



## Conference report

## Considerations for design and implementation of vaccine field trials for novel foot-and-mouth disease vaccines

Nicholas A. Lyons<sup>a,b,\*</sup>, Theodore J.D. Knight-Jones<sup>c</sup>, Chris Bartels<sup>a</sup>, David J. Paton<sup>b</sup>, Giancarlo Ferrari<sup>a,d</sup>, Meghan S. Vermillion<sup>e</sup>, Abdullah W. Brooks<sup>f</sup>, Roxann Motroni<sup>g</sup>, Elizabeth Parker<sup>h</sup>, Melissa L. Hefferin Berquist<sup>h</sup>, Keith J. Sumption<sup>a</sup>, Eyal Klement<sup>i</sup>

<sup>a</sup> European Commission for the Control of Foot-and-Mouth Disease (EuFMD), Food and Agriculture of the United Nations, Rome, Italy

<sup>b</sup> The Pirbright Institute, Ash Road, Pirbright, Woking, Surrey GU24 0NF, United Kingdom

<sup>c</sup> Dept for the Environment, Howard Davis Fm, Trinity, Jersey, United Kingdom

<sup>d</sup> Istituto Zooprofilattico Sperimentale del Lazio e Toscana 'M. Aleandri', Via Appia Nuova n. 1411, 00178 Rome, Italy

<sup>e</sup> Johns Hopkins Bloomberg School of Public Health, W. Harry Feinstone Department of Molecular Microbiology and Immunology, 615 N. Wolfe St, Baltimore, MD 21205, USA

<sup>f</sup> Johns Hopkins Bloomberg School of Public Health, Department of International Health, 615 N. Wolfe St, Baltimore, MD 21205, USA

<sup>g</sup> U.S. Department of Homeland Security, Science and Technology Directorate, Homeland Security Advanced Research Projects Agency (HSARPA), Chemical and Biological Defense Division, Washington, DC, USA

<sup>h</sup> Institute for Infectious Animal Diseases (IIAD), Texas A&M University System, College Station, TX, USA

<sup>i</sup> Koret School of Veterinary Medicine, Robert H. Smith Faculty of Agricultural, Food and Environmental Sciences, The Hebrew University, P.O.B. 12, Rehovot 76100, Israel

## ARTICLE INFO

## Article history:

Received 1 August 2018

Received in revised form 19 November 2018

Accepted 31 December 2018

Available online 23 January 2019

## Keywords:

Vaccine evaluation

Vaccine efficacy

Livestock

Foot-and-mouth disease

## ABSTRACT

Vaccines are commonly used to control Foot-and-Mouth Disease (FMD) in endemic regions and form an important part of contingency plans for FMD-free countries. Conventional FMD vaccines have numerous limitations, and the U.S. government supports the development of next-generation vaccines. In the U.S., vaccine efficacy is typically demonstrated through experimental vaccination and challenge of animals using the World Organization for Animal Health (OIE) standards. Although conventional challenge and immunogenicity studies provide useful information, they have limitations and results do not always accurately predict field performance. Consequently, there is a need to test next-generation vaccines under field conditions to gain a better understanding of field performance to inform policy decisions and support their viability as a commercial product.

In June 2017, an expert consultation was organised to discuss and define an optimal field study design for novel FMD vaccines. Cattle were the primary species considered, although parallel strategies for swine and small ruminants were also discussed. Many methodological and logistical considerations in the study design were identified, including: (1) study site selection and the importance of baseline studies to understand exposure risk, (2) ethics of using a placebo and assessing equivalence with conventional vaccines, (3) merits of using individual randomised versus cluster randomised trials, (4) preventive versus reactive vaccination, and (5) methods of randomisation and blinding.

The proposed optimal study design was a multicentre (i.e. farm), three-arm, double-blind randomised controlled trial comparing groups receiving the novel vaccine to a conventional vaccine group and a placebo group. Large farms in areas of high exposure risk were identified as ideal study sites, and the primary study outcome was susceptibility to disease or infection, during a six-month observation period, following a single dose of vaccine. This report provides a summary of the important issues to consider when designing a field efficacy study in livestock and proposes a study design that could be utilised for novel FMD vaccines.

### 1. Introduction

Foot-and-Mouth Disease (FMD) is a highly transmissible viral disease of cloven-hoofed livestock. The causal agent is a non-enveloped RNA virus within the family *Picornaviridae* and genus

\* Corresponding author at: European Commission for the Control of Foot-and-Mouth Disease (EuFMD), Food and Agriculture of the United Nations, Rome, Italy.

E-mail address: [nicholas.lyons@fao.org](mailto:nicholas.lyons@fao.org) (N.A. Lyons).

*Aphthovirus*. The virus can infect many domestic livestock and wild animal species but clinical disease mainly occurs in cattle and pigs. Although Europe, North America, Central America, much of South America and most of Australasia are officially free of the virus, it is still endemic through most of Africa, the Middle East and Asia. Although mortality is generally low, the high morbidity and costs associated with control make this a disease of high economic impact, and it is estimated to cost between US\$6.5–21 billion per year in endemic regions [1]. After acute infection, ruminants may become carriers by harbouring virus in the oropharynx, which can occur in vaccinated animals without apparent clinical disease [2]. Although African buffalo may become carriers and transfer infection to domestic livestock [3], a significant role for carrier cattle in FMD epidemiology has not been demonstrated. Nevertheless, the presence of FMD carriers has important implications for countries declaring freedom from FMD virus.

The virus exists in seven distinct serotypes (A, Asia-1, O, C, Southern African Territories [SAT]-1, SAT-2 and SAT3) with no or limited cross protection. The virus mutates readily at major antigenic sites leading to a diverse array of strains within serotypes. Viral lineages tend to cluster globally into geographical pools [4]. Emerging lineages are often associated with long-distance inter-pool transmission particularly from South Asia [5]. Vaccines are a commonly used tool in disease control and form an important part of contingency plans for FMD free countries including the U.S.

