Recommendation to AgResults on using serological indicators ("valency testing") of cross-protection for FMD vaccines

Summary

This document has been prepared in support of the AgResults Foot and Mouth Disease (FMD) Vaccine Challenge Project in Eastern Africa to outline options for the use of serological thresholds to provide a measure of antigenic relevance for FMD vaccines. A relationship between antibody titres and protection has been shown for vaccinated cattle that are challenged with either the same strain as is in the vaccine (homologous protection) or with a different virus strain (heterologous protection or cross-protection). However, predicting protection from antibody titres is problematic because: 1) titres that correlate with a specific level of protection are different between virus strains; 2) very few heterologous challenge studies have been done; 3) laboratories obtain different titres when testing the same serum; 4) due to animal-to-animal variation, the correlation is not reliable for small groups of animals.

This report considers (1) whether or not sera must be collected 21 days after one vaccination or 10 days after a primary course of 2 vaccinations? (2) how many animals must pass - 80% (4/5) or 60% (3/5) - or if the geometric mean titre should be used? (3) what serological cut-off should be used to indicate an acceptable likelihood of cross-protection?

The conclusions from the limited available data are as follows:

- Using VNT, an indicator of heterologous cross-protection is considered to be a $\log_{10}$ reciprocal titre of **1.5** (cut-off value) **after a single dose vaccination** with serum collected 21 days later.
- **Three out of five cattle** should have titres at or greater than this level for a pass.
- Due to limited data and the use of only five cattle, the precision of such an evaluation will be low and these threshold values should be regarded solely as a pragmatic indicator needed by and set for the purposes of the AgResults FMD Vaccine Challenge Project and not as a validated immunological standard.

Introduction

AgResults is seeking to promote the use of high-quality vaccines to improve the control of FMD in Eastern Africa where four serotypes of FMDV circulate (O, A, SAT1, SAT2). The effectiveness of vaccination against FMD is influenced by many factors, including vaccine quality (potency and antigenic relevance), the way vaccination is implemented (e.g. regime, cold chain and coverage), the weight of infection that must be blocked (e.g. livestock densities and contact structures) and how well the vaccination is supported by other control measures (e.g. movement controls, biosecurity).

The AgResults target product profile (TPP) sets out the minimum standards for FMD vaccine quality in the AgResults competition. Vaccines must have proven efficacy against serotypes O, A, SAT1 and

SAT2. Potency of each antigen must be at least 6 PD_{50}/dose (over 80% probability of protection (Goris et al., 2007; Jamal et al., 2008)), measured after single dose vaccination and homologous challenge. The antigenic relevance of each serotype must be demonstrated by serology involving the vaccination of five cattle and testing of their sera in virus neutralisation tests (VNT) against a panel of four regionally representative field strains per serotype. An acceptable immune response must be demonstrated against at least three of the four strains for all four serotypes. This report considers (1) whether or not sera must be collected 21 days after one vaccination or 10 days after a primary course of 2 vaccinations? (2) how many animals must pass 80% (4/5) or 60% (3/5) or if the geometric mean titre should be used? (3) what serological cut-off should be used to indicate an acceptable likelihood of cross-protection?

Previous research has shown that there is a correlation between the neutralising antibody titre and protection against homologous challenge (Cunha et al., 1957; Mackowiak et al., 1962; Pay and Hingley, 1986; Barnett et al., 2003; Maradei et al., 2008). There is, however, variability in the protection and VNT titre of individual animals receiving the same vaccine dose, as well as the relationship between titre and protection for individual animals (Paton et al., 2019). Furthermore, the amount of antibody needed to protect against different viruses varies per virus. This report considers what is the best method for conducting this serological evaluation of vaccine quality combined with antigenic relevance, taking account of the uncertainties created by the low precision inherent in testing only five cattle and the incomplete validation of serological methods of evaluation.

