Workshop

"FMD Cross-protection, Vaccine-matching and Vaccine Banks: Challenges and Opportunities"

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Guido König and Mariano Perez-Filgueira

Wednesday, **June 15th**

Vaccine and Antigen Banks

Coordinator: Eduardo Maradei (SENASA)

Presentations

- Foot and Mouth Disease Antigen and Vaccine Bank- SENASA- Argentina. **Eduardo Maradei** (SENASA).
- Foot-and-mouth disease Antigen Banks: MERIAL Experience. **Philippe Dubourget** (Merial).
- The Argentine FMD Antigen and Vaccine Bank (AFMDVB); 10 years of Experience. **Rodolfo Bellinzoni** (Biogénesis-Bagó).

Summary from talks and discussions:

1. Strategic stockpile options can be final vaccine stocks (formulated with the corresponding adjuvant) stored at +4°C or bulk antigens kept frozen at ultra-low temperatures, usually in liquid nitrogen (< -130ºC) that can be used to rapidly formulate vaccine for emergency use.
    a. Vaccine banks
        i. Advantages: immediate availability of the immunogens and the possibility of storage in different parts of the country in refrigerated rooms. Lower storage costs than ultrafrozen antigens.
        ii. Disadvantages: ready to use formulations have limited capacity to respond to antigenic variation in the field, vaccines have to be discarded and replaced at the end of shelf-life which also generates disposal costs of unused vaccine.
    b. Antigens banks
        i. Advantages: concentrated inactivated antigens may comprise a wider range of important epidemiologically relevant field strains which can be used for the formulation of "tailored" emergency vaccines. Longer shelf-life than formulated vaccines.
        ii. Disadvantages: trial vaccine blends need to be formulated for safety and potency tests, require sophisticated manufacturing process for concentration and storage, storage should be done in centralized, expensive and dedicated facilities and delay in formulation of final filled vaccine.
2. A combination of antigen banks and vaccine reserves can be used to source emergency vaccine supplies.
3. Vaccines produced from these controlled purified antigen banks have the potential to be used as DIVA vaccines to identify infected herds/animals following vaccination.
4. Emergency reserves should store high potency vaccines. At least for serotype A, it has been demonstrated that high potency may compensate a poor match.
5. The advantages of having an Antigen or Vaccine Bank in your own country are the quick availability of the immunogens, the flexibility to incorporate new strains and the possibility of keeping the banks updated for research and diagnostic projects like vaccine matching

studies. The main disadvantage is the cost, which is obviously higher than participating in regional banks.

6. The Argentinean FMD Antigen and Vaccine Bank played a major role in the scale up production of vaccines for the rapid control of the disease during the 2000-2002 regional FMD outbreaks. At current, the Argentinean bank has mono-, bi- and trivalent antigens for regional and non-regional FMDV strains. The stability of concentrated frozen antigens has been confirmed for up to 4 years.
7. The Hemispheric Program for the Eradication of Foot and Mouth Disease (PHEFA) Action Plan 2011-2020 recommends the creation of a regional bank in South America.
8. This bank should include extra-continental inactivated antigens. The handling of strains exotic to the continent should be authorized for by an official laboratory with BSL 4 OIE biosecurity conditions. Also, a risk analysis should be performed before the introduction of an exotic FMDV strain. Currently, each country decides which policies to implement to allow handling of the different strains, according to their capabilities and regulations.
9. Vaccine and antigen banks in Europe are held by Merial at Lyon and Pirbright, respectively. Financial resources are provided by the European Commission. Individual countries have restricted sets of vaccines. Since early 2000, due to terrorist's threats, no info is shared between banks.
10. In the EU, there are no defined procedures for incorporating new vaccine strains and the decision is reliant on the commercial sector.
11. The OIE/FAO FMD Laboratory Network coordinates the global strain surveillance using samples contributed by over 40 countries. The WRL generates quarterly reports and OIE/FAO RL Network annual reports.
12. Vaccine selection in practice: the WRL produces individual reports to affected countries and provides recommendations based on a list of internationally available vaccines.
13. A virtual 'global' vaccine bank could orchestrate additional emergency cover with vaccine or antigen from member's reserves, thereby (i) improving the effectiveness of reserves (ii) ensuring a better control of FMD in the event of an outbreak and (iii) reducing the cost of individual bank membership. However a number of regulatory and political hurdles prevent this accomplishment, even though many banks face similar issues (i.e., selecting strains, manufacturers and numbers of doses or testing and maintaining antigens/vaccines).