Conventional FMD vaccines are killed virus, inactivated and blended with either an aqueous (aluminium hydroxide/saponin) or oil based adjuvant. Such vaccines are produced worldwide, although the quality of vaccine production can be variable. A number of factors are important for FMD vaccine effectiveness, including sufficient match between the vaccine and field strain; capsid stability, which is dependent upon maintenance of a cold chain; adequate immunogenicity and an optimized dosing regimen. The duration of immunity following a primary course of vaccination is approximately six months. Maternally derived antibody, which can protect animals during the post-natal period can also interfere with the response to the vaccine [6–8]. As with all vaccines, adverse events/reactions can occur (for example injection-site granulomas in swine [9]) that have the potential to affect coverage and compliance with vaccination policy.

The accepted method of evaluating FMD vaccines is by experimentally challenging vaccinated and unvaccinated control animals in what is commonly known as a “potency test” [10]. These tests fulfil a regulatory requirement in measuring the vaccine protection to homologous virus challenge for the purposes of comparability and standardisation. Typically, they are performed for licensing new vaccine strains with batch release relying on small-scale immunogenicity studies. Although conventional challenge and immunogenicity studies used to evaluate FMD vaccines provide useful information, they suffer from several limitations such that the true effectiveness of vaccines cannot be directly deduced. These limitations include: an unnatural route of infection; challenge with a single and usually homologous strain only a short time after vaccination with no data provided on the duration of immunity and optimal revaccination schedules; no accounting for the complexity presented in natural settings (e.g. age, variable natural challenge, maintenance of cold chain); a small sample size limiting the statistical confidence in the results [11]. Therefore, care must be taken when interpreting the results, particularly when making inferences about predicted or expected performance in the field.

While the U.S. does not routinely vaccinate for FMD, in the event of an outbreak, vaccination could be employed [12]. However, manufacture of conventional FMD vaccines requires cultivation of live virus, which is not currently permitted on the U.S.

mainland for fear of virus escape. In 2004, a vaccine development programme started within Department of Homeland Security (DHS) Science and Technology Directorate (S&T), with a goal to support the commercialization of an alternative FMD vaccine platform. This vaccine platform, first described by the United States Department of Agriculture (USDA) Agriculture Research Service (ARS) in 2002 [13] utilizes a replication-deficient human adenovirus with FMDV inserts (AdFMD) that can be produced on a large scale without the need for live virus. In 2012, the USDA Center for Veterinary Biologics (CVB) granted a conditional license for an AdFMD monovalent vaccine for serotype A (A24 Cruzeiro) [14] based on satisfactory safety studies [15] and “potency tests” using the OIE recommended methods. To date, vaccines based on 16 additional priority strains have been developed [16]. Based on multiple, laboratory experimental challenge studies [14,17] using the A24 strain, a vaccine efficacy of up to 90% using certain adjuvants has been observed in cattle and is believed to protect them for up to 6 months following a single dose (unpublished results).

The purpose of a study based on the design proposed here would be to provide additional evidence to support full licensure of this product. Although the US regulatory body (USDA Center for Veterinary Biologics [CVB]) does not require a field study for full licensure, field efficacy data would influence vaccination policy in the event of an outbreak and would be important for demonstrating that vaccination is compatible with serological tests for infection (so-called “DIVA” testing – Differentiating Infected from Vaccinated Animals). For conventional vaccines that are purified of non-structural proteins (NSP), a single vaccine dose can be compatible with DIVA, although due to incomplete purification this may not be the case following administration of multiple doses [18]. For vectored vaccines that do not carry the NSP genes, the problem of using repeated doses may be avoided.

In June 2017, an expert consultation took place at FAO headquarters in Rome, organised by the European Commission for the control of FMD (EuFMD). This manuscript provides details of the final proposed study design for evaluating field-based vaccine efficacy divided into several relevant sections. Although the focus of this consultation was on the AdFMD vaccine, the study design aimed to produce a framework that could be used for any novel FMD vaccine.

## 2. Trial design objective and purpose

The purpose of this expert elicitation was to recommend a study design to demonstrate the efficacy of the novel AdFMD vaccine under field conditions in an endemic setting. Because FMD outbreaks are uncommon and difficult to predict in FMDV-free countries, undertaking the study in an endemic country is needed for adequate field exposure to demonstrate vaccine efficacy.

The primary objectives of the study would be to:

1. Demonstrate absence of clinical disease (i.e. vaccine efficacy for reducing susceptibility to disease)
2. Demonstrate absence of infection (i.e. vaccine efficacy for reducing susceptibility to infection).

Secondary objectives (to be performed in a subset of subjects) are:

1. Demonstration of seroconversion post-vaccination
2. Demonstration of DIVA compatibility of the vaccine
3. Demonstration that vaccination prevents the establishment of the carrier state (i.e. vaccine efficacy for reducing long term virus carriage) due to the theoretical risk that animals could continue to spread disease even post-vaccination

#### 4. Demonstration of decreased viral shedding following post vaccination infection.

Two primary objectives are specified, as they are considered equally important to estimate. In FMD-free countries or those wishing to reduce viral circulation (i.e. stage 3 onwards of the Progressive Control Pathway for FMD Control [PCP-FMD] [19]) preventing infection is considered important with an impact on virus transmission and indirect effects at the population level. In an endemic country, reducing the incidence of clinical disease and FMD impact may be the priority (i.e. PCP-FMD stage 2). For different vaccines, these effects can be different as suggested in the case of Polio, where inactivated vaccines have a greater vaccine efficacy for reducing infection, compared to oral Polio vaccines that have a greater vaccine efficacy for reducing susceptibility to disease [20].

### 3. Proposed study design

The consensus study design proposed to meet the above objectives is a multicentre, three-arm randomised controlled trial using the novel vaccine (first arm), a conventional vaccine (second arm) and a placebo vaccine (third arm). This study would compare the novel vaccine to its conventional equivalent and to a placebo over approximately six months.<sup>1</sup> The primary outcomes are to show the vaccine efficacy for susceptibility to disease and infection comparing the novel vaccine group versus the placebo based on a single dose of vaccine. Comparisons will also be made between the two vaccine groups, and between the conventional vaccine and the placebo group. The reason for inclusion of a third arm with a conventional vaccine is to provide evidence that it is at least equal (i.e. no worse than, or not inferior) to this product. This is important for decision makers to consider when weighing up options on which vaccines to choose along with other advantages and disadvantages such as the cost of production. The placebo group will allow the absolute efficacy to be estimated and therefore the potential impact of vaccination programmes with the two vaccine types. The vaccine would be used preventively prior to a period of high exposure risk (i.e. not “reactively” following a disease event). Reasons for using a preventive approach include: easier logistics by not having to detect outbreaks with active surveillance; greater certainty over time of exposure relative to vaccination which may be uncertain after reactive vaccination; reduced risk of vaccinators spreading infection in an area with known infection; relevance of the strategy to both prophylactic and reactive scenarios where the majority of animals are likely to be receiving vaccination prior to exposure.

