**OS**18

# **ONLINE** version
# Book of abstracts

Day

# 3

# SUPPORT

## Support | Institutional









## Support | Private






Dear colleagues,

Behind almost all of our problems with Foot-and-Mouth Disease are four things: the frequency of emergence of new strains, the exceptional virus infectivity and speed of spread, the impact on producers and the lack of security that comes from the limited and uncertain access to suitable vaccines.

Behind almost all our FMD mitigation nightmares is one simple fact: we do not have vaccine security.

Instead, we live with the fear that vaccine will not be available when needed, or if available, will not be effective when used, or achieve the outcomes desired. In FMD free regions, the "standard model" of vaccine banks is being challenged by the potential scale of need and the diversity of circulating FMD strains. In regions not free of FMD, the efforts being made for "risk based vaccination" and optimizing control measures relate to the same problem of lack of vaccine security. The lack of available quality vaccines results in ineffective vaccines being used, with disappointing results for the animal producers as well as nationally.

Without vaccine security, we need elaborate and well-drilled preparedness for an FMD emergency, to contain incursions before they outstrip vaccine supply. In endemic regions, millions of animals - and their owners - cannot access effective vaccines when they need them so lack of supply does matter for food security and livelihoods

Global security of supply of FMD vaccines therefore affects all countries – so what can be done about it? and how do we manage the risks without it? This is a big issue and needs all involved to have an overview of the scale of the problem, and the barriers and constraints to increased security. We must continue to cope with the risks posed by lack of vaccine, or to over-reliance on vaccination.

*Dr Keith Sumption*
Executive Secretary
European Commission for the Control of Foot-and-Mouth Disease

# YOUR AGENDA

DAY 3 BREAKOUTS. Morning sessions

| | ROOM 1 |
|---|---|
| 9A | AFRICA EPI-NET. *ANSES / Sciensano session* |
| 09:00 | SEROTYPES IN NIGERIA. *D. Lefebvre + A. Vleeschauwer + D. Ehizibolo* |
| 09:15 | SEROTYPING SEROPREVALENCE OF FMD IN CATTLE FROM UGANDA SURVEILLANCE 2014-2018. *K. Scott* |
| 09:30 | FMD IN BURUNDI. *K. de Clercq* |
| 09:45 | DOES THE AFRICAN BUFFALO REALLY SPREAD FMD? A SERO-SURVEY OF FMD IN CATTLE AROUND MANA POOLS CONSERVATION PARK OF NORTHERN ZIMBABWE. *W. Chikurunhe* |
| 10:00 | CONTROL METHODS OF FMD IN BENIN BY TRIAL VACCINATION AND MEDICINAL PLANTS. *E. Houndje* |
| 10:15 | MOLECULAR CHARACTERISATION OF FMD VIRUS DETECTED DURING 2015 – 2018 IN TANZANIA: INSIGHTS FOR VIRUS DIVERSITY AND EVOLUTION IN AFRICA. *C. Kasanga* |
| 10:30 | FMD SURVEILLANCE AND CONTROL IN MALI. *D. Diaoure* |
| 10A | DIAGNOSES & DIAGNOSTIC TOOLS |
| 11:00 | MULTIPLEX REAL-TIME RT-PCR FOR DETECTION OF FMDV, RIFT VALLEY FEVER VIRUS AND BOVINE VIRAL DIARRHEA VIRUS IN BULK TANK MILK. *M. Eschbaumer* |
| 11:15 | EMERGENCY SUPPLY OF FMD DIAGNOSTIC KITS: REAGENT BANKS AND IDVET SOLUTIONS. *L. Comtet* |
| 11:30 | RESULTS FROM A INTER-LABORATORY EXERCISE TO EVALUATE NON-STRUCTURAL PROTEIN ELISA KITS. *C. Browning* |
| 11:45 | RESULTS OF THE 2016 AND 2017 PROFICIENCY TESTING SCHEMES FOR FMD DIAGNOSTIC METHODS. *A. Ludi* |
| 12:00 | *Discussion* |

| | ROOM 3 |
|---|---|
| 10C | EuFMDiS DEMONSTRATION + DEBATE |
| 11:00<br>12:30 | **DEMONSTRATION**<br>*K. Mintiens*<br><br>**DEBATE** |

