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Fighting Foot-and-Mouth Disease together

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Can antigen determination replace animal experiments in foot-and-mouth disease vaccine control?

Aldo Dekker
Central Veterinary Institute, Lelystad, the Netherlands

Introduction

With FMD vaccines there is very good correlation between antibody response and protection. Consequently, a vaccine is often made available after performing a study in animals showing that there is a sufficient antibody response (Robiolo, La Torre et al. 2010; Robiolo, La Torre et al. 2010). However, modern vaccine production is much better controlled than it was in the past, so it can be argued that if a vaccine is produced with the same amount of antigen and production method, one would expect the same result.

In this paper we analyse whether protection can be equally or better explained by antigen amount than by the serological response.

Material and Methods

Data

The data used in this study is published data from potency tests performed in the United Kingdom (Pay and Hingley 1987) using Al(OH)3 adjuvanted vaccines. These 15 potency tests were performed according to the British Pharmacopoeia, which at the time prescribed dilution in adjuvant rather than dilution in buffer (as is prescribed by the European Pharmacopoeia) for decreasing the dose. In total, 360 cattle were vaccinated, with vaccines containing either A24 Cruzeiro, O1 BFS or C1 Noville. For each antigen, 5 batches were tested using 8 cattle vaccinated with a ½ dose, 8 with a 1/10 dose and 8 with a 1/50 dose. This design allows the analysis of the dose-response curve based on the amount of antigen, rather than the amount of vaccine, as is now prescribed by the European Pharmacopoeia. In addition to the antigen dose, the antibody responses were also documented (Pay and Hingley 1987).

Statistical analysis

To determine the relationship between the fraction of protected cattle and several explanatory variables, a generalized linear mixed effects model with a binomial distribution and a logistic link function was used. In these models the fraction of protected cattle was the response variable and the experiment was the random variable. Explanatory variables (Table 1) were included in a forward stepwise selection process. In each step the model that explained the outcome (protection) best was selected for the next step in which additional explanatory variables were included. A model was selected if it had a significant better explanation of the outcome ($p < 0.05$ based on likelihood ratio test) in comparison with a simpler model (Occam razor principle). All analyses were performed in R (www.r-project.org) using the lme4 package.

Results

The protection level in the experiments was high; 92.5% of the cattle vaccinated with A24 Cruzeiro were protected, 65.8% with O1 BFS and 90.8% with C1 Noville. Table 1 shows the univariate analysis of the explanatory variables.

Table 1: Univariate analysis of the relationship between protection and various explanatory variables using a logistic mixed model with batch number as a random variable.

Variable	Coefficient	Standard error	P value (likelihood ratio test)
VNT titre	5.76	0.73	< 0.001
Serotype	NA	NA	0.001
Antigen dose (^{10}log ng)	0.8	0.27	0.003

Normal linear regression showed that antigen dose and serotype were significantly related to the VNT titre, but the analysis in Table 1 shows that VNT titre had the strongest relation with protection. Therefore, in the forward stepwise analysis, VNT titre was first included in the model and, sequentially, serotype and antigen dose, as well as the interaction between the

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Table 2: Result of the multivariate logistic mixed model with batch number as a random variable

Variable	Coefficient	Standard error	P value (Wald test)
Intercept	-5.63	1.35	< 0.001
VNT titre	6.65	0.87	< 0.001
Serotype O_1 BFS	-6.38	1.24	< 0.001
Serotype C_1 Noville	-1.37	1.13	0.22
Antigen dose (^{10}log ng)	-1.01	0.43	0.02

different explanatory variables, were added. The final model included both serotype and antigen dose, but not the interactions between the various explanatory variables.

Discussion

In this paper we analyse whether or not protection can be equally or better explained by antigen amount than by the serological response. The analysis shows that VNT titre is the best explanatory variable for prediction of protection. In the univariate analysis it is shown that serotype and antigen dose are also related to protection. The difference in protection observed between the different serotypes results in a significant relationship between serotype and protection.

The multivariate analysis shows that protection is better explained by the VNT titre than by antigen dose (Table 2). This finding shows that the efficacy of an inactivated FMD vaccine is not only dependent on the amount of antigen, but also on the adjuvant. In the univariate model a higher amount of antigen is related with higher protection (positive coefficient), but in the final model a higher amount of antigen is related with lower protection (negative coefficient). This is because antibody response and antigen amount are not fully independent. Due to the effect of formulation on the antibody response, it is advised that each batch is tested for antibody response, unless a sufficient number of batches have shown consistency in formulation.

Interestingly the slope of the relationship between VNT titre and protection is the same for the different serotypes, which confirms previous reports (Hingley and Pay 1987; Pay and Hingley 1987). This finding makes the determination of the relationship between antibody response and protection easier. In a recent study, the ELISA titre and antibody response were evaluated with logistic regression separately for each serotype (Robiolo, La Torre et al. 2010). For one of the four serotypes insufficient data were available to get a valid estimate of the slope. However, if the results are analysed using a common slope for all serotypes and different intercepts for the different serotypes, then also for this fourth serotype the relationship between antibody response and protection can be established.

The analysis shows that until a producer has proven consistency of production, every vaccine batch has to be tested for antibody response. The analysis also shows that determination of antibody response is a very good predictor of protection which could negate the need for challenge experiments.

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Memories from Hanoi (David Paton)

Recent approaches to develop live attenuated FMD vaccines

V Umapathi, H J Dechamma, G R Reddy and A Sanyal

ICAR-Indian Veterinary Research Institute, Bengaluru, India

Vaccination is the most efficient method to control and prevent the spread of infectious diseases. Although vaccines have been used for centuries, there remains only three primary strategies to develop them, namely live attenuated vaccines (LAV), killed or inactivated vaccines and subunit vaccines. Out of these, LAVs are the most efficacious, in terms of disease prevention, due to their ability to replicate in relevant tissues, elicit strong cellular and humoral immune responses, and most importantly, confer immunity for life. Despite many LAVs being successfully used in the control and eradication of diseases, those vaccines were developed empirically by blind passaging in cell culture or unnatural hosts. There is always a risk of reverting back to virulence which could be catastrophic. In addition, RNA viruses such as FMDV are not suitable for developing conventional LAVs due to their capacity to exist as quasispecies because of the low fidelity of their RNA dependant RNA polymerase.

With the advances in molecular virology, our understanding of viruses, in terms of replication, expression of their genes, pathogenesis and control mechanisms in the host cell has improved. At the same time, utilising molecular tools such as cDNA technology and reverse genetics, new strategies for rational design of LAVs have been proposed. These approaches to viral attenuation and vaccine design include deleterious gene mutation, altered replication fidelity, codon deoptimization, and control by microRNAs or zinc finger nucleases. Currently the FMD Research Lab, IVRI Bengaluru campus is working on these first three approaches.

1. Deleterious gene mutation/deletion

The identification of genes essential for viral replication and assembly led to the first generation of rationally designed, live attenuated virus vaccines. Deletion or mutation of these genes results in a "defective virus," which cannot replicate in the host. The viruses are propagated in complementing cells that express the missing gene product. In normal cells, the replication cycle occurs for one progeny. However, these virions are non-infectious, so the infection does not spread to a second round of cells. In contrast to killed virus vaccines, the virus produced in the complementing cell line does not require inactivation but has the advantage of live attenuated virus as the virion may enter the cell as normal, produce one cycle of progeny and induce an immune response. A deletion in the C-terminal half of 3A in the FMDV genome has been associated with decreased virulence in cattle. Also the role of FMDV 3A in the viral cycle, such as RNA replication, rearrangements of intracellular membranes required for this process, pathogenesis, and lysis of host cells are reported. Therefore work is in progress to produce virus with deleted 3A FMDV cDNA in the 3A expressing BHK cell line.

2. Altered replication fidelity

Several RNA viruses with resistance to nucleotide analogues have been identified and linked to increased replication fidelity of their RNA-dependent RNA polymerase (RdRp). As viral quasispecies are important to the evolution and adaptation of RNA viruses, the increased replication fidelity, which restricts the amplitude of quasispecies, results in an unfit or attenuated phenotype. If successful in isolating such variants, they can be a candidate for an attenuated vaccine. Accordingly, the isolation of RdRp variants was carried out by passaging FMDV with mutagen selection pressure. The optimal non-toxic mutagen concentration that moderately reduces virus titres (approximately 0.5–2 log reduction) was identified. BHK-21 cell monolayers were pre-treated with the optimal non-toxic concentration of 5-Fluorouracil (FU) for 2 h, incubated with serotype O vaccine strain (IND R2/75) for 1h at a multiplicity of infection (m.o.i) of 0.01, and subsequently grown in the presence of the same concentrations of FU. Virus was harvested between 24 and 48h post-infection, and the progeny viruses were subjected to the next rounds of treatment for a total of 24 passages. Presence of the virus in every fifth passage was confirmed in serotype specific sandwich ELISA and multiplex PCR (Fig.1). Mean titres were determined by using a 50% tissue culture infective dose ($TCID_{50}$) assay in BHK-21 cells (Fig.2).

In order to understand whether or not selection of mutants by nucleoside analogues retains the antigenic properties of the virus, the antigenic relationship of the mutant viruses (P10 and P20) was analysed in two-dimensional micro-neutralization test (2D-MNT), as

per the standard method. Dilution of serum that neutralizes 100 $TCID_{50}$ of virus was determined. The one-way antigenic relationship (r-value) was calculated as the ratio between heterologous and homologous serum titre. All the virus showed an r-value in the range of 0.88–1.00, indicating that nucleoside analogue selection pressure did not alter the antigenic properties of the mutant viruses.

3. Codon deoptimization

A new strategy has been developed that has proven to be an effective route to the attenuation of viruses such as polio and influenza viruses to produce attenuated vaccines for FMDV by codon deoptimization process. The Indo-UK collaborative project (between University of St Andrews and IVRI Bangalore campus) was initiated to produce a stable and effective attenuated vaccine for FMDV serotype Asia 1 by using the synthetic biology and codon deoptimization process. A synthetic replicon was constructed with green fluorescent protein (GFP; replacing the structural protein genes), using advances in synthetic biology, which will act as benchmark for the comparison of the level of attenuation. Virus is rescued from this construct after replacing the GFP with the genes of structural proteins. A panel of clones with deoptimised codons have been constructed for FMDV using the benchmark construct and rescuing of the viruses and evaluation of the rescued viruses are in progress.