### 4. Site selection

The location of the study is likely to be critical. In particular, the exposure risk must be sufficiently high to provide field virus challenge within six months of vaccination (the length of the study), and it will be essential to have adequate follow-up of the study animals. It would also be important that vaccination is not routinely practiced in the study area and appropriate strains are available for the AdFMD vaccine using vaccine matching data. It is anticipated that a baseline sero-prevalence study would be required before commencing the study to indicate the high risk area and inform sample size equations (see Section 9).

A location with high levels of virus exposure in a naïve population would provide a suitable study location to demonstrate vac-

cine efficacy. Moreover, governmental approval to undertake the study is essential, with their involvement likely to be necessary to achieve the study objectives. Promising candidate locations include large dairy colonies in Pakistan, in which holdings are located adjacent to each other, and where, rather than relying on breeding for replacements, animals are introduced routinely (10–12% replaced monthly). In some of these colonies there is a very high rate (up to 80%) of seroconversion within two months of arrival.<sup>2</sup> Similar systems are present elsewhere (e.g. Iran). Another option would be to focus on a study area with animal markets with a high seasonal risk of exposure (for example related to Islamic festivals). Farm owners and animals could be recruited at purchase from the market and followed-up on the destination farm. Multiple farm owners would be recruited as animals are introduced to their farms, with these new animals being the subjects in the trial. These introductions could be normal farm purchases or provided as part of the study if there is farmer reluctance to participate. There would be a preference for larger farms that tend to be at relatively higher risk of exposure with more subjects available for recruitment. Moreover, fewer farms would then be needed, which minimises the variation in exposure risk that can contribute to a design effect.<sup>3</sup> This would also simplify logistics and costs of conducting the trial. The number of animals recruited on each farm would depend on the sample size (see Section 9 for a worked example) but the target number of recruited farms should be less than ten. Continual recruitment of new arrivals over time would increase the chance of having cattle experiencing exposure at different times since vaccination, and hence provide more information on how protection varies with time since vaccination.

### 5. Subject recruitment

Due to the numerous advantages in undertaking a trial in the aforementioned high-risk production systems, this is the basis of the proposed study design. Animals will be recruited going to farms in high-risk “hotspot” areas informed by the baseline study and considering spatio-temporal epidemiological information. Prior to the study being initiated, farmer meetings would be organised in the study area to understand if farmers would be willing to take part and to identify possible incentives to maximise study compliance and participation. Although no payment will be offered to the farmers, the incentive to participate will be free FMD vaccine in some of the animals (see Section 6) in an area where routine vaccination is not being used and is not freely available. The major risks are farmer withdrawal from the study before completion, non-compliance with study protocols and sales or deaths of recruited animals. This risk will be minimised by inflating the sample size (see Section 9) and by using the outcomes of the pre-study farmers meetings to identify incentives to ensure compliance and participation.

If a farm is eligible for the study, signed consent will be provided before proceeding with the recruitment process. All participating farms will be assigned a random ID code. Animals will only be recruited if there have been no clinical cases of FMD in the previous month, on the destination farm, to reduce the risk of infection occurring before the anticipated onset of immunity from the vaccine (infection may be continuing subclinically on the farm or virus may be present in the environment). This will be determined through a combination of farmer interviews and official outbreak data.

<sup>2</sup> [http://www.fao.org/fileadmin/user\\_upload/eufmd/docs/India\\_meeting\\_feb\\_2012/37\\_Afzal\\_Fmd\\_control\\_in\\_market.pdf](http://www.fao.org/fileadmin/user_upload/eufmd/docs/India_meeting_feb_2012/37_Afzal_Fmd_control_in_market.pdf)

<sup>3</sup> A “design effect” is a factor that the sample size needs to be increased by to account for clustering of observations. The greater the variance of the outcome (i.e. exposure) between clusters/farms, the more the sample size needs to be increased.

<sup>1</sup> Six months is the study length as this is the duration of immunity for the Ad5 vaccine after a single dose as indicated by challenge and immunogenicity studies. This is also generally considered the maximum duration of protection from conventional vaccines [29]

Inclusion criteria for including animals in the study include:

1. Aged older than 6 months of age (beyond the influence of maternally derived antibody)
2. No FMD non-structural protein (NSP) antibodies and no history of FMD vaccination
3. Owner consent to participation in the study.

Exclusion criteria include:

1. Any history of clinical FMD in the animal (based on verbal history and clinical examination) or on the farm of origin
2. Previous vaccination for FMD or evidence of previous exposure (i.e. the presence of NSP antibodies) in an individual animal
3. Any other illness present at enrolment
4. Destination farm uses routine vaccination for FMD
5. Destination farm has had clinical cases of FMD in the previous month.

Upon farm recruitment, newly introduced animals will be clinically examined to rule out any current or recent disease. Animals will be excluded if there is evidence of pyrexia, reduced appetite, depression or more specific signs of FMD, including hypersalivation and lesions in the mouth, feet or teats. Animals will have an ear tag placed with a unique number assigned. Data will be collected on each individual animal as indicated in section 8. To reduce the variation of risk that may be related to the animals' origin (which may introduce heterogeneity in the apparent efficacy of the vaccine), the intervention will be delivered once the animals have been on the destination farm for a two week period. Animals will be blood sampled on introduction, and the intervention applied if the animal is NSP antibody negative. To rule out the possibility of exposure soon after arrival on the farm or vaccination, animals will also be assessed for serological NSP antibody status at vaccination and two weeks after (i.e. one month after arrival on farm). Animals that are NSP positive at arrival or 14 days post vaccination will be excluded from the study due to the risk of infection having occurred before the onset of immunity from vaccination.