# YOUR AGENDA

| ROOM 2 | |
|---|---|
| **IMPROVING CONVENTIONAL VACCINES** | **9B** |
| A GVII-2015, A NEW HIGH POTENCY VACCINE WITH BROAD PROTECTION AGAINST A/ASIA/G-VII THREAT. *H. Gaude* | 09:00 |
| INTRADERMAL APPLICATION OF FMD VACCINES FOR PIGS. *J. Horsington* | 09:15 |
| EFFICACY OF A/MAY/97 FMDV VACCINE AGAINST HETEROLOGOUS CHALLENGE WITH A FIELD VIRUS FROM THE EMERGING A/ASIA/G-VII LINEAGE IN CATTLE. *P. Eble* | 09:30 |
| A SIMPLE UNIVERSAL TEST TO QUANTITATE 146S ANTIGEN DURING PRODUCTION OF FMD VACCINES. *T. Tuthill* | 09:45 |
| IMPROVING THE DURATION OF IMMUNITY FOR FMD VACCINES. *S. Parida* | 10:00 |
| THE USE OF REVERSE GENETICS TO FACILITATE THE GROWTH OF FMDV FOR THE PRODUCTION OF VACCINES. *S. Berryman* | 10:15 |
| **VACCINE QUALITY ASSURANCE** | **10B** |
| THE INTERDEPENDENCE OF FMDV PATHOGENESIS, CHALLENGE SYSTEM, AND OUTCOME OF VACCINE STUDIES. *C. Stenfeldt* | 11:00 |
| GOOD CORRELATION BETWEEN VACCINE MATCH IN POTENCY TESTS AND r1¬-VALUE. *A. Dekker* | 11:15 |
| POTENCY ASSESSMENT OF FMD VACCINES USING STANDARDISED SEROLOGICAL ASSAYS. *S. Jamal* | 11:30 |
| *Discussion* | 11:45 |

| ROOM 3 | |
|---|---|
| **9C SIGN-UP DISCUSSIONS** | |
| • PREDICTING VACCINE DEMAND (45 min)<br>• AESOP: A NEW OPTION FOR EMERGENCY RESERVES? (45 min) *Closed session* | 09:00<br>10.30 |

# YOUR AGENDA

DAY 3 BREAKOUTS. Afternoon sessions

| | ROOM 1 |
|---|---|
| 11A | MIDDLE EAST & ASIA EPI-NET. EuFMD Pillar II session |
| 14:00 | DETECTION OF FMDV O/ME-SA/IND-2001E IN JORDAN. *C. Van Maanen* |
| 14:15 | THE ASSOCIATION BETWEEN BORDER PROVINCES OF TURKEY AND FMD OUTBREAKS. *I. Keskin* |
| 14:30 | INTEGRATED RISK-BASED STRATEGIC PLANS FOR FIVE PRIORITY DISEASES IN THE PALESTINIAN AUTHORITY. *M. McLaws* |
| 14:45 | EMBEDDING PROGRESSIVE CONTROL FOR FMD IN THE POLICY AGENDA FOR LIVESTOCK PRODUCTION IN THREE COUNTRIES IN SOUTHEAST ASIA. *C. Bartels* |
| 15:00 | TRANS-POOL MOVMENT OF TWO FMDV SEROTYPE A LINEAGES: A/ASIA/G-VII AND A/AFRICA/G-IV. *K. Bankowska* |
| 15:15 | SOCIO-ECONOMIC IMPACT OF FMD OUTBREAKS AND CONTROL MEASURES AT DIFFERENT SCALES IN MONGOLIA: FROM NATIONAL LEVEL GROSS LOSSES TO HERDERS' FOOD SECURITY. *G. Limon-Vega* |
| 12 | CLOSURE AND ACKNOWLEDGEMENTS |
| 16:00 | *Wrap up + acknowledgements* |
| 17:00 | *Closure* |

| ROOM 2 | |
|---|---|
| VACCINE PERFORMANCE | 11B |
| RETROSPECTIVE FMD OUTBREAK REPORTS FROM UGANDA AND TANZANIA BORDER DISTRICTS (2011-2016): IMPLICATIONS FOR FMD CONTROL BY VACCINATION. *S. Kerfua (via Adobe)* | 14:00 |
| UPDATE ON FMD IN THE MAGHREB REGION: VACCINATION ISSUES. *S. El Azhari* | 14:15 |
| ASSESSMENT OF FMD VACCINES IN MONGOLIA AND THE ROLE OF BACTRIAN CAMELS. *G. Ilziibat* | 14:30 |
| *Discussion* | 14:45 |