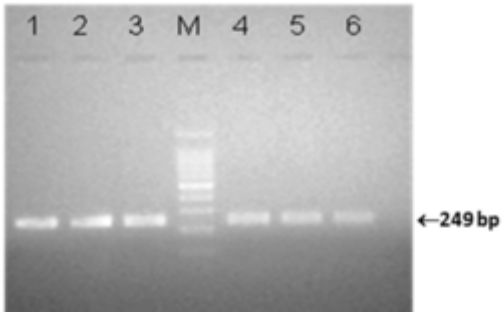


Figure 1: Multiplex PCR for confirmation of FMDV O serotype across the passages, showing 249 bp O serotype specific amplicons. Lanes 1, 2 & 3 : 5-FU treated @ 100 µg, 400 µg and control at 10th passage, Lane M: 100 bp marker and Lanes 4, 5 & 6: 5-FU treated @ 100 µg, 400 µg and control at 20th passage.

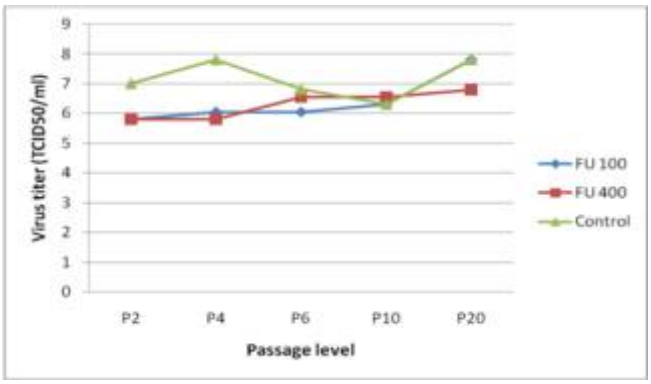


Figure 2: Mean virus titres determined as 50% tissue culture infective dose ($TCID_{50}$) assay in BHK-21 cells across the passage series.

Assessment of the relationship between serum neutralizing antibody titre and liquid phase blocking ELISA titre in immune response to FMDV Vaccine

Tamil Selvan, R.P., Sreenivasa, B.P., Madhusudan Hosamani, Saravanan, P, Basagoudanavar, S.H. and Venkataramanan, R.

ICAR-Indian Veterinary Research Institute, Hebbal, Bengaluru-560024, Karnataka, India.

Traditionally, quality assessment of FMD vaccine is carried out by live FMD virus challenge of vaccinated cattle. Replacing the use of animals for quality assessment with *in vitro* tests like serum neutralization test (SNT) or liquid phase blocking ELISA (LPBE), requires the establishment of logistic regression models from series of challenge experiments. Before establishing a logistic regression model, we did a preliminary investigation on the relationship between SNT and LPBE titre, to be used as cut-off titre for classifying cattle as protected or unprotected. Hence, the present study was conceived to elucidate the $LPBE_{50}$ titres corresponding to various classes of SN_{50} titres by deriving a linear regression model, and to compare the presumed protective titres of 1.5 and 1.8 $\log_{10}$ SN_{50} with the predicted $LPBE_{50}$ titre. The study would eventually help in complying with the 3R (replacement, reduction, refinement) strategy in animal testing for potency assessment.

SNT and LPBE were carried out on known negative (n=306) and positive serum samples against Indian vaccine strains of FMDV such as O/IND/R2/1975 (n=92), A/IND/40/2000 (n=16) and Asia1/IND/63/1972 (n=11) to evaluate the diagnostic and performance

characteristics by receiver operating characteristics (ROC) curves. Using 471 bovine trivalent FMD vaccinate serum, the relationship between SN_{50} and $LPBE_{50}$ titres were analyzed following linear regression modelling (Fig. 1 a, b ,c).

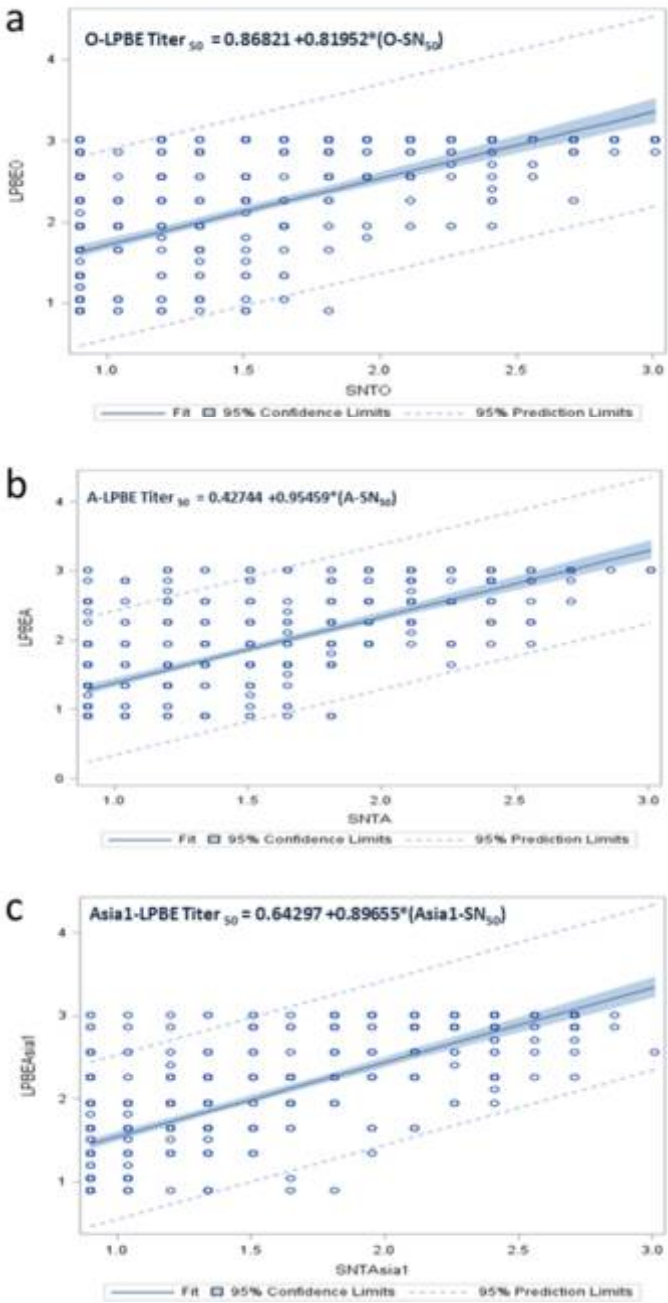


Figure 1: Scatterplot showing linear relationship between $Log_{10}LPBE_{50}$ titre and $Log_{10}SN_{50}$for serotype O (a), A(b) and Asia1(c) and corresponding linear regression model for predicting $Log_{10}LPBE_{50}$ titre.

Based on ROC curves, cut-off titres of 1.2 and 1.5 log_{10} were found to be suitable across all three serotypes with high sensitivity and specificity in SNT and LPBE, respectively. Compared to SNT, LPBE was found to be less variable, as indicated by the maximum standard deviation of 0.18 $log_{10}LPBE_{50}$ compared to 0.52 $log_{10}SN_{50}$ in SNT. Correlation of $LPBE_{50}$ titres with SN_{50} titres indicated that the $LPBE_{50}$ titres were greater than SN_{50} titres, with a significant positive correlation (Spearman correlation coefficient ρ=0.70, 0.75 and 0.76, (p=<0.0001) for FMDV O, A and Asia1, respectively). Simple linear regression modelling of $LPBE_{50}$ titres corresponding to the presumed protective SN_{50} titres (1.51 and 1.81) were shown to be 2.11, 1.87, 2.00 and 2.35, 2.16, 2.27 for O, A and Asia1 (Table 1), respectively. These results indicate that 2.1 $log_{10}LPBE_{50}$ is the minimum predicted titre for a presumed protective SN_{50} titre of 1.81, however, as with SNT this needs to be compared with *in vivo* protection status before being accepted as an alternate test to challenge methodology.

Table 1: Predicted $log_{10}LPBE_{50}$ $titre_{50}$ for each log_{10} SN_{50} titre based on linear regression.

$Log_{10}SN_{50}$	$Log_{10}LPBE_{50}$ Titre		
	O	A	Asia1
0.90	1.61	1.29	1.45
1.04	1.72	1.42	1.58
1.20	1.85	1.57	1.72
1.36	1.98	1.73	1.86
1.51	2.11	1.87	2.00
1.65	2.22	2.00	2.12
1.81	2.35	2.16	2.27
1.95	2.47	2.29	2.39
2.11	2.60	2.44	2.53
2.26	2.72	2.58	2.67
2.41	2.84	2.73	2.80
2.56	2.97	2.87	2.94
2.71	3.09	3.01	3.07
2.86	3.21	3.16	3.21
3.01	3.33	3.30	3.34

Vaccinating in northern Namibia (David Paton)

Changes in serum microRNA profile in FMDV infected Indian cattle (*Bos indicus*)

S.H. Basagoudanavar, M. Hosamani, R.P. Tamil Selvan, B.P. Sreenivasa and R. Venkataramanan

ICAR-Indian Veterinary Research Institute, Hebbal, Bengaluru-560 024, India

Although considerable advances have been made in understanding the pathogenesis of FMDV infection, there is still scope for delineating the complex interplay of host and virus interactions. FMDV pathogenesis in cattle is associated with a distinct profile of host microRNAs (miRNAs) that could influence cellular gene expression. The miRNAs are small RNA molecules which regulate gene expression by targeting messenger RNA and cause either mRNA degradation or translation repression, thus modulating gene expression and subsequent cellular pathways. Following infection, normal miRNA patterns can be altered to regulate disease pathogenesis, in turn determining the outcome of the infection. When we analyzed serum miRNA patterns in cattle infected with FMDV, we found that the host miRNA homeostasis is disturbed during the initial phase of FMDV infection resulting in differential patterns of miRNAs in the serum, with upregulation of numerous miRNAs on day 2 post infection (Fig. 1A). When compared to the uninfected state, 40 miRNAs were differentially upregulated (P <0.05) in the serum of the cattle. Three of these miRNAs were upregulated on all 3 days, while seven were upregulated on day 1 and 2 post infection (Fig. 1B). Bioinformatics analysis for the miRNA target prediction indicated that FMDV infection can trigger genes in various biological processes and pathways. The data suggest that FMDV pathogenesis in cattle is associated with a distinct profile of host miRNAs that could influence the cellular gene expression pattern and early events during infection.