The association between VNT titre and cross-protection

The relationship between serology and protection in FMD vaccinated cattle has been studied by correlating the antibody titres at the point of challenge with the outcome of challenge, i.e. protection or not. These studies have been done mainly at 21-28 days post vaccination with the same strain of FMDV as is incorporated in the vaccine (homologous challenge). The OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (Terrestrial Manual; OIE 2019) recommends the use of serology as an indirect measure of potency for vaccine batch control once the association between serology and protection has been established from such a challenge test. Some vaccine manufacturers already use these thresholds as indicators of cross-protection after changing the virus in the test to the field strain against which protection is needed.

Barnett et al. (2003) looked at the possibility of using generic serology thresholds as predictors of homologous protection using the WRLFMD VNT as the serological test (the test system proposed for AgResults). By studying the VNT results of 407 cattle from challenge tests, using 6 different serotypes, they estimated the titre associated with protection for the different serotypes, and evaluated the extent of animal-to-animal variability. From this and other work, it has become clear that although most serotypes behave in a similar way, the actual thresholds of protection are serotype and often strain-specific.

Cross-protection studies involving vaccination and heterologous challenges of cattle with a different strain of the same serotype as in the vaccine have been conducted infrequently and it is important to recognise that no empirical data is available to define protective responses for the FMDV lineages

that circulate in Eastern Africa. As part of the on-going OIE twinning project with AU-PANVAC, we have attempted to collect and test available sera from cross-protection challenge studies, using the WRLFMD VNT. This was done to test the hypothesis that the titres associated with protection after homologous challenge would be equivalent to those after heterologous challenge, provided that the heterologous virus was used in the VNT. These studies are not completed and further sera have been promised from additional studies. So far, a collection of 121 sera have been assembled and tested from studies with four FMDV serotypes as summarised in Table 1 below:

Sero-type	Vaccine	Challenge	Dose	Number vac/chall	Challenge time	Protection result	Reference
O	O Manisa	O/ALG/3/2014	various	15	21dpv	7/15	Fishbourne et al 2017, Vaccine, 35(20):2761-2765
O	O Manisa	O Campos	various	31	21 dpv	8/31	Nagendrakumar et al 2011, Vaccine 29: 1906–1912
A	AIrn05/ASau95	AIrn22/2015	Full	16	21dpv	9/16	Waters et al 2018, Vaccine 36 (14), 1901-1907
A	A22	AIrn22/2015	Full	7	21 dpv	2/7	Dekker et al, 2020
A	AMay97	AIrn22/2015	Full	7	21 dpv	5/7	Dekker et al, 2020
A	AMay97	AIrn22/2015	various	15	21 dpv	13/15	Dekker et al, 2020
Asia1	Asia1 Shamir	Asia1 Tur49/11	various	15	21 dpv	13/15	Li et al, unpublished
SAT2	SAT2 Sau/2000	SAT2 Lib/2012	various	15	21 dpv	11/15	Dekker et al, unpublished

Table 1. Cross-protection studies where point of challenge VN titres have been correlated with protection outcomes. NB In the AMay97 experiments, the same vaccine strain and the same challenge strain are used.

A summary of the underlying relationships between heterologous VNT titre and protection that were revealed by modelling is shown in Figure 1 below:

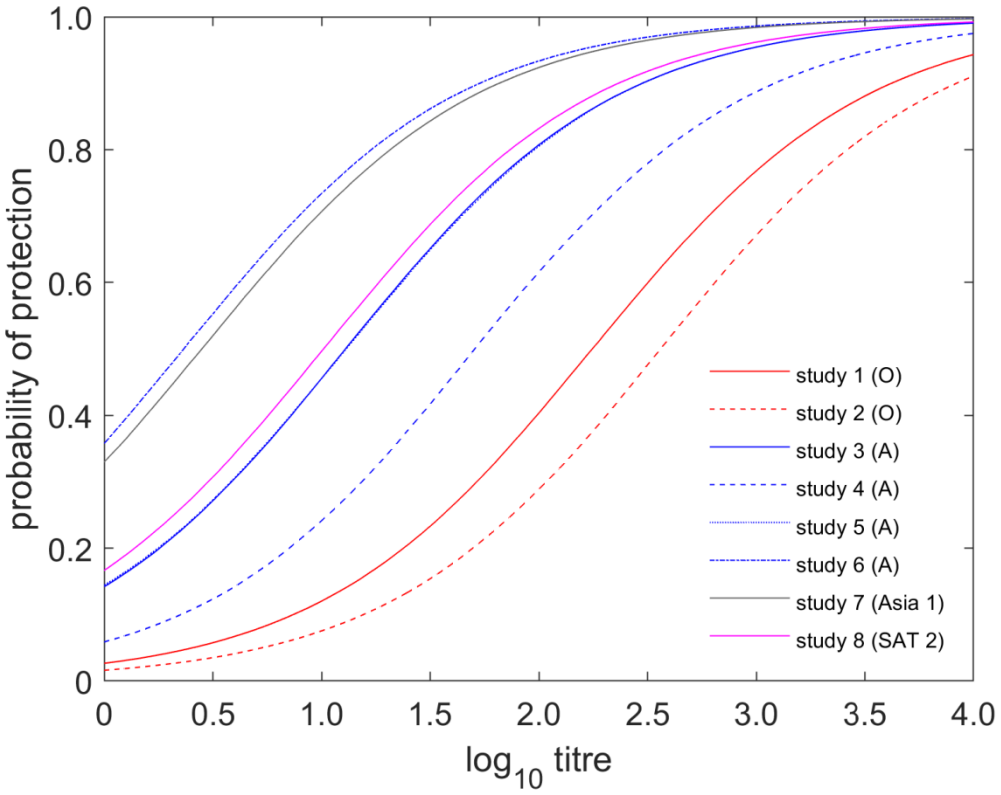

Figure 1 shows the probability of protection as a function of $\log_{10}$ titre for each of the eight studies. The lines show the posterior median for the probability of protection for each study. Colour indicates serotype: O (red), A (blue), Asia 1 (grey) and SAT 2 (magenta).

The serological thresholds associated with different probabilities of protection are summarised in Figure 2, which also compares the thresholds for heterologous protection to those previously associated with homologous protection by Barnett et al (2003).