# YOUR AGENDA

| DAY 3 morning / SESSION 9A |
|---|
| FMD OUTBREAK IN LUKULU DISTRICT, EVIDENCE OF VIRAL SPREAD OUTSIDE THE KNOWN ENDEMIC AREAS. *F. Banda* |
| GENETIC CHARACTERIZATION OF FMD VIRUSES RESPONSIBLE FOR OUTBREAKS IN NIGERIA DURING 2016: RESURGENCE OF THE NOVEL FMD- SAT1 TOPOTYPE. *D. Ehizibolo* |
| CHARACTERIZATION OF FMDV ISOLATES CANDIDATE STRAINS FOR POLYVALENT VACCINE DEVELOPMENT IN NIGERIA. *H. Ularumu* |
| **DAY 3 morning / SESSION 10A** |
| FMD VIRUS ADSORBED TO GENOTUBE SWABS REMAINS INFECTIOUS AT HIGH TEMPERATURE. *M. Eschbaumer* |
| COMPARATIVE PERFORMANCE OF MONOCLONAL AND POLYCLONAL-BASED ANTIGEN ELISAS FOR FMDV DETECTION. *L. Henry* |
| INACTIVATION OF FMDV IN TISSUE SAMPLES TO ENSURE SAFE TRANSPORT FROM INFECTED PREMISES TO DIAGNOSTIC LABORATORIES. *J. Horsington* |
| DETECTION OF EARLY FMD VIRUS INFECTION IN PIGS USING IgA AND IgM ASAAYS. *S. Parida* |
| PRODUCTION OF SWINE SEROLOGICAL PANEL FOR THE VALIDATION OF FMD ANTIBODY TEST. *H.M. Pyo* |

| DAY 3 morning / SESSION 10B |
|---|
| INSIGHTS AND OPTIMISATION OF THE FMD VIRUS NEUTRALISATION TEST FOR R1 ANTIGENIC MATCHING. *F. Feenstra* |
| USE OF SEROLOGICAL TESTS FOR CHECKING NSP PURITY OF FMD VACCINES. *D. Paton* |
| WHAT CAN WE SAY? HARMONY OR DISHARMONY BETWEEN VACCINE MATCHING AND CHALLENGE STUDY. *P. Tuncer-Göktuna* |
| **DAY 3 afternoon / SESSION 11A** |
| REGIONAL COOPERATION BETWEEN TRANSCAUCASIA AND NEIGHBOURING COUNTRIES ON PREVENTION AND CONTROL OF FMD. *F. Rosso* |
| FMD OUTBREAKS DUE TO AN EXOTIC VIRUS SEROTYPE A LINEAGE (A/AFRICA/G-IV) IN ALGERIA IN 2017. *G. Pezzoni* |

# DAY 3

## BREAKOUTS

9:00h / Room 1*
9A. Africa EPI-NET

9:00h / Room 2*
9B. Improving conventional vaccines

11:00h / Room 1
10A. Diagnoses and diagnostic tools

11:00h / Room 2
10B. Vaccine Q&A

11:00h / Room 3
10C. EuFMiS Demonstration + debate

14:00h / Room 1
11A. Middle East and Asia EPI-NET

14:00h / Room 2
11B. Vaccine performance

* Rooms will be notified during the conference

## PLENARY ROOM

12. Closure and acknowledgements

SEROTYPES IN NIGERIA

# GENETIC CHARACTERIZATION OF FMDV RESPONSIBLE FOR OUTBREAKS IN NIGERIA DURING 2016: RESURGENCE OF THE NOVEL FMD-SAT1 TOPOTYPE

*D.O. Ehizibolo[1], I. Fish[2], B. Brito[3], S. Pauszek[2], C. Stenfeldt[2], M. Bertram[2], G.H. Ularamu[1], Y.S. Wungak[1], D.D. Lazarus[1], A.G. Ardo[4], C.I. Nwosuh[1], and J. Arzt[2]*

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## Introduction

It is critical to obtain and report up to date information on circulating foot-and-mouth disease virus (FMDV) strains and epidemiology to support future control strategies in West Africa and support risk assessment and legal international trade. These data are required to select appropriate vaccine strains and prioritize vaccine deployment.

## Materials and methods

Epithelial tissue samples (45) collected from suspected FMD-infected cattle during 2016 outbreaks in Nigeria, and an additional three samples (epithelial) retrieved from archival samples from 2014 outbreaks yet to be sequenced were shipped to PIADC, USA for analyses. Consensus sequences were obtained by Illuminaplatform NGS.

## Results

Using rRT-PCR, FMDV genome was detected in 93% (42/45) of epithelial tissue samples tested, and 40% (20/45) of these samples produced cytopathic effect (CPE) in cell culture after 48h in one or two passages. Four FMDV serotypes (O, A, SAT1 and SAT2) were identified. Phylogenetic evaluation showed that FMDV serotypes O/East Africa-3 and West Africa; A/AFRICA genotype IV (G-IV); SAT1 topotype X and SAT2 lineage VII were recorded to be in circulation during the study period. Regarding recently identified SAT1 viruses in Nigeria, two distinct groups within a cluster circulating in Nigeria and Cameroon were identified which have a common ancestor in 2007. The two Nigerian SAT1 topotypes from 1970's and 1980's were not identified and are apparently extinct. Divergence was identified within the serotype A viruses suggesting that there may have been more than one introduction in recent years.