Animals will be continually recruited as they arrive onto the farm until the desired sample size is attained. Since only new arrivals will be recruited and the selected area is not using routine vaccination, the farm level vaccination coverage will be low if large sizes are preferred, which will minimise any indirect protection at the herd level.

## 6. Trial intervention

The primary trial intervention is a single dose of AdFMD vaccine which has passed routine OIE prescribed tests for potency based on challenge studies and safety studies. For the latter, these have been published for the A24 strain [15] but would be repeated if alternative strains are used in the proposed study. The vaccine will be administered by a trained livestock worker. The dose will be based on experience from challenge studies undertaken in experimental containment facilities that also indicated an assumed vaccine efficacy of 80% [17] (see Section 9).

The second arm of the study will use a conventional vaccine which will be oil adjuvanted, killed, NSP purified vaccine. An oil adjuvant is important as the appearance will be similar to the AdFMD vaccine ("skimmed milk" colour). In addition, the oil adjuvant leads to a relatively longer antibody response compared to an aqueous equivalent, and is the favoured adjuvant in vaccines that would be formulated in the event of a disease incursion in a free county. The same strain will be used in the conventional and novel

vaccine to allow a fair comparison. The strain that is selected will depend on the dominant lineages detected in the area following a one year period of active surveillance before the study period (and on supporting in-vitro  $r_1$  values, a serological measure of match between field and vaccine strains [21]) combined with expert consultation at the WRLFMD and regional OIE reference laboratories and relevant local animal health experts.

The placebo will be saline based and will look identical to the AdFMD and conventional vaccines. No adjuvant will be included which may lead to non-specific effects such as immune enhancement.

All of these vaccines will be delivered intramuscularly at identical volumes to permit blinding of the intervention. Storage will be in local refrigeration units (with backup generators to ensure consistent temperatures) and the cold chain will be monitored to the point of delivery using digital thermometers with automated temperature recording and data storage.

## 7. Randomisation

Randomisation is essential to ensure a fair comparison between groups and assure the even distribution of confounders.

Simple randomisation may lead to imbalances in the number of animals allocated to the different study arms on each farm, which may affect the results. To minimize the impact of this effect, at recruitment into the study animals will be randomised into one of the three arms using a variable block randomisation. This ensures equal numbers of study subjects are allocated to each arm per farm but the order of randomisation varies between the farms [22]. A two-stage process is suggested where the size of the block for each farm is also randomly chosen (Appendix A). A randomisation table will be generated prior to recruitment by someone not involved with the vaccination or follow up [23]. This will be used by the vaccinator who follows the assigned arm order on the list (with ear tag numbers corresponding to a vial containing either of the vaccine types or a control). Ideally pre-dose loaded syringes will be used with labels matching the randomisation table. If this is not possible, several vials will be used by each vaccinator, which will have plain labelling and the vial number cross referenced to the randomisation table. The vial/syringe number will be recorded alongside the assigned animal unique ID number.

## 8. Blinding

The study will be double blinded with neither the vaccinator nor farmer knowing the identity of the products. Attempts will be made to make all three products (novel vaccine, conventional vaccine, placebo) visually identical. If the vaccinator is blinded, they can also be the observer to follow the animals through the study. If vaccinator blinding is not possible, vaccine administration will be performed out of sight of the farmer and anyone providing care or evaluating the animal for infection or disease (i.e. the vaccinators have no role in following up the animals - "observer-blinded").

## 9. Data collection and analysis

All data will be collected by project staff after appropriate training in trial protocols. Data will be stored securely with access only by project staff. The codes associated with the farms will not be shared beyond field staff.

At recruitment, data will be collected on the individual animals as indicated in Table 1. All data and samples will be collected according to the unique ear tag number. For ease of data collection and storage, using a mobile phone application for data entry (e.g.

**Table 1**  
Suggested data collected from individual animals and farms at initial examination and recruitment.<sup>a</sup>

| Individual animal data             | Clinical examination | Farm data   |
|------------------------------------|----------------------|---|
| Ear tag ID                         | Mouth lesions        | Farm ID and georeference  |
| Farm ID                            | Feet lesions         | Number of cattle present  |
| Age                                | Teat lesions         | Number of sheep/goats/pigs present  |
| Breed                              | Rectal temperature   | Number of other species present   |
| Sex                                | Lethargy             | Production systems (dairy/beef/other)                                     |
| Body condition score               |                      | Grazing practices   |
| Purpose                            | Drooling             | Water access  |
| – Dairy                            | Anorexia             | Last outbreak of FMD  |
| – Beef                             | Lameness             | Frequency of FMD outbreaks on yearly basis (and approximate attack rates) |
| – Breeding                         |                      | History of other diseases on farm   |
| Origin <sup>b</sup>                | Other                | Vaccine types currently in use (and when given)                           |
| Pregnancy status (gestation stage) |                      |   |
| Vial/syringe assignment            |                      |   |

<sup>a</sup> Data collected may vary depending on the study location.

<sup>b</sup> Location prior to arrival on new farm.

EpiCollect [24] is preferable. The results of the clinical examination will also be recorded as well as farm level data collected through interviews with the farmers. All animals will be blood sampled at arrival on the farm, at the time of vaccination (two weeks after arrival), two weeks post vaccination, and monthly thereafter, until the end of the study (6 months post recruitment). These samples will be tested for NSP antibodies as evidence of infection with FMD, assessing crude results and those adjusted for the test performance that may vary between the three groups. The reason why test performance may vary is that conventional vaccines are NSP purified to varying degrees whereas for recombinant vaccines NSP is absent. Therefore a higher rate of false positives may occur in conventionally vaccinated animals which needs to be accounted for when estimating the true infection incidence. A subset of the samples collected one month after vaccination will also be tested for FMD structural protein (SP) antibodies to characterize the immunogenicity of the vaccines. It is suggested that this be done through virus neutralisation tests using the strain used for the vaccine. Laboratories utilised for testing these samples should be internationally accredited for the specific tests being undertaken (for example according to the International Organization for Standardization (ISO) 17,025 requirements).