Further studies are needed to investigate the miRNA and mRNA co-expression to identify regulatory networks by establishing miRNA-mRNA correlations. These studies would provide insight into the molecular mechanism of virus-host interactions and disease pathogenesis, thus aiding in rational design of therapeutic intervention. Also, the differentially expressed signature of circulating miRNAs could serve as non-invasive biomarkers for preclinical diagnosis of FMDV infection.

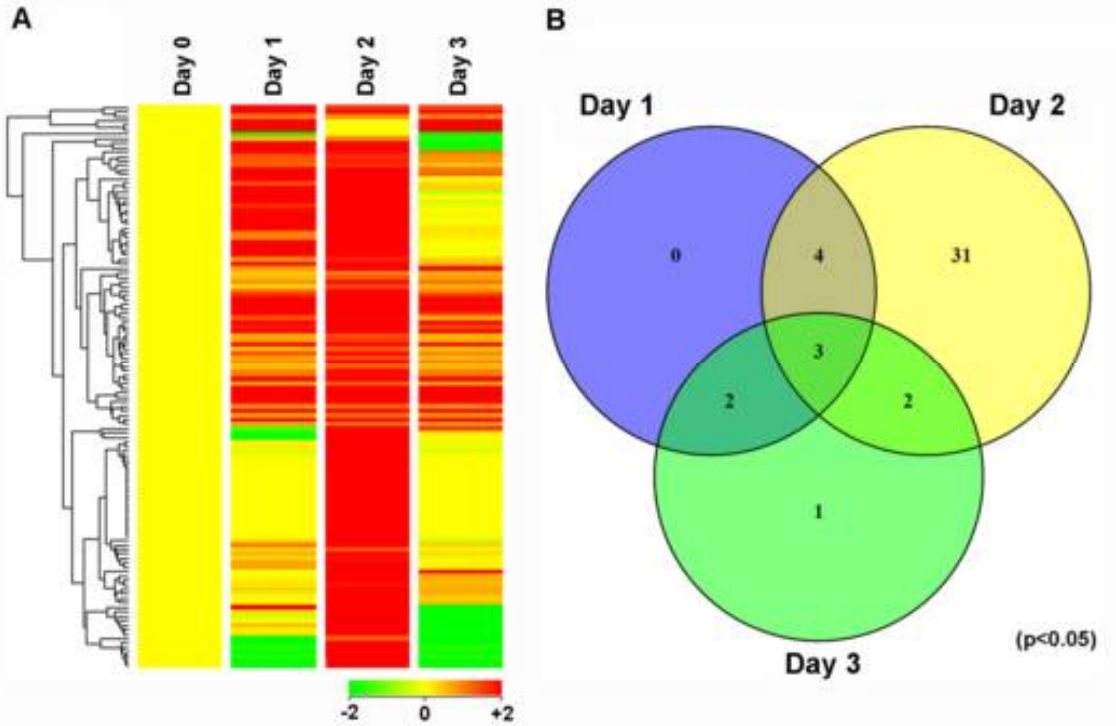


Figure 1: (A) Hierarchical clustering of differentially expressed serum miRNAs in cattle on different days following FMDV infection. (B) Venn diagram showing the overlap of significantly upregulated (p<0.05) miRNAs in cattle on different days following FMDV infection.

Recent research activities on foot-and-mouth disease in the Exotic Disease Research Division, National Institute of Animal Health (NIAH), NARO, Japan

Abstracts of Scientific Papers

1) Development and evaluation of a rapid antigen detection and serotyping lateral flow antigen detection system for foot-and-mouth disease virus. Morioka K, Fukai K, Yoshida K, Kitano R, Yamazoe R, Yamada M, Nishi T, Kanno T. 2015. PLoS ONE. 10(8), e0134931.

We developed a lateral flow strip using monoclonal antibodies which allows for rapid antigen detection and serotyping of FMDV. This FMDV serotyping strip was able to detect all 7 serotypes and distinguish serotypes O, A, C and Asia1. Sensitivity ranged from 10^3 to 10^4 of a 50% tissue culture infectious dose of each FMDV stain; this is equal to those of the commercial product Svanodip (Boehringer Ingelheim Svanova, Uppsala, Sweden), which can detect all seven serotypes of FMDV, but does not distinguish between them.

Our evaluation of the FMDV serotyping strip using a total of 118 clinical samples (vesicular fluids, vesicular epithelial emulsions and oral and/or nasal swabs) showed highly sensitive antigen detection and accuracy in serotyping in agreements with ELISA or RT-PCR results. To the best of our knowledge, this is the first report on any FMDV serotyping strip that provides both rapid antigen detection and serotyping of FMDV at the same time on one strip without extra devices. This method will be useful in both FMD-free countries and FMD-infected countries, especially where laboratory diagnosis cannot be carried out.

2) Comparative performance of fetal goat tongue cell line ZZ-R 127 and fetal porcine kidney cell line LFBK-αvβ6 for Foot-and-mouth disease virus isolation. Fukai K, Morioka K, Yamada M, Nishi T, Yoshida K, Kitano R, Yamazoe R, Kanno T. 2015. J Vet Diagn Invest, 27(4), 516-521.

The fetal goat tongue cell line ZZ-R 127 and the fetal porcine kidney cell line LFBK-$\alpha_v\beta_6$ were recently reported to have high sensitivity to various FMDV strains. The suitability of ZZ-R 127 cells for FMDV isolation not only from epithelial suspensions but also from other clinical samples has already been confirmed in a previous study. However, the suitability of LFBK-$\alpha_v\beta_6$ cells has never been evaluated using clinical samples other than epithelial materials. In addition, both cell lines have never been compared, in terms of use for FMDV isolation, under the same conditions. Therefore, in this study, the virus isolation rates of both cell lines were compared using clinical samples collected from animals infected experimentally with FMDV.

Viruses were successfully isolated from clinical samples other than epithelial suspensions for both cell lines. The virus isolation rates for the two cell lines were not statistically different. The Cohen's kappa coefficients between the virus isolation results for both cell lines were significantly high. Taken together, these results confirmed the suitability of LFBK-$\alpha_v\beta_6$ cells for FMDV isolation from clinical samples other than epithelial suspensions. The levels of susceptibility of both cell lines to FMDV isolation were also confirmed to be highly similar.

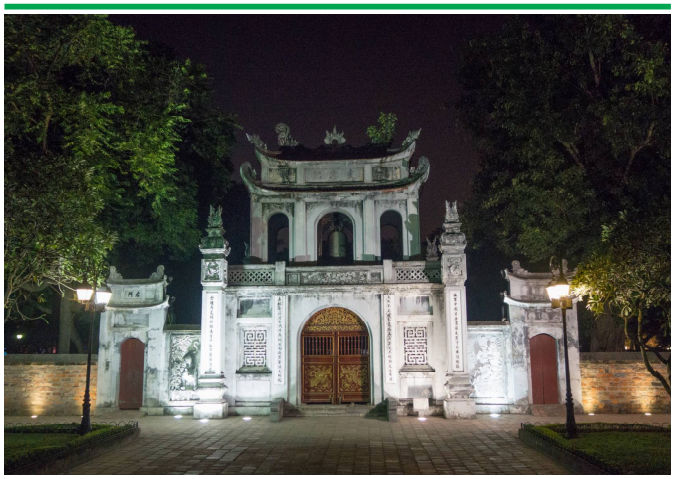
3) Further evaluation of an ELISA kit for detection of antibodies to a nonstructural protein of foot-and-mouth disease virus. Fukai K, Nishi T, Morioka K, Yamada M, Yoshida K, Kitano R, Yamazoe R, Kanno T. 2016. J Vet Med Sci 78(3), 365-373.

An ELISA kit for detection of antibodies to a nonstructural protein of FMDV was further evaluated using sequentially collected serum samples from experimentally infected animals. In a previous study using samples from field animals, the sensitivity of the kit was low. Antibodies to nonstructural proteins were detected in all unvaccinated, infected animals, using the kit. However, these antibodies were detected at a later time point than antibodies to structural proteins of FMDV, detected using the liquid-phase blocking ELISA that is used for serological surveillance in the aftermath of outbreaks in Japan.

Additionally, although the kit effectively detected antibodies in infected cattle with vaccination, there were several infected pigs with vaccination for which the kit did not detect antibodies during the experimental period. Taken together, the kit may not be suitable for serological surveillance after an FMD outbreak either with or without emergency vaccination in FMD-free countries.

4) Efficacy of Expired Foot-and-mouth Disease O Type Vaccines in Cattle and Buffalo.
Sakamoto K, Morioka K, Fukai K, Yamamoto T, Tsutsui T, Muroga N, Ojima N, Mago J, Katagiri Y, Aviso, S R, Phouaravanh M, Soukvilay V, Keokhamphet C, Buranathal C, Khounsy S, Yamada M. 2016. JARQ 50 (2), 163 – 168.

Lao People's Democratic Republic (Lao PDR) submitted a request to Japan for 200,000 doses of expired FMD O type vaccines that were in storage for emergency use. Approximately 100,000 animals, consisting of both cattle and Asian water buffalo (*Bubalus bubalus bubalis*), received the same vaccine twice within one month in Xieng Khouang province in the northeast area of Lao PDR. The efficacy of the three-month expired FMD O type vaccine ($6PD_{50}$ O Manisa) was assessed in serum samples from 90 cattle and 31 buffalo from the field using a Liquid Phase Blocking-ELISA assay. Of these samples, 75 cattle (83.3%) and 24 buffalo (77.4%) were seropositive against FMDV O type before vaccination. Testing for non-structural protein using the PrioCHECK FMD NS kit showed that many of the animals with high titres in the screening test were FMDV-infected before vaccination. Fifteen cattle and seven buffalo with titres 1:32 or under before vaccination exhibited high titres of antibody (1:45-1:1448) one month after the first vaccination and further increased titres (1:362-1:5792) one month after the second vaccination. Nearly all of the cattle (97.6%) had high titres to FMDV 14 months after the second vaccination. To date, no outbreak of FMD has been reported at the study site. Three-month expired FMD O type vaccines induced appropriate immune responses against FMD in both cattle and buffalo.