Issues to be addressed:

a. Definition of exotic strains: not defined by OIE. Currently, it is taken as a local rather than a regional concept. Even locally, it is not clear whether it refers to an exotic strain which has not been active for a certain time or if it is related to vaccine cross-protection issues.
b. Harmonised matching tests and procedures for serological tests and improved access to virus strains, reference sera and vaccines.
c. Optimise the collection protocols of field data (field evaluation of vaccine induced protection=ideal sets of data) during outbreaks or from endemic areas.

d. Generation of procedures to introduce changes in the vaccine formulations (especially in South America and following the European example) agreed by Reference Laboratories, Regulatory Authorities and Vaccine manufacturers.
e. The creation of a virtual 'global' vaccine bank.

Reference Reagents for Vaccine Matching

Led by Yanmin Li (IAH-WRL)

Presentations

- The vaccine matching studies and the reagents from the WRL/EURL for FMD. **Yanmin Li** (IAH-WRL).

Summary from talk and further discussions:

1. The similarity between the r1 values from the pooled sera and the mean of the individual serum was discussed: The r1 values from the infected pooled SAT 1 sera didn't represent the mean of the r1 values by using the individual serum. Whereas, the r1 values from the vaccined pooled sera provided the similar pattern to the mean of the r1 values derived by using the individual serum.
2. The information on the materials for the ring trial for FMD vaccine matching studies 2008 and 2009 was requested and provided: The inter-laboratory comparative test 2008 and 2009 was for FMD type A. The vaccine virus was the prototype A_{22} Iraq. The field isolates were selected to give a range of high, medium and low r1 values. All viruses were sent in the same passage and ready for use. Hence they are homogeneity for the exercise. However, there are differences in virus in terms of the passage numbers from different laboratories when they perform the routine diagnosis.
3. The number of field isolates to be tested for r1 values and how would they be chosen in WRL was asked and the answer was: WRL normally choose only a few representative field isolates from a submission for the r1 value testing. The phylogenetic tree can be used as a starting point for choosing a virus from the same cluster as the representative. But this wasn't always the case for two reasons: the sequencing work and the vaccine matching studies most time have been performed at the same time to speed up the reporting of the results. Also we are fully aware that the antigenic relationship doesn't always agree with the antigenic analysis. But due to the limitation of the resources, WRL isn't practically possible to test r1 values for all virus samples submitted. The vaccine to be tested was chosen based on the knowledge on if it is useful in the area and if it is available from the vaccine bank.
4. The correlation between r1 values and the protection results has been discussed and this correlation was validated from 15 or more accumulated potency tests and published in 1970's from WRL.
5. The decision maker on the incorporation of a new vaccine strain and the role of the reference laboratory has been asked and the information was provided: It will be a

communal decision on the incorporation of a new vaccine strain into the vaccine bank from the Europe. The discussion will involve CVOs, EUFMD, OIE, WRL and the vaccine bank. As the reference laboratory, WRL will provide the recommendation on the vaccine selection on the request to the customer or the local authorities. The recommendation will be made based on the vaccine matching studies on the circulating virus and the monitoring on the performance of the virus from the area. EURL has conducted a heterologous challenge experiment to test the vaccine efficacy on the prevalence virus from a region. The field information on the efficiency of the vaccine recommended was asked from WRL but they hardly received the information requested.

6. The usage of the Mab was discussed and it was agreed that the FMD Mab will provide more specific results than the polyclonal antibodies. However, a well characterised a panel of Mab, which isn't currently available, should be build before it can be used for the FMD vaccine matching studies.