Each animal in the study will be visually observed every other day and the farmer asked about health and production status. If any clinical sign is observed, this will be followed up with a closer examination with samples taken if necessary. All clinical scoring and examinations will be performed by people trained in livestock health and after appropriate training in study protocols. Any animals with disease detected by clinical monitoring will receive a daily full clinical examination for the duration of the illness. A suggested clinical scoring sheet is provided in Appendix B. Serum samples and nasal and/or oropharyngeal swabs will be taken during each examination. If lesions are presented consistent with FMD, epithelium samples will be taken for virus detection and isolation. All animals will have an oropharyngeal fluid sample taken using a probang at the end of the study to assess the presence of virus carriage. Appendix C has a schematic of the sampling events after study recruitment based on a CONSORT (Consolidated Standards of Reporting Trials) Flow Diagram.<sup>4</sup> Swabs will be used to compare the level of virus shedding in clinical cases that occur in vaccinated and non-vaccinated animals. A similar comparison will be made for virus carriage based on the oropharyngeal fluid samples.

The definition used for estimating vaccine efficacy is the relative decrease in disease or infection incidence comparing vaccinated and non-vaccinated animals:

$$\text{Vaccine efficacy} = \frac{I_u - I_v}{I_u} = 1 - RR$$

where  $I_u$  is the incidence in unvaccinated subjects,  $I_v$  is the incidence in vaccinated subjects, and  $RR$  is the risk ratio [25]. Interim analysis will be periodically performed every month and the study terminated if the anticipated efficacy is demonstrated before the scheduled trial completion (6 months after the last animal is vaccinated). If insufficient cases occur by this time, there should be scope for continuing the rolling participant recruitment until a sufficient number of cases occur. Cases should be related to the same lineage of virus so that if multiple outbreaks occur with different lineages, these will be considered separate incursions for which independent efficacy estimates will be generated. Since there may be insufficient cases associated with a specific lineage to provide a statistically valid estimate of efficacy, it would be justifiable to estimate a pooled estimate at the serotype level. The field strains should also be sufficiently matched to the vaccine strain for the efficacy estimate to have validity. This will be demonstrated by judging regularly generated one-way comparative relationship ( $r_1$ ) values by an expert panel [21].

## 10. Statistical considerations

The sample size calculation will be based upon demonstrating efficacy of the novel vaccine compared to the placebo using the equation provided by Carpenter (2001)<sup>5</sup>:

$$n = \frac{(Z_{\alpha/2} \sqrt{2\bar{p}(1-\bar{p})} + Z_{1-\beta} \sqrt{p_v(1-p_v) + p_{nv}(1-p_{nv})})^2}{(p_{nv} - p_v)^2}$$

where

$n$  = number of animals required per group

$\bar{p}$  = mean proportion

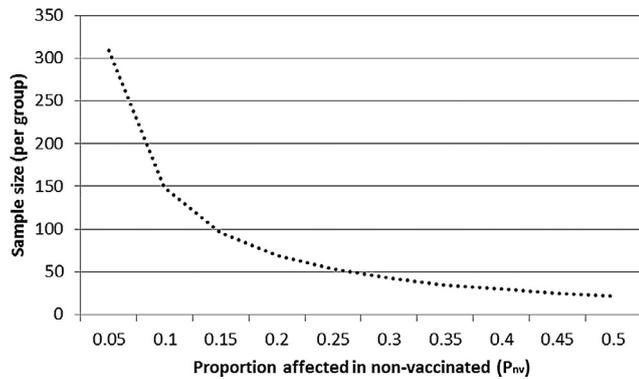
$p_v$  = proportion with outcome in vaccinated

$p_{nv}$  = proportion with outcome in unvaccinated

$Z_{\alpha/2}$  = Two-sided percentage from standardized normal distribution corresponding to required significance level. For 5% this is 1.96.

<sup>5</sup> Equation in original publication has an error. The equation in this document is corrected and verified using citations and data within the publication.

<sup>4</sup> <http://www.consort-statement.org/consort-statement/flow-diagram>



**Fig. 1.** Sample size per group assuming a vaccine efficacy of 80% varying with the expected proportion affected in the non-vaccinated group [26]. Assumes 80% power 95% confidence intervals.

$Z_{1-\beta}$  = One sided percentage point from standardized normal distribution corresponding to 100-power ( $\beta$ ). For 80% power this is 0.84.

A correction factor of  $2/|p_{nv}-p_v|$  is also included for a more conservative estimate as suggested by Carpenter (2001) [26]. A vaccine efficacy of 80% will be assumed based on knowledge from laboratory challenge studies. The sample size will depend on the expected incidence in the non-vaccinated population which will be informed by the baseline study (Fig. 1).

The sample size will be increased to account for the following:

1. Expected proportion of animals being NSP positive at arrival
2. Potential exposure within 7 days of vaccination (the assumed onset of immunity post vaccination)
3. Loss to follow up (death, sales etc.)
4. Clustering with a design effect based on the intra-class correlation coefficient.

The impact of these issues and magnitude of clustering will be informed by the baseline study.

As an example, if the vaccine efficacy is assumed to be 80% and the proportion affected in the non-vaccinated population during the study period is estimated at 30%, 48 animals are required per group (i.e. a total of 144 for the three groups combined). If 5% of animals are removed due to being NSP positive or exposed in the first 14 days of arrival and there is a further 20% loss to follow-up through death, sales and culling, this increases the sample size by 25% to 180 animals with 60 animals assigned to each group.

Another consideration is the comparison between the conventional and traditional vaccines. This comparison can be based on non-inferiority, a demonstration that the novel vaccine is no worse than a conventional equivalent. An equation for equivalence [27] can be used with a one-sided hypothesis test to estimate the sample size using a pre-specified efficacy margin:

$$n = \frac{2p(100 - p)}{\delta^2} (Z_{(1-\alpha)} + Z_{(1-\beta)})^2$$

where

$n$  = required size of each group,

$p$  = overall percentage incidence of disease if the two groups are equivalent

$\delta$  = acceptable difference in equivalence (percentage)

$Z_{1-\alpha}$  = One-sided percentage from standardized normal distribution corresponding to required significance level.

$Z_{1-\beta}$  = One sided percentage point from standardized normal distribution corresponding to 100-power ( $\beta$ ).