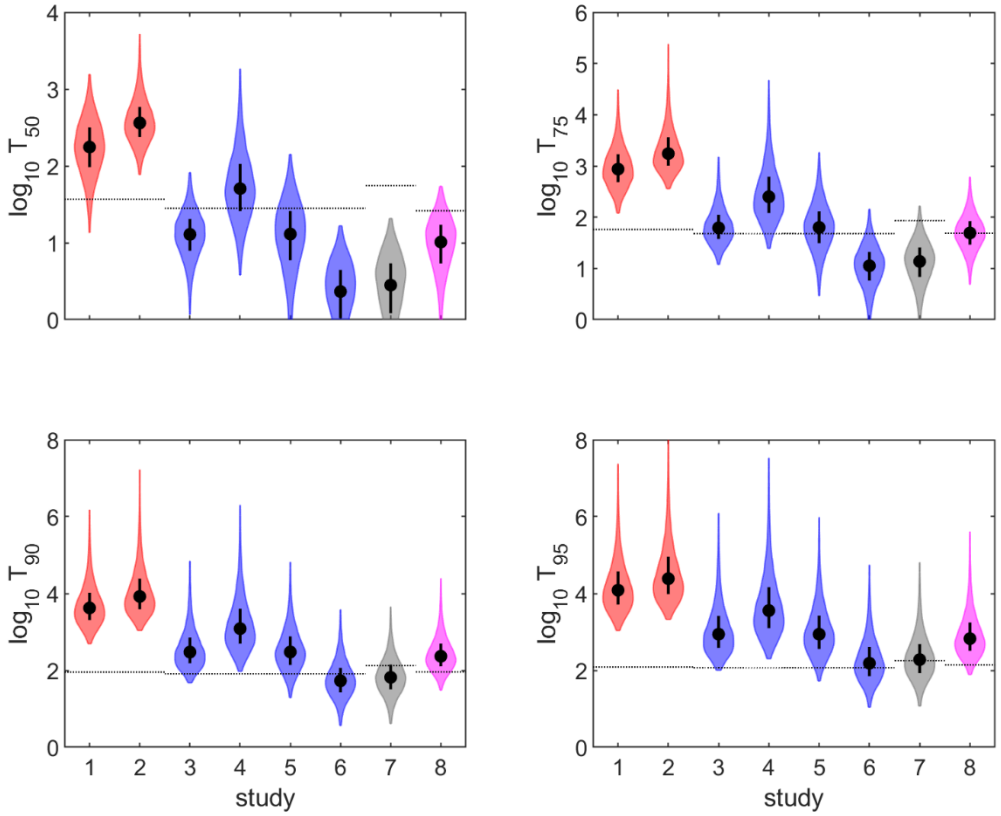

Figure 2. This shows posterior median (black circles), interquartile range (black line) and density (up to 95%) (shape) for the $\log_{10}$ titre required for 50% (T_{50}), 75% (T_{75}), 90% (T_{90}) or 95% (T_{95}) of cattle to be protected, estimated for each study. Colour indicates serotype: O (red), A (blue), Asia 1 (grey) and SAT 2 (magenta). The black dotted lines indicate the thresholds for protection from homologous challenge reported in Barnett et al. (2003).

The main conclusions are:

1) There are wide credible intervals due to the small numbers of animals in each study. This means that a system based on serological evaluation of only five vaccinated cattle will always lack precision (i.e. tend to under or over score the performance of some vaccines).

2) There is also considerable study-to-study variability but the numbers are too small to ascertain whether or not the differences are due to vaccine/virus specific effects.

3) The results indicate that more studies are required to properly establish the thresholds for heterologous protection and to judge whether or not virus substitution in the VNT can lead to an equivalent titre predictive of homologous and heterologous protection.

Discussion modality

Prior to finalizing this report, the results of the testing and analysis of sera from cross-protection studies and the possibility of setting a threshold for predicting cross-protection have been discussed with colleagues who have expertise in evaluating FMD vaccines.

The effect of booster vaccination

As for most other killed vaccines, FMD vaccines are more effective if given as a two dose primary course, which results in a stronger, broader, and more durable protection and this is recommended by most, if not all, FMD vaccine manufacturers. However, the potency tests required at registration for proof of efficacy usually involve challenge after a one dose vaccination and it is easier to distinguish a poor vaccine from a good one after a single rather than a double dose primary course. In contrast, a two-dose vaccination is generally used to demonstrate duration of immunity - usually of at least six months. Due to the extra effort and cost involved, many vaccine users only give one dose of vaccine to naive animals. Whether or not this approach will be sufficient will depend upon many factors, such as the potency of the vaccine, its antigenic match to the field strains, the age structure of the target livestock population, the timing of subsequent revaccination and the timing and weight of challenge.

Conclusions and recommendations

1) Thresholds of predictive protection.

The preliminary data that we have so far gathered on the relationship between heterologous VNT and cross-protection are insufficient to judge the hypothesis that similar thresholds are indicative of homologous and heterologous protection, so long as the appropriate test virus is used. Considering that the homologous thresholds are much better validated, it makes sense to use them rather than those established in our preliminary studies of cross-protection. However, it would be unreasonable to expect a vaccine to have the same probability of protection when confronted with a heterologous challenge compared to a homologous one. Therefore, a slightly lower threshold is required. If the AgResults TPP requires vaccines to be at least 6 PD_{50} (>80% probability of protection after homologous challenge), then a somewhat lower threshold should be set for heterologous protection (50% probability, which equates to 1 PD_{50}). This approximates to the approach used in vaccine matching, where a one-way relationship value of 0.3 between a vaccine and a field strain is considered sufficient (an r_1 value of 0.3 corresponds to around a 0.5 log10 reduction in titre). For the fitted responses in Fig 1 this corresponds to a change from 80% protected to about 60% protected. Barnett et al (2003) found that the log_{10} reciprocal VNT titres that correlate with 50% probability of protection were 1.57, 1.45, 1.15 and 1.41 for serotypes O, A, SAT1 and SAT2 respectively. Given the similarity of these values and the lack of precision in any estimate based on five animals, we therefore suggest a generic heterologous cut-off for the East Africa Reference antigens of log_{10} 1.5 or 1 in 32 dilution (i.e. log_{10} 1.5 or greater is a pass). This should be corrected in light of new data that may become available from on-going projects.