Ring-test Based on LP-ELISA for Determination of "r" Indexes for Serotype A Strains

Led by Tom Willems (CODA-VAR).

Presentations

- Ring-test based on LP-ELISA for determination of "r" indexes for serotype A strains. **Tom Willems** (CODA-VAR).

Summary from talk and further discussions:

A mini ring-test was carried out to determine serological relationships between serotype A strains by lpELISA. Six Laboratories were involved but only the results from 4 of them were received on time. Three pools of 5-10 bovine sera of high and medium homologous titers were sent to every Lab, as well as homologous (A24/Cruzeiro) and heterologous (A/Arg/01, A/Arg/87 and A/Arg/00) viruses.

Bovine sera was originated in PPG trials carried out in the years 2006-2007 on behalf of the Contract Project No RT-05/06-ALTANDI-2, between RIIDFA and Veterinary and Agrochemical Research Center (VAR).

Two standard control sera (SCS) from multivalent revaccinated cattle were also distributed to be used as controls, with the idea of introducing a correction factor to increase comparability of r1 values determined by the different Labs.

Virus strains were selected based on known low r1 values determined by VNT (r1< 0.2), as well as absence of cross protection in PPG trials.

The results obtained were as follows:

Lab. 1: 27 correct scores (no cross protection)

Lab. 2: No correct scores (cross protection)

Lab. 3: 24 correct scores (no cross protection) and 3 incorrect scores (cross protection)

Lab. 4: 18 correct scores (no cross protection) and 9 incorrect scores (cross protection)

- Different r1 results were obtained in different labs, ranging from low to high cross protection
- The lowest r1 values were obtained with the pool of sera with highest titers, but no significant differences were found between pools of 5 or 10 sera. Moreover, no significant differences were found between pools made of high or medium titer sera.
- The SCS were cross reactive with all strains, as expected, but a correction factor could not be applied

Different general reagents and different protocols were used by the Labs, as well as different concentrations of antigen and capture antibodies.

All labs found difficulties to capture the three heterologous antigens using sera to A24/Cruzeiro. Therefore, high concentrated antigen was used in the assays and in many cases the results could not be repeated for confirmation.

One of the Labs used cross-reactive monoclonal antibodies, allowing the capture of all strains.

Overall, the results showed low comparability.

After discussion, some recommendations arose, such as:

- Monovalent sera should be used as SCS
- A more related virus strain, with an r1 value higher to 0.4, should be included
- lpELISA r1 might become comparable if all labs had used identical reagents

Future work will estimate r1 values by VNT using this same set of reagents.

Thursday, **June 16th**

Recent and Ongoing Cross-Protection Experiments: *In Vivo* Results and Predictive *In Vitro* Assays

Led by Nora Mattion (CONICET)

Presentations

- FMD estimation of vaccine potency and cross protection. **Tom Willems** (CODA-VAR)
- Evaluation of cross protection within FMD virus A and O strains. **Madhan Mohan** (IIL).
- Custom-engineered chimeric FMD vaccines elicit protective immune responses in hosts. F**rancois Maree** (OVI).
- Predicting vaccine protection: the experience of PANAFTOSA. **Rossana Allende** (PANAFTOSA)
- Vaccine matching results on recent "O" field isolates. **Eduardo Maradei** (SENASA).

Summary from talks and discussions:

1. *In vivo* vaccine matching experiments (n=5) were performed in cattle (n=75) following the PD50 protocol, using FMDV A96 Iran as vaccine strain and A22 Iraq as challenge strain *[CODA-VAR in collaboration with Intervet (Germany), FLI (Germany), FGI-ARRIAH (Russia)]*

- ✓ Results showed low cross-protection (mean PD_{50}= 1.03) with high variability among experiments: PD_{50} values ranged from 0.50 to 3.48 (95% CI=0.59-2.12), with an accordance = 72.6% and a concordance = 70.4%.