Memories from Hanoi (David Paton)

Queensland's biosecurity preparedness program for FMD, Australia

Dr Hendrik Camphor and Dr Mark Cozens

Biosecurity Queensland, Department of Agriculture and Fisheries, Queensland State Government, Australia

The Queensland Department of Agriculture and Fisheries has invested in a three-year biosecurity preparedness program with a focus on FMD. The program has worked to enhance Queensland's FMD preparedness across ten key areas including strategy, policy and legislation; surveillance; laboratory preparedness; animal destruction and disposal; and training. Collateral benefits have extended to whole of government, industry and community, particularly in terms of enhanced response capability to biosecurity incidents through improved partnership arrangements.

Notable activities and products developed as part of the program include, but are not limited to:

- An FMD vaccination strategy which aims to ensure consistency and transparency in state-level decision making processes to enhance Queensland's preparedness to implement an emergency FMD vaccination program. This strategy is the culmination of 14 months' of work which involved close collaboration between Biosecurity Queensland and disease modelling experts from the Australian Government Department of Agriculture and Water Resources.

Collectively, vaccination and control strategies for twelve hypothetical FMD incidents in Queensland were developed which can be used as a 'ready-reference' for vaccination planning during an incident. The Queensland strategy provides a framework to support transparent risk-based decision making around emergency vaccination and used disease modelling to test and validate these decisions using key metrics to measure the success of the various control strategies employed. Findings did not support the use of emergency FMD vaccination for moderate incidents, while there was a demonstrated benefit from the use of vaccination for some severe incidents. Several national policy issues including between-jurisdiction vaccine distribution and adjunctive disease control measures were identified. Should similar work be completed in other jurisdictions, decision making around sharing limited vaccine supplies and a truly national approach to vaccination could continue to be progressed.

- Development of FMD response and proof of freedom surveillance plans for Queensland. The former is an operational plan which details the expected surveillance to be undertaken on a range of premises during an FMD emergency response, including approaches to decision making and risk management around prioritisation of surveillance activities, alignment of surveillance activities with available resources and the clinical, serological and/or virological surveillance activities that may be required. The latter is a strategic level plan aimed at incorporating surveillance activities conducted within Queensland into the broader plan for Australia to demonstrate freedom from FMD virus infection in accordance with requirements set out by the World Organisation for Animal Health (OIE).

- Enhancements to laboratory preparedness to service scalable FMD incidents through review, enhancement or establishment of policies and procedures on specific testing requirements, sustainable sample throughput, surge capacity solutions including human and physical resourcing and a supporting document management system.

- Development of mass animal destruction and disposal decision support tools, a live animal, carcass and animal product transport risk management guidance document, and processing industry consultation and analysis to support operational decision making around large-scale livestock depopulation to enhance preparedness for implementing a stamping-out policy.

- Development of an FMD awareness campaign linked to two public facing eLearning training courses. The first course is targeted towards producers and the livestock supply chain with a focus on enhancing early detection of FMD and reducing the high-risk behaviour of swill feeding and supply among pig owners, food outlets and suppliers. The second course, designed for veterinarians and veterinary paraprofessionals, focuses on early FMD recognition, reporting procedures under Queensland legislation, conducting an epidemiological investigation, sampling, sample packaging, laboratory diagnosis and on-farm biosecurity risk management. These short online training modules will be freely available online by the end of May 2016.

Development and sharing of these deliverables with other jurisdictions has enhanced Queensland's and Australia's preparedness for an FMD incident. Whilst Queensland's biosecurity preparedness program will conclude in June 2016, ongoing preparedness activities will continue to be part of Queensland's biosecurity preparedness agenda.

A decision tool for FMD

Alasdair King

MSD Animal Health, The Netherlands

Why Develop a Decision Tool?

Much of the current modelling for FMD control focusses on the epidemic situation in previously free areas/zones. However, endemic countries need help to develop strategies to control the disease. Key factors such as the correct use of limited resources and long-term planning are important to consider. Yet, without tools to assist, often decisions are driven by short-term needs. Working with LEI Wageningen University, MSD Animal Health has developed an interactive modelling tool that can provide governments with important information on the economic impact of different strategies. This model was presented at the GFRA meeting last year in Vietnam and also at the SEACFMD meeting in Thailand this year where it received significant interest.

Using the Tool

The tool allows countries to enter their own specific data in parameters such as livestock population, disease prevalence, mortality rates, and the costs involved in production loss (Figure 1). It is best to use true data but if these don't exist then estimates can be used.

Parameters	Unit	Value
Epidemiological parameters		
Total size of livestock population (N)	number of animals	1,000,000
Time step	number of quarters	1
Birth rate (B)	new born animals per year per animal	0.7
Replacement culling rate (X)	fraction of animals replaced (sales)	0.2
Detection rate of FMD	detection rate of FMD	0.5
Contact rate (C)	rate	1.80
Infection rate (R)	rate	0.80
Initial prevalence (I_0)	%	5.00
Fraction infectious (T)	fraction	0.85
Mortality of infected (M)	fraction	0.1
Economic parameters		
Vaccine cost	USD per dose	
Production loss	USD per animal	100
Value culled animal	USD per animal	400

Figure 1: Country parameters

Parameters	Unit	Default Value	High coverage high potency	High coverage low potency	Low coverage high potency	Low coverage low potency
Vaccination coverage (V)	fraction of animals vaccinated per year	0	0.85	0.85	0.5	0.5
Frequency of vaccination (F)	Number of quarters before next vaccination	0	2	2	2	2
Vaccination efficacy (E)	fraction of animals protected by vaccination	0	0.85	0.65	0.85	0.65
Duration of immunity (P)	number of quarters	0	3	1	3	1
Economic parameters						
Vaccine cost	USD per dose	1	11.5	10.7	11.5	10.7

Figure 2: Vaccination scenarios

Having defined the situation in the country, data from up to four different vaccination scenarios can be entered and the results compared. For instance, in our example we have entered combinations of high vs low coverage and high vs low potency (Figure 2). We have assumed that the vaccination campaign will cost $10/animal and that a low potency vaccine costs $0.70/dose, compared to $1.50/dose for a high potency vaccine. It is important to remember that individual countries can adjust these figures to suit their own situation.

Planning

Once all the parameters are entered, then the model can be run. This generates the data and graphs for analysis. For instance, Figure 3 demonstrates the cumulative costs of disease and vaccination over time. By visually representing the costs over a period of five years it is easier to see the real impact of any investment being made. Often when developing strategies, the procurement process can focus on the short-term cost of vaccine. However, the costs of the disease itself going unchecked massively outweigh any potential cost of vaccine, and this becomes clear when both are taken into account. One of the really interesting findings of running different scenarios is that the second best alternative to high coverage and high potency vaccine is actually using a high potency/ low coverage combination. This opens the door for developing targeted campaigns.

The usual dogma is that vaccination campaigns need to have a coverage of over 80%, and for eradication of disease this is certainly true. However, if finances are limited, then economically it may be better to use a high quality vaccine with lower coverage, thus achieving control of the disease. The message from this tool appears to be that if you can't afford to vaccinate everything, then the compromise should be on the coverage, not the quality of the vaccine.

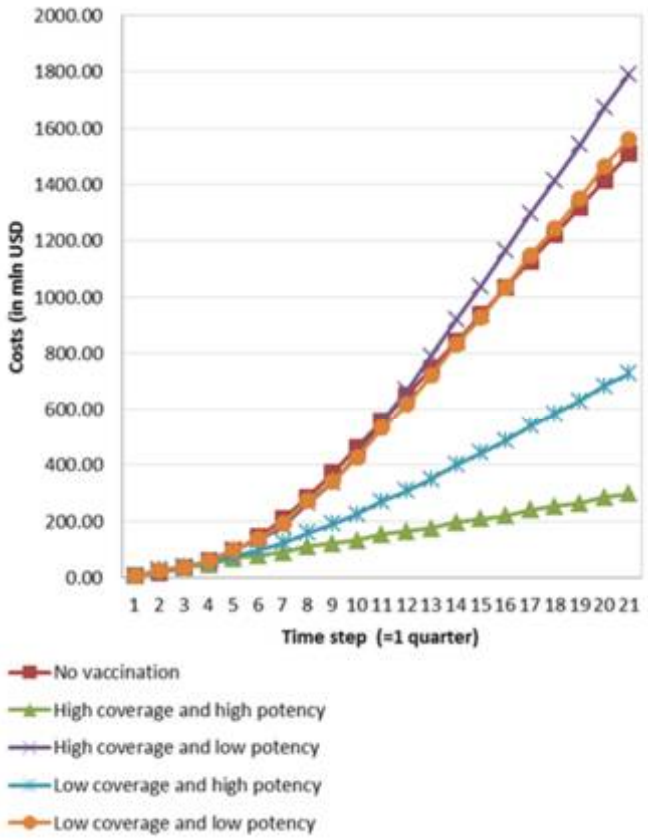


Figure 3: Cumulative costs of disease and vaccination

A Dynamic Approach

FMD strategies need to be dynamic. A single strategy will not work over a long period and it is therefore important to adapt to changes in conditions over time and, to help that, the FMD situation can be seen as a "subway map" (Figure 4).

This approach allows the policy makers to identify the possibilities and to switch from one option to the other. The "switch" points are identified as changing conditions, such as:

- The uncertainties about initial FMD prevalence;
- The uncertainties about contact rate or infection rate between herds;
- Practical constraints;
- Improved surveillance;
- Changes in field strains of virus.

These switching points can be explored by running the simulation model with varying parameters. This 'what-if' type of analysis can be used to assess the

uncertainties of future FMD situations and provide insight into implementing different vaccination campaigns.

Supporting Strategy and Planning

The goal of this research was to support decision making with regard to vaccination campaigns in an endemic situation by highlighting the options and the considerations. For decision support, it is necessary to assess the social-economic consequences of FMD and FMD intervention strategies following the objectives of the country. Choosing the right options at the right time should be based on practical resource constraints and uncertainties about FMD prevalence and spread. The aim of this tool is to help governments make informed decisions.

Thanks

With thanks to Dr Lan Ge, Dr Marcel van Asseldonk, and Dr Ron Bergevoet of LEI Wageningen UR for developing the model for this work.