For antigenic relevance testing of vaccines, an argument can be made to include sera derived from either single dose or double dose vaccinated cattle, according to the regime that will actually be

used. However, we consider that the benchmark should be set using only single dose vaccinated cattle (at 21 days post-vaccination) as: (1) vaccine potency is usually assessed after a single vaccination and our proposed measurement of heterologous responses will be a proxy measurement of vaccine potency and antigenic match combined in one experiment, (2) it is simpler and less expensive to generate sera from cattle given only one dose, (3) we do not know what threshold is appropriate after a double dose, (4) it is easier to discriminate between good and poor vaccines after a single dose based on the metrics that are available for single dose potency studies, and (5) we know that users do not always follow recommendations for two doses to be used. As with the current potency test by challenge, this does not contradict the argument that a double dose course is likely to be beneficial.

From a preliminary analysis of sera collected from groups of five cattle vaccinated once with candidate vaccines (confidential data not shown), we see variability in antibody responses, signifying that the proportion that have to respond at or above the threshold will influence the stringency of the evaluation. An option is to require that the geometric mean titre for the group should pass the threshold. However, given the small numbers, the mean can be sensitive to outliers. For example, four animals might have titres well below the level expected to give protection, whilst one has a very high (and protective) titre. This might result in a geometric mean titre above the threshold, despite only one out of five cattle expected to be protected. An alternative that is less sensitive to outliers would be to consider the median titre. For a group size of five this would be equivalent to requiring three out of five cattle to have titres above the threshold and this is therefore our recommendation.

2) Precision and validation and limitations of testing

It must be understood that although the threshold has been selected based on the best evidence that is available, these cut-offs are not properly validated and the numbers of animals proposed are too small to provide a high confidence in any predictions that are made as to whether or not animals would actually be protected (especially where titres are close to the acceptance threshold). Aside from the accuracy with which vaccine quality can be predicted, it is also important to remember that this type of testing will also not provide strong evidence that a vaccine will protect against infection in the field, where neither the quality of the vaccination campaign (timing of vaccination and cold chain) nor the antigenic differences of the actually circulating strains are known.

This document considers an approach where sera from only 5 animals are tested. However, if instead, the manufacturers supplied a larger number of sera (for example from a set of fifteen cattle involved in a potency study by challenge) then this would improve the precision of testing for antigenic relevance. Furthermore, if the sera came from a challenge test, the actual data on protection could be used to help set a specific threshold for that vaccine rather than relying on a generic one. Use of additional sera from manufacturers should therefore be strongly considered.

Other options exist for interpretation and use of thresholds. With this level of uncertainty, a comparative rather than an absolute judgement on titres could avoid mistakes in categorisation and instead users might be allowed to see for themselves whether or not different vaccines induce similar or widely different antibody responses. We recommend that AgResults consider publishing

the results from heterologous testing, as an aid to vaccine selection by users. It would also be possible to consider giving advice on interpretation of published results, as is done currently when providing antigenic matching results based on r_1 values (also known to be unreliable when used in isolation as one-off indicators). Three titre ranges could be considered, defining three levels of predicted probabilities for cross-protection: low, uncertain and high. Vaccines in the low category could be rejected, those in the uncertain category could be considered only acceptable if regularly boosted including a double dose primary course.

Since the chosen threshold cannot be fully backed up scientifically (due to lack of precision and validation), it is recommended to make it clear that this generic cut-off has been set to enable AgResults to meet a specific practical need for competitive discrimination and is not an immunologically validated standard. Furthermore, evaluation criteria can and should be updated over time as further knowledge is accumulated.

Finally, it should be emphasised that the confirmation of antigenic relevance does not replace the need to check actual match between candidate vaccines and current field strains on an ongoing basis.

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