2. Alternative *in vitro* methods (EPD_{50}) based on VNT or LPB-ELISA were also applied in this experiment. Assays were performed by 3 different labs (VAR, ARRIAH and BHC)
   - ✓ EPD_{50} values were similarly low to in vivo PD_{50} values (0.6 to 1.4) by both LPB-ELISA and VNT.
   - ✓ r1 values estimated by VN or ELISA were also similar. Values obtained from pooled sera were lower than those from individual samples (and thus closer to *in vivo* results). For VNT, variability of 16-sample pools was lower than from 5-sample pools.
   - ✓ Pools of FMDV A Iran 1996 vaccinal sera (21 dpv) were also tested against 4 different heterologous isolates: FMDV A22 Iraq 24/64, A IRN19/95, A IRN 32/2001 and A IRN 1/2005. Pools comprised: 2 pools of high titres, 1 pool of intermediate titres and 3 pools of mix of high and intermediate titres with different numbers of sera (5, 10, and 15). Performance of the assays was as described above: VNT r-values ≈ LPBE r-values; grouping decreases r-values (as observed for in vivo experiments) and variability of 16-sample pools was lower than for 5-sample pools.
3. Also working with the A serotype, monovalent vaccines were prepared using the A IND 40/00 (8 µg/dose) and A APS 66/05 (6 µg/dose) and challenged with third heterologous strain (A RAJ 21/96). All of them presented several differences along the VP1 aa sequence and poor *in vitro* cross-neutralization. Challenge trials were performed following the PD50 protocol *(IIL)*
   - ✓ The A APS 66/05 vaccinated animals showed high homologous PD50 values (15.85) as well as high heterologous PD50 values (7.94 and 19.95 against A IND 40/00 and A RAJ 21/96, respectively). On the contrary, A IND 40/00 vaccinated calves presented good homologous PD50 values (7.94) but modest or low scores for the heterologous strains (3.98 and 2.00 against A APS 66/05 and A RAJ 21/96, respectively).
   - ✓ As reported earlier, these results indicate that high payload serotype A vaccines can protect against heterologous challenge, although intraserotypic differences also exists for this serotype.
4. Working with the O serotype, animals vaccinated O IND R2/75 formulations (10 µg/dose) were challenged (PD50 protocol) with the O APKr 94/2005, showing good homologous and heterologous PD50 (7.01 and 15.85 for the homologous and heterologous challenge, respectively). These results were in contrast with previous experiments showing limited cross afforded by O1 Manisa vaccines in cattle further challenged with FMDV O1 (Nagendrakumar et al., 2011). (IIL)
   - ✓ Further experiments are needed to substantiate the hypothesis that high payload vaccines can provide heterologous protection for strains within same serotype and similar geographical origin (same topotype).
5. Chimerical viruses were constructed using reverse genetics carrying sequences from the same (SAT2/SAT2) or different (SAT1/SAT2) strains. Recombinant viruses retains properties of the parental virus and can be successfully propagated, inactivated, purified and formulated as vaccines *(OVI).*

- ✓ Similar immune responses induced in guinea pigs for inter-serotype chimera vaccines, indicating similar immunological profiles for the respective viral capsids.
- ✓ Vaccine containing inter- or intra-chimera antigen induced significant immune responses in pigs or cattle to protect animals against homologous virus challenge. However, no direct link between neutralising antibody titers and protection was found in these experiments.