The full paper can be viewed at http://edepot.wur.nl/378019

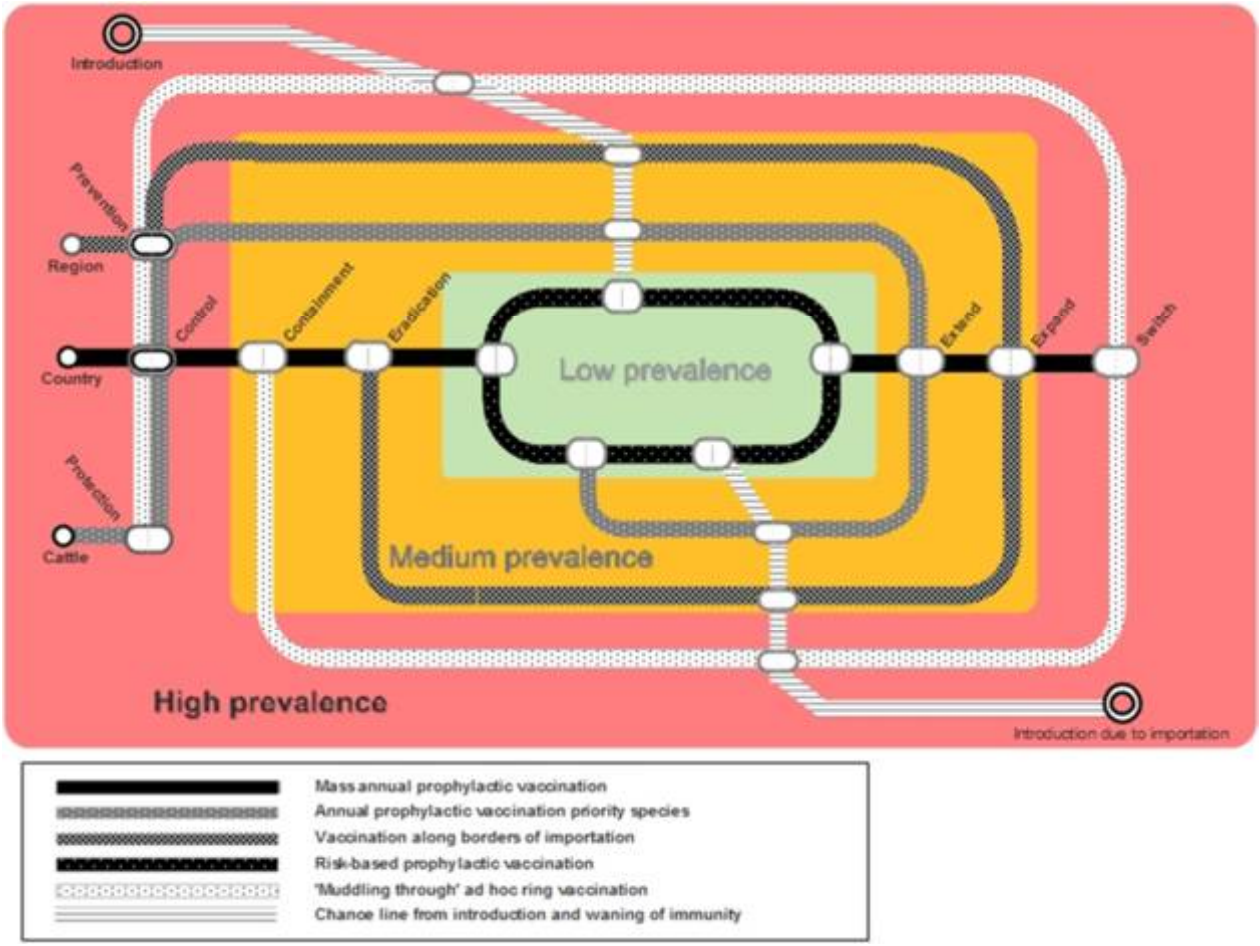


Figure 4: An FMD subway map

Memories from Hanoi (David Paton)

Ministry for Primary Industries (MPI), New Zealand

Richard Spence

New Zealand involvement in control of FMD in SE Asia

Over the next five years the New Zealand Ministry of Foreign Affairs and Trade has committed considerable funds to the South East Asian region. Funding is centred around control of FMD. The programme is aimed at building on successes from Australian funding and is focused on FMD control in Laos and Myanmar and includes vaccination of cattle in high risk areas. There are number of key pieces of information that are required if control is to be successful; these include why disease occurs in certain areas, what factors influence its occurrence, the impact it has on households and suitable mitigating response steps that can applied. This information will be gathered by a number of epidemiological projects carried out with the assistance of New Zealand expertise.

The development challenge for the livestock production sector in SE Asia is to control FMD. The disease has a significant negative effect to rural economies and the incomes of rural households. The Activity will work in South East Asia in the context of the South East Asia and China Foot and Mouth Disease (SEACFMD) Campaign run by the OIE (World Animal Health Organisation). With adaptation to local conditions, the Activity will broadly follow methodology laid out in the OIE and FAO framework Progressive Control Programme for FMD.

National Biocontainment Laboratory Project

In late 2015 MPI started construction of New Zealand's new highest containment laboratory on the existing site of the Animal Health Laboratory (AHL) in Upper Hutt. The laboratory represents a major investment by the New Zealand government and when operational in 2019 will provide state of the art laboratory facilities to support diagnosis and management of high risk diseases such as FMD. For further details refer to the following link: http://www.mpi.govt.nz/protection-and-response/finding-and-reporting-pests-and-diseases/laboratories/national-biocontainment-laboratory/

New Project – Development and application of a field deployable diagnostic test to aid rapid differential diagnosis of FMDV and endemic causes of vesicular disease

The AHL was recently awarded internal funding from MPI's Operational Research Fund to undertake a three year project to develop and investigate the utility and performance of a field deployable test to diagnose FMDV and other endemic causes of vesicular disease in New Zealand cattle. This is a collaborative project between MPI's AHL, The Australian Animal Health Laboratory and Massey University. A detailed project plan is currently being developed and the project is due to begin in July 2016.

CODA-CERVA, Belgium

David Lefebvre

GFRA scientific meeting – Hanoi, Vietnam (October 20th-22nd 2015)

At the GFRA Scientific Meeting in Vietnam the CODA-CERVA presented "Laboratory validation of two real time RT-PCR methods for foot-and-mouth disease virus with an increased diagnostic specificity". In this work, so called "flap" modifications, consisting of 5'AT-rich overhangs, were made to the forward and reverse primers of the reference 3D and 5'-UTR real time RT-PCR methods from Callahan et al. (2002) and King et al. (2006). Cycling conditions were likewise adapted to ensure a higher specificity without adverse effects on the sensitivity of the assays. The adaptation of primers and PCR protocols resulted in a reduction of doubtful or false positive test results without affecting the diagnostic sensitivity of the assay. It was concluded that the amended 3D and 5'-UTR real-time RT-PCR methods are reliable tools to declare negative field samples free from FMD virus. Experimental data were recently finalized and publication of a research paper is expected in the course of 2016.

International collaborations

In the framework of the bilateral collaboration between CODA-CERVA, an OIE Collaborating Center, and the Botswana Vaccine Institute (BVI), an OIE Reference Center, 2 scientists from BVI visited the CODA-CERVA for 2 weeks for a hands-on training with

a particular emphasis on genome sequencing and analysis. As a result, 3 FMD virus strains from Southern Africa were isolated and characterized by full genome sequencing and phylogenetic analysis. Based upon the generated Neighbor-joining (NJ) and Maximum Likelihood (ML) trees, FMD virus strains were classified as follows:

Strain name	*Accession number*	*Genotype*
O/ZAM14/2010	KU821591	EA-2
SAT1/NAM01/2010	KU821590	III (WZ)
SAT2/ZAM18/2009	KU821592	III

These data will be published in the course of 2016.

Since June 2014, the CODA-CERVA has been involved as a parent collaborating center in an OIE Laboratory Twinning Program for capacity building via a technical and scientific collaboration with the National Veterinary Research Institute (NVRI) from Vom, Plateau State, Nigeria. The main aims are 1) to identify key gaps in the laboratory practices with recommendations to improve current practices, 2) to strengthen and enhance safe and secure diagnostic laboratory practice and skills and 3) to improve laboratory surveillance and disease reporting in Nigeria.

In 2016, the CODA-CERVA provided 2-week laboratory training courses to 3 scientists and 2 technicians from the NVRI. Main topics included FMD virus isolation on different cell cultures, viral RNA extraction from blood, serum and tissue samples, 3D and 5'-UTR real time RT-PCR for FMD virus, serotype-specific FMDV antigen ELISA, concentration and quantification of the 146S antigen content and VNT.
Another scientist from the NVRI visited the CODA-CERVA for a 3-month period and subsequently for a one-month-period. In this way he continued his work to characterize serological profiles from FMD screening surveys in Nigeria and to characterize FMD virus isolates from disease outbreaks in Nigeria. Particular attention was given to extensive molecular characterisation including sequencing, sequence analysis and phylogeny.

International meetings

The CODA-CERVA organized the annual OIE/FAO FMD Reference Laboratory Network Meeting from November 24th-26th 2015.

Science reports

Scientific publications on foot-and-mouth disease since January 1st 2015 (in alphabetic order)

De Vleeschauwer AR, Lefebvre DJ, Willems T, Paul G, Billiet A, Murao LE, Neyts J, Goris N, De Clercq K. A Refined Guinea Pig Model of Foot-and-Mouth Disease Virus Infection for Assessing the Efficacy of Antiviral Compounds. Transboundary and Emerging Diseases, 2016 Apr;63(2):e205-12. doi: 10.1111/tbed.12255.

van der Linden L, Vives-Adrián L, Selisko B, Ferrer-Orta C, Liu X, Lanke K, Ulferts R, De Palma AM, Tanchis F, Goris N, Lefebvre D, De Clercq K, Leyssen P, Lacroix C, Pürstinger G, Coutard B, Canard B, Boehr DD, Arnold JJ, Cameron CE, Verdaguer N, Neyts J, van Kuppeveld FJ. The RNA Template Channel of the RNA-Dependent RNA Polymerase as a Target for Development of Antiviral Therapy of Multiple Genera within a Virus Family. PLoS Pathogens, 2015 Mar 23; 11 (3):e1004733. doi: 10.1371/journal.ppat.1004733

Group photo from the 2015 OIE/FAO FMD RefLab meeting hosted by CODA-CERVA.

