak. There is, therefore, an obvious need to underline these previous observations and to try and quantify whether there is indeed a relationship between potency and the ability of emergency FMD vaccines to reduce or even inhibit viral persistence. In countries with the disease and under controlled field conditions, research in this area could help authorities in evaluating the time needed before vaccinated animals can move from an area with restricted movement to limit the spread of the infection.
4. There should also be more research aimed at developing FMD vaccines that are capable of inducing sterile immunity preventing infection and the carrier status. Such a vaccine could dramatically reduce movement and international trading restrictions in the aftermath of an outbreak of FMD.
5. The duration of immunity following emergency vaccination of cattle should be examined and both this species and sheep should undergo challenge experiments to establish the duration of protection following a single injection of vaccine.
6. Manipulation of the vaccine formulations to produce smaller volume doses per animal, while retaining the same antigen payload, would be a useful exercise. Providing the response was comparable to that observed with the current dose volumes, using smaller dose volumes would potentially allow larger numbers of doses to be produced in a single batch for emergency purposes and could reduce the time from formulation to dispatch.
7. Recent research into prolonging the storage of fully formulated vaccine by a novel approach of layering the individual components of FMD vaccine in the same primary container and then storing the product at ultra-low temperature (Patent no. 00 12 817) has met with some success and should be explored further.
8. Research should be actively financed toward improving response time between the initial outbreak and the application of vaccine. Technical aspects within manufacturing process for improving the rapidity of output are limited, but by no means impossible. Therefore, guidelines and effective computational models for most outbreak scenarios should be established to assist objective decision-making about whether or not to vaccinate and minimise bureaucratic delays.
9. Where possible, if a FMD emergency vaccination programme is indeed implemented, a well-designed surveillance programme should be also established to estimate

the effectiveness of the vaccine to prevent further infections and to reduce local virus replication.

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