6. The experience gathered during the last decade in South America was presented by PANAFTOSA through 4 field cases: 2001 outbreaks in Uruguay (A serotype), 2003 outbreaks in Bolivia (O serotype), 2004 outbreaks in Brazil (C serotype) and 2010 outbreaks in Ecuador (O serotype) *(PANAFTOSA)*
   - ✓ Vaccine matching assessments were made in all cases based on serological methods (complement fixation, CF; LB-ELISA and virus seroneutralization, VN), using serum panels of single vaccinated and revaccinated cattle.
   - ✓ Vaccine matching assessments performed with the panel from single vaccinated cattle by CF (expressed as r1 values) showed discrepancies with those obtained by LP-ELISA and VN (expressed as expected protection percentage, EPP) in 3 out 4 examples (Brazil, Bolivia and Ecuador): r1 values indicated significant antigenic relatedness (r1>0.4) while EPP values were < 75%.
   - ✓ However, the EEP estimated using a 30 dp revaccination sera panel indicated that revaccinated animals would be protected against field virus in all 4 examples presented. Field evolution of the outbreaks after revaccination coincided with these serological results.
7. Results from *in vitro* and *in vivo* experiments performed in Argentina with samples from the 2009-2010 FMDV O strain outbreaks in Ecuador were also presented. Epithelial samples from infected cattle were analyzed by ELISA typing, RT-PCR and VP1 sequencing. Virus was also isolated by tissue culture and assayed by reactivity against a panel of 20 MAb against FMDV O strains. Vaccine matching was studied *in vitro* by VN (EPP and r1 estimation) and *in vivo* by protection against podal generalization (PPG) challenge experiments. *(RIIDFA)*
   - ✓ Sequence studies revealed that all viruses belonged to a single lineage within the Euro-South American topotypes (>98% identity among 2009 isolates and ~ 100% identity among 2010 isolates). Most of the 2010 isolates showed on average 96% identity with the 2009 virus.
   - ✓ MAb reactivity profiles confirmed sequence results: field isolates clearly differed from vaccine strain O1/Campos, including loss of reactivity against three O1 Campos neutralizing MAb. Moreover, isolates from 2009 differed from most of the 2010 samples thus revealing antigenic changes during virus circulation in the field.
   - ✓ Vaccine matching assessed using the single vaccinated serum panel (O1 Campos) against one of the 2010 isolates (# 46-2010) presented r1 values $\leq$ 0.1. In this case, EPP values obtained against this isolate using the revaccinated serum panel were also low (56% to 62%)

- ✓ Finally, *in vivo* experiments using single vaccinated and revaccinated cattle (O1 Campos monovalent vaccine, 20 µg/dose) challenged with the 46-2010 O Ecuador isolate resulted in 6% and 18% of protected animals, respectively.

Relevant remarks of the session

a. Standard assays, reagents and protocols to evaluate cross-protection are different depending on the laboratories. This usually leads to different conclusions regarding matching between vaccine and field isolates.
b. Moreover, correlation curves between serology and protection are build for vaccinal strains and may not be applied for heterologous field strains.
c. Epidemiological information must be considered when analyzing vaccine matching data. However, this information should be carefully obtained and interpreted to avoid confusing data on the temporal and geographical progression of the outbreaks.
d. Vaccines are useful tools if used in well defined vaccination programs and together with field control strategies. As an example, the overall situation of the disease in South America showed a clear improvement during the last two decades.
e. Weakness of vaccination programs and epidemiological field strategies may be related to outbreak occurrence in some cases.
f. However, matching results obtained under controlled experimental conditions also pointed out that for some cases, update of vaccine strains should be also considered. Circulating field strains may accumulate mutations that result in antigenic differences with current vaccine strains that were isolated many decades before.

Immunological Bases of Cross Protection

Led by Alejandra Capozzo (CONICET) and Mariano Pérez-Filgueira (INTA)

Presentations

- Overview of the homologous and heterologous acquired immune responses against FMDV in cattle. **Mariano Perez-Filgueira** (INTA) .
- FMD vaccine induced correlation in protection with humoral and cellular immunity. **Satya Parida** (IAH).
- Antibody-based effector mechanisms relating to vaccine. **Nils Lannes** (IVI).
- Alternative parameters of specific humoral responses against FMDV: Immunoglobulin avidity and subtypes. **Alejandra Capozzo** (CONICET)

Summary from talks and discussions:

1. Initial approaches to the study of heterologous responses to FMDV strains were conducted using peptides comprising discrete immunogenic regions within structural proteins. These early studies revealed the importance of the passive epitopes, sequences surrounding the energetic epitope that provide surface complementarities around the residues that form the energetic epitope.