National Centres for Animal Disease, Canadian Food Inspection Agency, Winnipeg, Manitoba and Lethbridge, Alberta, Canada

Charles Nfon, Oliver Lung, Ming Yang and Aruna Ambagala

Rapid detection of FMDV is critical to prevent and contain an FMD outbreak. Availability of a fast, highly sensitive and specific, yet simple and field-deployable assay would support local decision making and reduce the diagnostic burden on central laboratories during an FMDV outbreak. We have validated a novel reverse transcription-insulated isothermal PCR (RT-iiPCR) assay with the 3D region of the FMDV as target, which can be performed and results displayed as "+" or "-" on a commercially available, compact and portable POCKIT™ analyser. The assay detected 9 copies of *in vitro* transcribed RNA standard and accurately identified 63 FMDV strains belonging to all seven serotypes, with no cross-reactivity with other viruses considered as differentials for FMD. The assay also detected FMDV genome in oral, nasal and lesion swabs, epithelial tissue suspensions, vesicular (without RNA extraction) and oral fluid samples. Clinical sensitivity of the assay was comparable or slightly higher than the laboratory-based real-time RT-PCR assay in use. This assay provides a potentially useful field-deployable diagnostic tool for rapid detection of FMDV in an outbreak in FMD-free countries or for routine diagnostics in endemic countries with less structured laboratory systems.

We have evaluated the use of oral fluids (OF) as an alternative to swabs, serum and/or blood for the detection virus and antibodies to FMDV and its differentials (SVD and/or VS) in pigs. FMDV, SVDV or VSV genome and specific antibodies were detected in OF from groups of experimentally infected pigs by real-time RT-PCR and ELISAs, respectively, demonstrating the potential for OF in testing and surveillance of swine for FMD, SVD and VS.

We are currently working on the molecular engineering of antibodies for improving FMD diagnosis. Many antibodies suffer from low binding affinity for specific antigens. This characteristic may greatly limit their applications in different immunoassays. Increasing antibody binding affinity can lead to the development of highly sensitive assays for *in vitro* diagnosis. We previously developed two FMDV serotype independent monoclonal antibodies (mAb) which recognised all seven FMDV serotypes individually, but with low binding affinity. By modifying the immunochemical properties of mAbs through recombinant technologies, it is possible to create antibodies with optimal affinity. Therefore the development of recombinant FMDV specific antibodies with high affinity using antibody engineering technologies could enhance their use in FMD diagnosis.

We are developing a fully automated and integrated multiplex assay for detection of the nucleic acid of FMDV and other high consequence livestock viruses that does not require user handling after sample introduction. The assay is designed to detect a number of viruses that include FMDV, SVDV, VSV, CSFV and ASFV. To date, the fully integrated and automated assay accurately detected a panel of 28 laboratory amplified viral strains representing all 7 FMDV serotypes, 2 VSV serotypes and 3 CSFV genotypes. No cross reactivity was observed with 6 other viruses associated with livestock. The assay also detected FMDV in OF samples from pigs infected with FMDV as early as 1 day post infection.

Australia's FMD preparedness activities

Dr Corissa Miller
Australian Government Department of Agriculture and Water Resources

Australia continues to maintain globally competitive and sustainable livestock industries within a strong biosecurity framework. Maintaining freedom from FMD and enhancing national preparedness remains a high priority. Australian governments and industry engage in regular FMD prevention, planning and preparedness activities to ensure the state of Australia's FMD preparedness is continually reviewed and strengthened.

Exercise Odysseus

The Australian Government, state and territory governments, and industry collaborate to conduct regular scenario exercises to train personnel and assess FMD response plans and procedures. In 2014

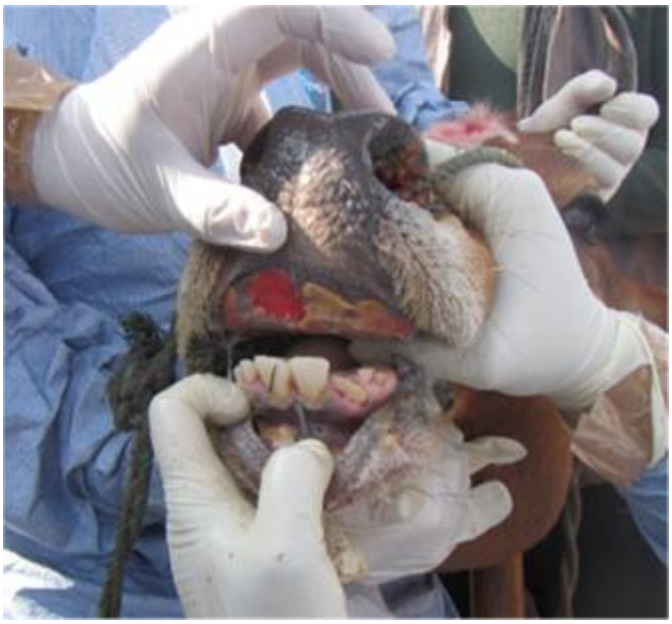
and 2015, a series of 48 discussion exercises and field activities, known as Exercise Odysseus, were conducted with the aim of enhancing preparedness for, and implementation of, a national livestock standstill following the suspicion or diagnosis of FMD. Key learnings from Exercise Odysseus will be used to strengthen Australia's capability to implement a livestock standstill and enhance national FMD preparedness.

For more information see: http://www.agriculture.gov.au/biosecurity/emergency/exercises/exercise_odysseus.

FMD Outbreak Modelling

In the event of an FMD outbreak, Australia's policy is to eradicate the disease as rapidly as possible, while minimising the socio-economic impacts. Emergency vaccination may be implemented, in combination with stamping out, if considered likely to improve outbreak management and expedite eradication. The Australian Government Department of Agriculture and Water Resources (DAWR) has undertaken collaborative modelling research with several research partners including the Australian National University, the University of New England and the University of Melbourne. With funding from the Centre of Excellence for Biosecurity Risk Analysis, factors that may influence the severity of an outbreak of FMD were assessed, in order to assist decision-making on appropriate response strategies, including vaccination.

FMD Real-Time Training in Nepal



It is well recognised that early detection of FMD is important to minimise the adverse impacts of an outbreak. As few Australian veterinarians and livestock workers have first-hand experience with FMD, DAWR has engaged the European Commission for the Control of FMD (EuFMD) to deliver practical training in Nepal, where the disease is endemic. The courses, which have been running since 2012, are co-funded by the Australian Government, state and territory governments, peak industry bodies and the New Zealand Ministry for Primary Industries. To date, 40 veterinarians from Nepal as well as 152 Australian and 23 New Zealand veterinarians, producers and stock handlers have received practical hands-on training in the rapid detection, investigation and response to outbreaks of FMD.

EuFMD Online FMD Emergency Preparation Course

In addition to real-time training in Nepal, DAWR has funded EuFMD to develop an online FMD Emergency Preparation course for veterinarians. The course is designed to enhance the preparedness of Australia's veterinary services for recognition and response to an FMD emergency, through interactive online discussions, live webinars and self-directed coursework over a four week period. The course was piloted in March 2016 and to date, 118 veterinarians from the Australian Government, state and territory governments, private practice and industry have been trained in key aspects of FMD preparedness, including lesion ageing, clinical and laboratory diagnosis, vaccination and Australia's FMD response policy.

Update from the OIE/FAO FMD Laboratory Network

Don King and Anna Ludi
The Pirbright Institute, on behalf of the Network Partners
donald.king@pirbright.ac.uk
anna.ludi@pirbright.ac.uk

The OIE/FAO FMD Laboratory Network coordinates global surveillance activities for FMD to identify new threats, and to ensure that appropriate diagnostic tools and vaccines are available for detection and control. In addition to continuing to collect samples from field outbreaks in the well-described endemic pools in Asia and Africa, members of the OIE/FAO FMD Laboratory Network have recently detected the emergence of a number of viral lineages in locations distant from their normal geographical distribution. These unexpected events were discussed at a recent meeting of the Network in Brussels during November 2015 and are briefly highlighted below.

Since 2013, laboratories have been monitoring the spread of the O/ME-SA/Ind-2001 lineage in the Gulf States of the Middle East and countries in North Africa. These viruses originate from the Indian sub-continent, where they now comprise the most dominant circulating lineage. In 2015/16, this lineage has also been detected in Laos and Vietnam, representing the first introduction of this FMDV lineage into Southeast Asia. Based on the movement of other viruses in the region, we should now anticipate that this lineage may spread more widely into other endemic countries, and could even move northwards into East Asia (China and other countries in the neighbourhood).

In the Middle East, the spread of another new FMD virus lineage (A/ASIA/G-VII) continues to raise concerns. This lineage has also recently emerged from the Indian sub-continent and closely related viruses have caused outbreaks in Saudi Arabia, Iran, Armenia and Turkey. Recent data provided by colleagues at the Şap Institute, (Turkey) dramatically show how quickly this virus lineage has spread, from the index case in September 2015 (occuring in the east of the country), to the situation in January/February 2016 when outbreaks have occured in the west of Anatolia.
The changing situation due to these two lineages (O/ME-SA/Ind-2001 and A/ASIA/G-VII) highlight threats posed via the trade of livestock and increased movement of people between endemic regions. The circulation of these new viral lineages raises obvious questions about the availability of suitable vaccines that might be deployed for control, and reinforces the importance of a global FMD Network to share laboratory data that monitor FMD outbreaks in different endemic settings, and combine resources to evaluate the performance of vaccine candidates.

The meeting delegates also heard feedback from two new Working Groups that have been recently established (i) to improve the formal nomenclature of the different FMD virus topotypes, lineages and isolates, and (ii) to provide practical guidance for the selection of appropriate vaccines, particularly focusing on the challenges that arise in FMD endemic settings. The Network partners also debated the status of serotype C, which has not been detected anywhere in the world since the last clinical samples representing this serotype were collected in 2004 (in Kenya and Brazil). A number of recommendations were agreed to: (i) highlight research projects that could be undertaken to provide evidence to determine whether or not serotype C is still circulating in South America and Africa, (ii) consider risk-based approaches that should be implemented to govern whether or not laboratories continue to undertake *in vivo* and *in vitro* work with "live" viruses from this serotype, and (iii) reconsider the continued use of serotype C in vaccines (in South America) and their inclusion in vaccine antigen banks held in FMD-free countries.

Acknowledgements
We thank the OIE and the European Commission (via EuFMD support to WRLFMD) for providing financial support for delegates to travel to the meeting. This meeting was kindly hosted by CODA-CERVA, Brussels, Belgium and the hospitality of Drs Kris De Clercq, David Lefebvre and colleagues in the face of the prevailing "difficulties in the city" was very much appreciated by the delegates. The OIE/FAO FMD Laboratory Network also warmly thanks Dr Thomas Struckmeyer and Thermo Fisher Scientific for kindly hosting the evening meal. Thanks also go to Sarah Belgrave who provided assistance to organize this meeting at WRLFMD.