For human vaccines, there is a lack of clear guidance on defining the margin, although 10% is often used [28]. For this study design,

the greater estimated sample size from the two approaches (efficacy compared with a placebo and non-inferiority between the two vaccine types) will be used.

The number of animals used per farm will be variable as per the variable block randomisation design. For example, if ten farms are recruited, the mean number of animals used per farm will be 18. The number of animals on each farm will be a multiple of three to fit with the randomisation scheme.

## 11. Safety studies

The AdFMD vaccine has undergone extensive safety testing in compliance with U.S. regulatory requirements (Title 9, U.S. Code of Federal Regulation [9CFR]) and international standard guidelines (International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products [VICH] Topic GL-44) for veterinary live vaccines [15]. As part of an efficacy study, further data on the safety of the vaccine will be generated through the clinical screening process outlined in section 8. Due to the international nature of the trial and potential use of the vaccine, VICH guidelines will be used in conjunction with national requirements for the host country.

## 12. Ethical considerations and regulatory requirements

All participating farmers will provide written consent prior to recruitment and be informed about the use of a placebo. Ethical approval will be granted by relevant within-country review committees as appropriate. Particular regulatory requirements for the host country will be sought including: (1) the use of an unlicensed vaccine; (2) necessary trial protocols for demonstrating vaccine efficacy; (3) any necessary approvals for using genetically modified organisms (GMO). It is possible that in some circumstances those receiving a GMO vaccine without licensure will need to be culled which should be borne in mind when planning the logistics and financing of the project. The trial should also conform to international standards of Good Clinical Practice as detailed in VICH GL9 international guidance document.

## 13. General consideration for other species: Swine and small ruminants

Although the primary species considered in this study design is cattle, considerations for assessing the vaccines in other FMD susceptible species are also required (in particular swine and small ruminants), since the U.S. swine and sheep industries are also pushing for an effective vaccine to be available in the event of a U.S. FMD outbreak. However, production systems and the nature of FMD in these species are different from cattle, which may influence study location and design.

For example, in swine production there is a high turnover of animals, particularly in intensive fattening systems. Due to their short lifespan (e.g. six months) and maternal immunity that persists until three months of age, there is a limited time for the study to be performed. Breeding animals have relatively longer lifespans, and, therefore, may be a better target population in intensive systems depending on the exposure risk, but animals in these systems are usually vaccinated routinely (for example South Korea). Backyard swine production systems (e.g. smallholders in Nepal) represent alternative trial sites where routine FMD vaccination is uncommon, but FMD epidemiology is poorly described in these types of systems necessitating thorough baseline surveillance to inform the study design. Such surveillance would include seroprevalence studies and targeted surveillance for clinical disease

to establish the baseline clinical disease incidence at the individual animal level in addition to circulating viral lineages.

Compared with swine and cattle, small ruminants demonstrate relatively mild, and often subclinical disease following FMD infection. Consequently, seroconversion to NSP antibodies (and therefore infection) should be the primary outcome in the vaccine efficacy study in small ruminants. Otherwise, the study design as suggested for cattle may be possible.

**14. Limitations of the approach**

There are numerous limitations to the proposed study design that should be highlighted. Although strong consideration has been given to ensuring a sufficient number of cases to demonstrate the desired efficacy, there is a risk that they may not occur within the anticipated timeframe of the project. As a consequence, the study duration would have to be prolonged through enrolling additional animals into the study. No revaccination would take place of the study animals. The flexibility of the suggested approach would accommodate this need.

The cost of implementing the study is potentially high, depending in part on the required local capacity development and production costs of the novel vaccine in addition to the costs associated with the trial itself including the examination of samples. The generalizability of the study to other settings may also be questioned. Despite these limitations, the disadvantages of conventional approaches are largely overcome with a field efficacy study.

**15. Conclusion**

The outcome of this meeting was to propose a study design for evaluating the efficacy of novel FMD vaccines. Although estimates of vaccine efficacy are important, only certain study designs and study settings were considered to be economically and logistically feasible. Baseline studies are essential with appropriate randomisation and blinding systems in place to minimise bias. Comparing the relative efficacy of novel FMD vaccines to pre-existing ones and considering protection against natural infection and disease should guide the use of next-generation vaccines in both endemic and free countries. Here we propose a particular design suited to our needs, however, the recommendations in this paper can be used by other researchers as part of their planning to evaluate novel FMD vaccines in field conditions.

**Acknowledgements**

The authors of this report would like to thank staff at EuFMD for organising the logistics of the meeting and FAO for use of the facilities at the headquarters in Rome.

**Conflict of interest**

None of the authors declares a conflict of interest.

**Funding**

This material is based upon work supported by the U.S. Department of Homeland Security task order HSHQDC-14-J-00335. The views and conclusions contained in this document are those of the authors and should not be interpreted as necessarily representing the official policies, either expressed or implied, of the U.S. Department of Homeland Security.

**Appendix A. – Variable block randomisation**

Table demonstrating block randomisation for a three arm study with a block size of three. There are six combinations possible.

| Farm | Animal 1 | Animal 2 | Animal 3 |
|------|----------|----------|----------|
| 1    | A        | B        | C        |
| 2    | A        | C        | B        |
| 3    | B        | A        | C        |
| 4    | B        | C        | A        |
| 5    | C        | A        | B        |
| 6    | C        | B        | A        |

Where A is vaccine 1, B is vaccine 2 and C is control.

For a three arm trial, to ensure equal numbers are assigned to each arm, the block size has to be a multiple of three.

In variable block randomisation, the farm is also assigned a variable number of blocks (each of size three). The number of blocks assigned per farm can be random. The order of arm assignment in each farm is random but the numbers assigned for each arm is always equal because the block size is equal to the number of arms in the study.