2. Several results also point out the heterologous T-cell responses are more frequent to find than cross-reactive humoral responses.
3. A review of previous papers highlighted a set of strategies to induce heterologous responses and cross-protection: multiple vaccinations, multivalent vaccines, route of administration high payload vaccines and silencing of immunodominant epitopes.
4. The role T-cell mediated immunity in protection was analysed and T CD4+ T-lymphocytes were identified as the subset that proliferated and produced IFN-γ after *in vitro* stimulation of peripheral blood from vaccinated animals.
5. The evaluation of the T-cell responses was proposed as an alternative parameter for indirect assessment of protection.
6. Two complementary serological parameters were proposed to study cross-protection: avidity of specific antibodies induced and IgG subtyping.
7. Vaccinated animals that failed PPG-test with homologous or heterologous strain have low avidity antibodies (avidity indexes < 25%). Low amounts of high avidity antibodies against epitopes shared by different strains were found in monovalent vaccinated animals (A and O strains)
8. Animals vaccinated with monovalent vaccine and protected against challenge with a heterologous strain have higher IgG1/IgG2 values than non protected animals. Protected animals with low heterologous VN titers have high IgG1/IgG2 ratios
9. The development steps of an alternative potency test based on the evaluation of the presence of opsonising Ab was presented.
10. The strategy is based on a mouse macrophage cell line (RAW 267.7) transduced with bovine CD32 (FcR) which becomes susceptible to FMDV only when complexed with bovine antibodies. This would allow evaluating bovine sera by their ability to promote opsonisation-related virus phagocytosis.

Issues to be addressed:

a. Development of experimental strategies to detect cross-protective B-cell epitopes. Evaluate the role of the deceiving imprinting in cross-reactive responses in the FMDV model.
b. Apply new approaches and techniques (some of them currently in development) to study cross-protective humoral and cellular immune responses.
c. Study the role of T-independent and T-dependent responses to the virus in the heterologous protection.

Informatics-Based Approaches for Cross-Protection

Led by Francois Maree (OVI) and Richard Reeve (UoG)

Presentations

- Introduction: History and homologous protection. **Richard Reeve** (UoG)
- Mapping the antigenic variation of FMDV serotype A. **David Paton** (IAH)
- Using antigenic data on the FMDV capsid for the selection of new vaccine candidates for the SAT types. **Francois Maree** (OVI).
- Bioinformatics approaches to predict vaccine match and further antigenic sites for serotype O FMDV. **Mana Mahapatra** (IAH)

Summary from talks and discussions:

1. Existing methods for calculating protection from challenge date back to 1908, and existing methods are largely unchanged since 1980s. There are now Bayesian methods for examining challenge experiments and choosing new protocols that require fewer animals to obtain the same information.
2. Challenge-based methods have been extended by examining serological correlates of protection over the last 20 years, and there are 3Rs pressures to move to such methods. However, problems are known to exist with inter-strain variability in protective titres.
3. Techniques developed for Influenza have been extended to allow 'mapping' of (serotype A) strains in an antigenic landscape that improves our ability to visualise variability in antigenicity of strains. This will hopefully allow us to better visualise selection of new vaccine candidates.
4. Predictive methods based on measuring sequence variation at epitopes may allow us to identify cross-reactivity and hence cross-protection from sequence data. This is achieved by the combination of sequence and structural data with virus neutralisation assays through a mathematical model.
5. This is particularly important for SAT viruses where there is high antigenic and sequence variability. FAO and SADC funding has now provided a more antigenic data for SAT1, and there are indications that 3 vaccine strains may be sufficient to cover the serotype. More work is needed on SAT2 – UK funding may provide some of the samples for this work.
6. A serotype O modelling/prediction study has been carried out by IAH/UoG. Models are showing ability to identify epitopes, but r1-values > 1 causing problems with predicting cross-reactivity, as unexplained in literature.
7. Structural modelling software also used to identify epitopes – Epitopia seems to be most reliable, but also Discotope, Seppa. Seem to be reliable predictors, but sensitivity-specificity trade-off inevitable in this kind of more theoretical model
8. There is significant overlap between serology-based epitope prediction, mAb-based epitope identification studies and structural prediction. More work (reverse genetics) needs to go into understanding inconsistencies.