Vaccinating in northern Namibia (David Paton)

Sokoine University of Agriculture, Tanzania

Christopher Jacob Kasanga

Announcement of research project on foot-and-mouth disease

Project title: Full genome sequencing to identify determinants that impact upon foot-and-mouth disease virus strains with a potential for enhanced transmission in endemic settings in Africa

Duration: Five years (2015 – 2020)
Principal investigator: Christopher Jacob Kasanga (PhD), Department of Microbiology and Parasitology and Southern African Centre for Infectious Disease Surveillance, Sokoine University of Agriculture (SUA), P. O. Box 3019, Chuo Kikuu, Morogoro, Tanzania.
E-mail: chrisskasa@gmail.com; christopher.kasanga@sacids.org;
Tel: +255 786 181 444

Project type: Intermediate Fellowship in Public Health and Tropical Medicine
Source of funds: Wellcome Trust

Hosting institution: Sokoine University of Agriculture

Brief project description:
The control of FMD outbreaks in endemic settings requires better understanding of the epidemiology of the disease as well as new tools that can rapidly detect and characterise the strains responsible. These approaches can identify viral features and risk factors that might be linked to the occurrence of the disease. Factors and early signs that trigger evolution and/or virulence and antigenic changes of the field FMDV strains in endemic regions have not been clearly investigated. The epidemiological factors and virus evolutionary characteristics that contribute to the sporadic outbreaks of FMD in different locations in time and space in endemic settings are not known. Often, FMD outbreaks also occur in vaccinated stock regardless of vaccinations.

This project aims to investigate the evolutionary characteristics of FMDV field strains in endemic settings of Africa that could be associated with future epidemics/spread. The main focus is to understand the molecular basis and factors associated with overt antigenicity and/or transmission ability of FMDV in different geographic areas within endemic settings. The outcomes of project will be identification of early genomic changes that are likely to lead to broader antigenicity and virulence, the knowledge of which could be crucial in defining early interventions, including vaccine strain selection before such changes result in wide-scale epidemics of FMD. In this study, molecular epidemiology and virus evolution data will be generated from persistently and/or naturally infected animals (cattle and buffalo) and following FMD outbreaks in specified geographic locations, whereas pathogenicity and transmission dynamics will be assessed through targeted experimental infections of animals. This study will also generate whole genome sequence data of the circulating FMD viruses and unravel viral genotypes and/or phenotypes in specific geographic areas, the information that could be linked with persistence, pathogenicity and phylogeography of the field strains. The in-depth genetic analysis of sequences will further unravel the molecular determinants of FMDV endemicity in specific geographic locations in Africa. This information, together with antigenicity data, could be used for recommendation of appropriate vaccines to use in specific geographic areas with overt FMD outbreaks.

Current collaborators: Tanzania Veterinary Laboratory Agency (TVLA), Tanzania Wildlife Research Institute (TAWIRI), Onderstepoort Veterinary Institute (OVI), The Pirbright Institute (TPI) and Royal Veterinary College (RVC).

Mentors and scientific advisors: Professors Mark Rweyemamu and Philemon Wambura (Sokoine University of Agriculture), Professors Donald King and David Paton (The Pirbright Institute), Professor Joe Brownlie (University of Bristol) and Professor Wilna Vosloo (CSIRO Australian Animal Health Laboratory).

OTHER ARTICLES OF INTEREST

- A special issue of Frontiers in Veterinary Science focussing on FMD in pigs will be out in 2016.
- A special issue of Transboundary Animal Diseases with papers covering the global research report for 2011–2015 done by GFRA on behalf of EUFMD will be published later in 2016.

**We will announce the publications to all GFRA partners and collaborators when available.*

- An interesting report on FMD vaccines entitled "NAHEMS Guidelines: Vaccination for Contagious Diseases, Appendix A: Foot-and-Mouth Disease" can be found at http://lib.dr.iastate.edu/vmpm_reports/2/

GFRA Scientific meeting, 20–22 October 2015

The biennial GFRA scientific meeting was held from 20 –22 October 2016 in Hanoi, Vietnam. The meeting was attended by 150 people from >35 countries and consisted of two days of scientific talks and posters that covered themes such as 1) Epidemiology, transmission and FMD ecology, 2) Vaccines, immunology and FMD pathogenesis, and 3) Surveillance and diagnostics. The third day was dedicated mostly to a workshop with 3 break-away groups that discussed issues such as 1) Control measures following vaccination without slaughter, 2) Optimal regime for emergency vaccination of pigs, and 3) Post vaccination monitoring and proof of freedom. David Paton kindly facilitated the workshop (the outcome is available in this newsletter).
A special word of thanks goes to our sponsors and the organising committee as well as other willing helpers (see below). Without their commitment and contribution the meeting would not have been possible. Hoabinh Tourist and Convention, based in Vietnam, was very helpful and took care of all the logistics.

The meeting allowed people to interact and build relationships and most of all, share their experiences with FMD control and results from research. As in the previous GFRA meetings, it was clear how much the world can learn from experience in endemic situations and how increased knowledge regarding epidemiology and basic research can assist with better control. This is all in line with the Global Control Strategy driven by the OIE and FAO.

The GFRA partners also had a meeting to discuss the management structure for the next 2 years. It was unanimously agreed that GFRA will remain in South East Asia for the next 2 years to allow more research groups in this region to become involved in GFRA activities. Dr Long stood down as president elect and Dr Dung was elected in the role. Our sincere thanks to Dr Long for the role he played and we welcome Dr Dung on board the Executive Committee. Both gentlemen work for the Department of Animal Health in Vietnam.

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USDA/ARS Plum Island Animal Diseases Center (PIADC)
The Pirbright Institute, UK
ARC-Onderstepoort Veterinary Institute, RSA
Ministry of Agriculture and Rural Development, Vietnam

Organising committee
Wilna Vosloo – CSIRO-AAHL
Luis Rodriquez – PIADC
Bryan Charleston – The Pirbright Institute, UK
Cyril Gay – Agricultural Research Service, USA
Do Huu Dung - Ministry of Agriculture and Rural Development, Vietnam
Ngo Thanh Long – Regional Animal Health Office no. 6, Vietnam
Francois Maree - ARC-Onderstepoort Veterinary Institute, RSA
Jonathan Arzt - PIADC
Elizabeth Rieder - PIADC

Assistance
Melanie Peterson - Agricultural Research Service, USA
Ashley McFadden - Agricultural Research Service, USA
Madeleine Clark - The Pirbright Institute, UK
Phuong Tran - Ministry of Agriculture and Rural Development, Vietnam

Abstract book
Carla Bravo de Rueda - PIADC
Jacquelyn Horsington - CSIRO-AAHL

Awards Presented to Four Young Scientists at the Global Foot-and-Mouth Disease Research Alliance 2015 Scientific Meeting

MSD Animal Health (known as Merck Animal Health in the USA and Canada) sponsored awards to four young scientists at the Global Foot-and-Mouth Disease Research Alliance (GFRA) 2015 Scientific Meeting, in honour of their research in disease-endemic areas. Syed Jamal of Pakistan and Nguyen Van Long of Vietnam received the awards for best oral presentations, and Raphael Sallu of Tanzania and Truong Dinh Bao of Vietnam were honoured for best poster presentations.

One favourite was the award for the largest poster.

One of the best oral presentations was **"Evidence for multiple recombination events within genomes of FMDVs circulating in West Eurasia"** by Syed M. Jamal (Department of Biotechnology, University of Malakand, Pakistan), M. Nazem Shirazi (Iran Central Veterinary Laboratory,Tehran, Iran), Fuat Ozyoruk (FMD Institute, Ankara, Turkey), Unal Parlak (FMD Institute, Ankara, Turkey), Preben Normann (National Veterinary Institute, Technical University of Denmark, Lindholm, Denmark) and Graham J. Belsham (National Veterinary Institute, Technical University of Denmark, Lindholm, Denmark)

Abstract: Phylogenetic studies on FMDVs circulating in the West EurAsian region have largely focused on the genome sequences encoding the structural proteins that determine the serotype. We have generated and compared near complete genome sequences of FMDVs representative of the three serotypes (O, A and Asia-1) which are circulating in this region and presented our findings as an oral presentation during the GFRA 2015 Scientific Meeting, held in Hanoi, Vietnam. Comparison of different regions of the FMDVs genomes revealed evidence for multiple, inter-serotypic, recombination events between some of the FMDVs belonging to the serotype O, A and Asia-1 viruses. The study concluded that in addition to genetic drift, more dramatic changes in FMDV genomes can occur in the field through recombination between different FMDV genomes. The analyses provide information about the ancestry of the serotype O, A and Asia-1 FMDVs circulating within the West EurAsian region.

FMD Policy Meets Science

Scenario development and session moderator: David Paton

During the GFRA Scientific Meeting 2015, a session was held involving interactive discussions on topical policy issues. The aim was to discuss whether there are gaps in knowledge that could be filled by research to the benefit of policy development.

Participants were invited to join one of three break-out groups discussing the disease control scenarios below. A facilitator introduced each topic, collated the outcome of group discussions and fed this back to the full meeting in plenary.

Scenario	Background	Facilitator
1) Following use of emergency vaccination without slaughter in a FMD affected area of a PCP stage 3 country, what other control measures are most important and for how long should they be maintained?	It is a truism that FMD vaccination alone is rarely sufficient without other measures to reduce transmission, but in many countries with communal herds, conventional movement controls are very difficult to implement	Nick Lyons Aldo Dekker
2) What is the optimal regime for emergency vaccination of pigs in response to an outbreak of FMD?	In Korea, there has been discussion over whether incomplete protection of vaccinated pigs stems from poor vaccine match or sub-optimal vaccination regime. Many FMD experts outside Asia are unfamiliar with vaccination of pigs in the field.	Young Lyoo, Don King
3a) As a country that buys vaccine via a tender process, how do you establish what is an acceptable post-vaccination serology titre for post vaccination monitoring? 3b) What post vaccination monitoring (PVM) is appropriate for use in a FMD free zone without vaccination after use of emergency vaccination?	Vaccines are invariably used to protect against heterologous virus challenge. But in most cases the correlation between heterologous antibody titre and protection is not known. PVM can improve confidence in the effectiveness of emergency vaccination and provide additional confidence to trade partners complementing or replacing the need for NSP serosurveys.	David Paton Wilna Vosloo

Recommendations arising from scenario 1

Key considerations: (i) What are the risk factors for ongoing transmission in an area using emergency vaccination? (ii) What other measures are needed for control? (iii) How do we understand how to target these control measures?