For example if there are 10 farms in the trial, with the number of blocks ranging from 1 to 4, the allocation may look like:

| Farm | Block number | Animal |   |   |   |   |   |   |   |   |    |    |    |  |
|------|--------------|--------|---|---|---|---|---|---|---|---|----|----|----|--|
|      |              | 1      | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |  |
| 1    | 1            | A      | C | B |   |   |   |   |   |   |    |    |    |  |
| 2    | 2            | B      | A | A | C | B | C |   |   |   |    |    |    |  |
| 3    | 1            | A      | B | C |   |   |   |   |   |   |    |    |    |  |
| 4    | 4            | B      | C | A | A | B | B | C | C | A | B  | C  | A  |  |
| 5    | 3            | C      | B | A | B | C | A | A | B | C |    |    |    |  |
| 6    | 1            | C      | B | A |   |   |   |   |   |   |    |    |    |  |
| 7    | 4            | B      | A | C | A | B | C | C | B | A | A  | B  | C  |  |
| 8    | 2            | C      | B | A | B | A | C |   |   |   |    |    |    |  |
| 9    | 1            | C      | B | A |   |   |   |   |   |   |    |    |    |  |
| 10   | 1            | B      | C | A |   |   |   |   |   |   |    |    |    |  |

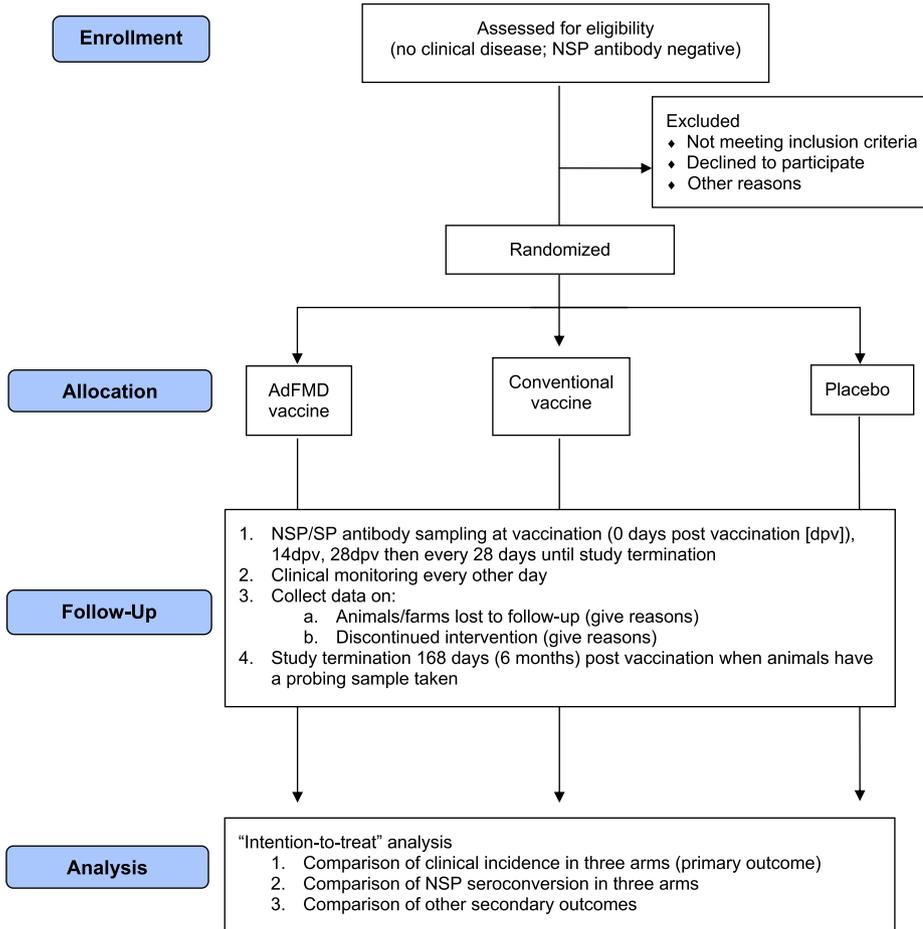
Where A is vaccine 1, B is vaccine 2 and C is control.

**Appendix B. . Example of clinical scoring sheet.**

|               |                |                      |                 |   |   |           |       |
|---------------|----------------|----------------------|-----------------|---|---|-----------|-------|
| Farm ID _____ |                |                      |                 | Date: _____                             |   |           |       |
| Animal ID     | Clinical signs |                      |                 |   |   |           |       |
|               | Lameness       | Increased salivation | Lesions on teat | Lesions on feet (+estimated lesion age) | Lesions in or around mouth/muzzle (+estimated lesion age) | Milk drop | Fever |

## Appendix C. . CONSORT flow diagram

CONSORT Flow Diagram (<http://www.consort-statement.org/consort-statement/flow-diagram>) representing allocation and data collection in proposed vaccine field trial. Animals will be allocated to each group using variable block randomisation with a rolling enrolment on multiple farms in a high risk area. Follow-up time is six months following enrolment. NSP = Non-structural proteins. SP = Structural proteins. PB = Probang.



emulsified with oil adjuvant. Strategies of vaccination. *Vaccine* 1995;13:909–14.

- [8] Doel TR. Natural and vaccine-induced immunity to foot and mouth disease: the prospects for improved vaccines. *Rev Sci Tech* 1996;15:883–911.
- [9] Lyons NA, Lyoo YS, King DP, Paton DJ. Challenges of generating and maintaining protective vaccine-induced immune responses for foot-and-mouth disease virus in pigs. *Front Vet Sci* 2016;3:1–12. <https://doi.org/10.3389/fvets.2016.00102>.
- [10] OIE. Foot and mouth disease. *OIE Terr Man* 2017;2.1.8:1–32.
- [11] Goris N, Merkelbach-Peters P, Diev VI, Verloo D, Zakharov VM, Kraft HP, et al.