Considering:

- Current potency tests in cattle and efficacy tests in sheep and pigs provide good evidence of vaccine efficacy
- The relation between antibody response and protection has been shown in various species, with most data available in cattle
- Most countries applying prophylactic vaccination do not use serological tests to evaluate vaccine potency, nor vaccination efficacy in the field
- The level of outbreak reporting is low in most FMD endemic countries
- Emergency vaccination around outbreaks is often not practiced in countries using prophylactic vaccination
- Strategic revaccination of animals that are moved from areas that are facing outbreaks in a country that is using prophylactic vaccination is often not practiced.

The group recommends:

- Countries using prophylactic vaccination should test every batch of vaccine in a small group of animals before it is applied in the field.
- Countries should apply currently available tools are needed to help with the identification of outbreak strains, such as serotype specific penside tests to identify if the outbreak is caused by a serotype not present in the vaccine. But also collaborate with reference laboratories that can do genetic and antigenic characterisation.
- Serological surveys are needed in vaccinated areas to determine if the vaccine response in the field is similar to the response at batch testing. When an outbreak occurs, serological studies in risk areas (neighbouring villages) are necessary to determine the vaccination status and the need for revaccination.
- Research on risk assessment is needed in the context of movements of animals, networks, hubs, modelling, where are the hot spots and to review value chain analysis, taking into account the

implemented control measures, vaccine induced antibody titres in the field, number of outbreaks and spread due to various transmission routes (see points 1 - 3). Based on these data an indication of the antibody titre needed in the field can be determined in relation with the antibody titre determined in potency tests.

- Socioeconomic studies are needed to develop suitable incentives for implementing movement controls, taking into account the possibility of early movement after revaccination.
- Studies in risk communication will help to maximise awareness of stakeholders

Recommendations arising from scenario 2

Key considerations: Observed low efficacy of vaccines in swine could reflect (i) Regime used (timing and frequency of vaccination); (ii) Formulation of oil vaccines; (iii) Inadequate match of antigen used in the vaccine. A literature review of pig vaccination could be helpful.

Considering:

- There are almost no published studies comparing adjuvants for pigs with respect to protection.
- Although the best antibody response is seen when vaccinating in absence of maternally derived antibodies, there are studies that indicate that active immunisation is possible in the presence of maternally derived antibodies.
- All vaccine matching tests are performed in cattle, matching of vaccines could be different in pigs
- Due to limited availability of data on vaccine efficacy in pigs, limited data are available on the relation between antibody response and protection. The limited studies available show however that similar to cattle there is a significant relation between antibody response and protection.
- Limited studies on use of alternative routes of vaccination in pigs are available.
- No standard test for vaccine efficacy in pigs is available
- Limited studies into passive immunisation in pigs show limited effect of passive immunisation.
- Disinfection works only at temperatures above 0 ° C, no data are available on combinations of antifreeze and disinfectant at low temperature.

The group recommends:

- Research on evaluation and screening of alternative adjuvants for vaccination in pigs
- Research on effective (improved) vaccination regimes to generate optimum protection in pigs to accommodate maternal antibody responses (to reduce the immunity gap)
- Research on optimized (or calibrated) vaccine matching tests for infection in pigs
- Research on reliable lab tests (ELISA) to measure protective immune responses in pigs (particularly heterologous responses to field viruses - see scenario 3)
- Research on validation of alternative routes (IM, SC, ID) and sites of vaccination (to minimize local tissue granulomas in valuable meat cuts) and even multiple sites (with divided dose)
- Research on impact of interference between components in multivalent vaccines?
- Research on develop specific parameters for vaccine batch release for use in pigs
- Research on explore passive immunisation in pigs to generate transient immunity?
- An additional gap not related to vaccination that became apparent during recent Korean outbreaks:
- Research on effective disinfection protocols for low temperatures?

Recommendations arising from scenario 3

Key considerations: Protection is influenced by many variables relating to the properties of the vaccine used, the vaccination regime and the nature of virus challenge that occurs in the field. Measuring protection requires use of serological tests for which the correlation to protection is also affected by multiple factors. Scenario 3b was not discussed due to lack of time. The soon-to-be-released post vaccination monitoring guideline from OIE/FAO will provide advice and options to help address some of these uncertainties

Considering:

- Serology provides very useful correlates of protection as determined in potency tests. But the relation between antibody response and protection is different for different vaccines, different vaccine formulations, different routes of vaccination and different times after vaccination
- The relation between antibody response and protection in potency tests is different from the relation between antibody response and protection in the field.
- There is limited data on the effect of concurrent infections on protection induced by FMD vaccines.
- The relation between antibody response and protection is often not validated in newly developed serological tests.
- Countries that use prophylactic vaccination do not

sufficiently evaluate the antibody response in the field.

- Countries that use prophylactic vaccination do not sufficiently evaluate the duration of the immunity after vaccination.

The group recommends:

- Research on the actual determinants of immunological protection for better prediction of protection under different circumstances without the need to establish correlations in advance using specific potency tests that match these circumstances.
- Comprehensive field studies on antibody response and vaccine efficacy, e.g. a detailed longitudinal follow-up of vaccination campaigns in endemic settings.
- Research on the impact of other diseases and vaccinations on the development of FMD immunity.
- Vaccine users should work closely with vaccine producers and would benefit if as well as advice, they received appropriately calibrated reference sera or test kits to help determine the expected threshold of vaccine-induced immunity.
- Research on relation between protection and antibody response in newly available, commercial serology kits that detect structural protein antibodies.
- Countries that use prophylactic vaccination should determine what proportion of the population has responded in the expected way to a vaccine. This type of straightforward survey should always be performed on a large scale to identify problems in vaccine delivery, whilst complementary studies to look at aspects of protection such as its duration and the impact of antigenicity should be performed on a small scale to augment understanding of the main survey.

UPCOMING EVENTS

2016::Open Session of the EuFMD::

*The **EuFMD O**pen **S**ession (OS) is the world's largest regular gathering of FMD technical experts*

OS'16::The Practice of Innovation*

26-28 October 2016
Portugal – Cascais

OS'16 is focussed on **innovation**, **innovative practice** and the challenges and lessons learnt from the field, of translating science into improved disease management.

Innovators in the private and public sectors, leaders in FMD management, science and policy **get together at OS'16!**

Email: OS16-FMD-General@fao.org

eofmd
european commission for the control of foot-and-mouth disease

EuFMD e-Learning

FMD Emergency Preparation Course

Who is the course for?

Veterinarians or those involved in the livestock industry who would be involved in diagnosing and investigating an outbreak of foot and mouth disease.

What does the course cover?

- FMD aetiology and pathogenesis
- Clinical diagnosis, lesion ageing
- FMD epidemiology, outbreak investigation
- Biosecurity

What does the course involve?

The course is studied entirely online, and will take approximately **10 hours to complete**. **75–100 participants** take the course at the same time, and it is open for **4 weeks**.

The course opens with **a live interactive webinar**, where trainees meet their trainers, and are introduced to the course, and to foot-and-mouth disease.

Trainees then study **four interactive online modules**, which include lots of **photographs, videos and self test questions**.

During the course, **EuFMD expert trainers are available** through a discussion forum to answer questions from the trainees, and to lead interactive discussions.

Towards the end of the course there is a second **live interactive webinar,** to discuss interesting topics raised during the course in more detail.

At the end of the course all trainees must complete a **comprehensive assessment**. Successful trainees are provided with a certificate, and records of completion passed to the sponsoring body.

What do trainees say about the course?

80% of 40 trainees surveyed during a recent course rated the course as “very good” and “very relevant” to their needs. 100% would recommend others take part in the course in future.

Comments included *“this is a great opportunity for those who cannot travel to obtain training”, “very good, well prepared, very informative video parts”, “very interesting course” “great and interesting discussions”.*

For more information e-mail: eufmd-training@fao.org

WEBINARS

The EuFMD organises a number of informal networks which hold regular webinars. This includes a webinar series on Practical FMD Management, organised through the Contingency Planning Network, webinars on using disease spread modelling to assist contingency planning, and on vaccination as a control measure for FMD. **Please e-mail eufmd-training@fao.org for more information, or to join the network(s) and receive updates.**

Already available for viewing:

- Data driven models of foot-and-mouth disease: reviewing outbreak models and highlighting new research in an endemic setting
- Modelling spread of highly infectious diseases in the EU before detection Model development - issues and best practices: Foot and mouth disease transmission models and the estimation of parameters
- Ensemble modelling for use in epidemiology: a foot and mouth disease case study
- Optimising foot-and-mouth disease control: the role of clear objectives and real-time updating
- Introduction of the strategy and role of EuFMD, EU, OIE, FAO for the control of Foot and Mouth Disease in the European neighbourhood and globally; legal frameworks and introduction to the Progressive Control Pathway
- Construire une stratégie régionale pour le contrôle de la fièvre aphteuse en Afrique du Nord
- Evaluation of surveillance for freedom of disease versus early detection across countries: Case study adapting scenario tree methodology using Reed Frost models

Want to know more?

The Global Foot-and-Mouth Disease Research Alliance (GFRA)

A worldwide association of animal health research organisations to assist the global control and eventual eradication of foot-and-mouth disease.

www.ars.usda.gov/gfra

Australian Animal Health Laboratory

Global Foot-and-Mouth Disease Research Alliance

The GFRA Executive Committee

Wilna Vosloo	Chief Executive Officer (Australian Animal Health Laboratory, Australia – Wilna.vosloo@csiro.au)
Long Thanh Ngo	Outgoing President (Onderstepoort Veterinary Institute, South Africa – mareef@arc.agric.za)
Do Huu Dung	President Elect (Department of Agriculture, Hanoi, Vietnam – dung.dah@gmail.com)
Cyril Gay	Executive Secretary (Agricultural Research Service, USA – Cyril.Gay@ARS.USDA.GOV)
Luis Rodriguez	Science Director (Plum Island Animal Diseases Centre, USA – luis.rodriguez@ars.usda.gov)
Bryan Charleston	Finance Director (Pirbright Institute, UK – bryan.charleston@pirbright.ac.uk)

Newsletter compiled and edited by Jacquelyn Horsington, FMD Risk Management Project, CSIRO-AAHL

**Please note the contents of this newsletter are not peer reviewed.*