## References

- [1] Knight-Jones TJD, Rushton J. The economic impacts of foot and mouth disease – what are they, how big are they and where do they occur? *Prev Vet Med* 2013;112:161–73. <https://doi.org/10.1016/j.prevetmed.2013.07.013>.
- [2] Hedger RS. Observations on the carrier state and related antibody titres during an outbreak of foot-and-mouth disease. *J Hyg (Lond)* 1970;68:53–60. <https://doi.org/10.1017/S0022172400028497>.
- [3] Dawe P, Flanagan F, Madekurozwa R, Sorensen K, Anderson E, Foggin C, et al. Natural transmission of foot-and-mouth disease virus from African buffalo (*Syncerus caffer*) to cattle in a wildlife area of Zimbabwe. *Vet Rec* 1994;134:230–2. <https://doi.org/10.1136/vr.134.10.230>.
- [4] Paton DJ, Sumption KJ, Charleston B. Options for control of foot-and-mouth disease: knowledge, capability and policy. *Philos Trans R Soc Lond B Biol Sci* 2009;364:2657–67. <https://doi.org/10.1098/rstb.2009.0100>.
- [5] Paton DJ, Gubbins S, King DP. Understanding the transmission of foot-and-mouth disease virus at different scales. *Curr Opin Virol* 2018;28:85–91. <https://doi.org/10.1016/j.coviro.2017.11.013>.
- [6] Kitching RP, Salt JS. The interference by maternally-derived antibody with active immunization of farm animals against foot-and-mouth disease. *Br Vet J* 1995;151:379–89.
- [7] Späth EJ, Smitsaart E, Casaro AP, Fondevila N, Fernández F, Leunda MR, et al. Immune response of calves to foot-and-mouth disease virus vaccine

European Pharmacopoeia foot-and-mouth disease vaccine potency testing in cattle: between test variability and its consequences. *Vaccine* 2007;25:3373–9. <https://doi.org/10.1016/j.vaccine.2006.12.049>.

- [12] Foot-and-mouth USDA. Disease response plan. *The Red Book 2014*.
- [13] Moraes MP, Mayr GA, Mason PW, Grubman MJ. Early protection against homologous challenge after a single dose of replication-defective human adenovirus type 5 expressing capsid proteins of foot-and-mouth disease virus (FMDV) strain A24. *Vaccine* 2002;20:1631–9. [https://doi.org/10.1016/S0264-410X\(01\)00483-2](https://doi.org/10.1016/S0264-410X(01)00483-2).
- [14] Schutta C, Barrera J, Pisano M, Zsak L, Grubman MJ, Mayr GA, et al. Multiple efficacy studies of an adenovirus-vectored foot-and-mouth disease virus serotype A24 subunit vaccine in cattle using homologous challenge. *Vaccine* 2016;34:3214–20. <https://doi.org/10.1016/j.vaccine.2015.12.018>.
- [15] Barrera J, Brake DA, Kamicker BJ, Purcell C, Kaptur R, Schieber T, et al. Safety profile of a replication-deficient human adenovirus-vectored foot-and-mouth disease virus serotype A24 subunit vaccine in cattle. *Transbound Emerg Dis* 2018;65:447–55. <https://doi.org/10.1111/tbed.12724>.
- [16] Barrera J, Brake DA, Schutta C, Etyyreddy D, Kamicker BJ, Rasmussen MV, et al. Versatility of the adenovirus-vectored foot-and-mouth disease vaccine platform across multiple foot-and-mouth disease virus serotypes and genotypes using a vaccine dose representative of the AdA24 conditionally licensed vaccine. *Vaccine* 2018;36:7345–52. <https://doi.org/10.1016/j.vaccine.2018.10.031>.
- [17] Barrera J, Schutta C, Pisano M, Grubman MJ, Brake DA, Miller T, et al. Use of ENABL® adjuvant to increase the potency of an adenovirus-vectored foot-and-

- mouth disease virus serotype A subunit vaccine. *Vaccine* 2018;36:1078–84. <https://doi.org/10.1016/j.vaccine.2018.01.026>.
- [18] Paton DJ, de Clercq K, Greiner M, Dekker A, Brocchi E, Bergmann I, et al. Application of non-structural protein antibody tests in substantiating freedom from foot-and-mouth disease virus infection after emergency vaccination of cattle. *Vaccine* 2006;24:6503–12. <https://doi.org/10.1016/j.vaccine.2006.06.032>.
- [19] Sumption K. The progressive control pathway for FMD (PCP-FMD): a tool for developing sustainable long term national and regional FMD Control. Bangkok, Thailand: FAO/OIE Glob Conf Foot Mouth Dis; 2012.
- [20] Chendon Y, Robertson SE. Interrupting the transmission of wild polioviruses with vaccines: immunological considerations. *Bull World Health Organ* 1994;72:973–83.
- [21] Paton DJ, Valarcher JF, Bergmann I, Matlho OG, Zakharov VM, Palma EL, et al. Selection of foot and mouth disease vaccine strains – a review. *Rev Sci Tech* 2005;24:981–93.
- [22] Vickers AJ. How to randomize. *J Soc Integr Oncol* 2006;4:194–8. <https://doi.org/10.2310/7200.2006.023>.
- [23] Suresh K. An overview of randomization techniques: an unbiased assessment of outcome in clinical research. *J Hum Reprod Sci* 2011;4:8–11. <https://doi.org/10.4103/0974-1208.82352>.
- [24] Aanensen DM, Huntley DM, Feil EJ, Al-Owaini F, Spratt BG. EpiCollect: linking smartphones to web applications for epidemiology, ecology and community data collection. *PLoS One* 2009;4. <https://doi.org/10.1371/journal.pone.0006968>.
- [25] Halloran ME, Struchiner CJ, Longini IM. Study designs for evaluating different efficacy and effectiveness aspects of vaccines. *Am J Epidemiol* 1997;146:789–803. <https://doi.org/10.1093/oxfordjournals.aje.a009196>.
- [26] Carpenter TE. Use of sample size for estimating efficacy of a vaccine against an infectious disease. *Am J Vet Res* 2001;62:1582–4. <https://doi.org/10.2460/ajvr.2001.62.1582>.
- [27] Jones B, Jarvis P, Lewis JA, Ebbutt AF. Trials to assess equivalence: the importance of rigorous methods. *BMJ* 1996;313:36–9. <https://doi.org/10.1136/bmj.313.7048.36>.
- [28] Donken R, de Melker HE, Rots NY, Berbers G, Knol MJ. Comparing vaccines: a systematic review of the use of the non-inferiority margin in vaccine trials. *Vaccine* 2015;33:1426–32. <https://doi.org/10.1016/j.vaccine.2015.01.072>.
- [29] Parida S. Vaccination against foot-and-mouth disease virus: strategies and effectiveness. *Exp Rev Vacc* 2009;8:347–65. <https://doi.org/10.1586/14760584.8.3.347>.