## Discussion

The study provides an update on the FMD situation in Nigeria considering samples from outbreaks during 2014 and 2016. Highlights include serotypes/topotypes continuity, resurgence of the novel FMD-SAT1 topotype X in Nigeria and evidence of strong association between FMDV serotypes/topotypes in Nigeria and North Africa. Continuous molecular epidemiologi-

cal studies like this are important to create awareness and understanding of the trans-border movement of FMDV.

SEROTYPES IN NIGERIA

## SEROLOGICAL AND MOLECULAR EPIDEMIOLOGY OF FMD VIRUSES IN AGRO-PASTORALIST LIVESTOCK HERDS IN THE KACHIA GRAZING RESERVE, NIGERIA

*D.O. Ehizibolo[1], A.R. De Vleeschauwer[2], A. Haegeman[2], D. Lefebvre[2], C.I. Nwosuh[1], J.U. Umoh[3], E.C. Okolocha[3], H.M. Kazeem[4], S. Van Borm[5], K. De Clercq[2]*

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### Introduction

Foot-and-mouth disease virus (FMDV) is endemic in Nigeria, where livestock keeping is dominated by Fulani pastoralists. In the 1960s, grazing reserves were established to encourage pastoralist sedentarisation. The Kachia Grazing Reserve (KGR) consists of 6 contiguous blocks housing 744 defined households (HH), all engaged in livestock keeping, and it is considered a homogenous epidemiological unit.

### Materials and methods

In 2012, serum was collected from all cattle and sheep of 40 selected HH in KGR to determine sero-prevalence of antibodies to FMDV and of FMDV genome. In 2012 and 2014 serum, epithelium and probang samples were collected from cattle in reported FMD outbreaks and analyzed by virus isolation, antigen ELISA, RT-qPCR, VP1 sequencing and phylogeny.

### Results

The sero-prevalence of antibodies as detected by NSP-3ABC ELISA was 28.9% (380/1315) (30.6% cattle; 16.3% sheep), and in 4.5% (62/1380) (5% cattle; 0.6% sheep) of the sera FMDV RNA was detected by RT-qPCR. Half (50.9%) (27/53) of the 2012 outbreak sera reacted positive in NSP-3ABC ELISA, and 88% (52/59) of the outbreak sera contained detectable viral RNA. Overall, antibodies against five FMDV serotypes (O, A, SAT1, SAT2 and SAT3) were detected by solid phase competitive ELISA with combinations of two or more serotypes being common. Of the 21 FMDVs that could be isolated 19 were sequenced and 18 confirmed as SAT2 (lineage VII) while one was characterized as serotype O (EA-3 topotype). Phylogenetic analysis revealed close relationship between Nigerian FMDV strains and strains in this region

and even with strains in North-Africa.

### Discussion

Our findings indicate that KGR has not become a separate epidemiological unit with distinct circulation and evolution of FMDV strains nor is it a closed system. They highlight the importance of structured surveillance in support of an FMD control policy, with consideration of husbandry practices and potential role of small ruminants.

SEROTYPES IN NIGERIA

## COMPLEX CONCOMITANCE OF FMDV STRAINS IN NIGERIA

*H.G. Ularamu[1], D. Lefebvre[2,*], A. Haegeman[2], Y.S. Wungak[1], D.O. Ehizibolo[1], D.D. Lazarus[1], A. De Vleeschauwer[2], K. De Clercq[2]*

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### Introduction

In Nigeria FMD virus (FMDV) serotypes (st) O, A and SAT2 are endemic since long and SAT1 was re-introduced in late 2015. We previously described the presence of topotypes O/WA, O/EA-3, A/Africa/G-IV, SAT2/VII and SAT1/X (Ehizibolo et al., Transbound Emerg. Dis., 2017a and 2017b). Additional samples from late 2012 till late 2017 were investigated to gain more in-depth knowledge on circulating FMDV (OIE Twinning laboratory project).

### Materials and methods

Eighty-one tissue samples were collected from diseased cattle in 6 States in Northern Nigeria and 1 State in South-West Nigeria and analyzed by virus isolation, antigen ELISA, RT-qPCR, VP1 sequencing and phylogeny.

### Results

In Plateau State (North-Central), st SAT2 was isolated in 2013 and O in 2014. From September to November 2015 st O, A and SAT1 were isolated while in August and September 2017 st O and SAT2 were isolated.
Further in 2017, st A was isolated in Kaduna (North-West), Benue (North-Central) and Oyo (South-West) and st O was isolated in Bauchi (North-East).

### Discussion

The results demonstrate that FMDV is omnipresent and highly dynamic in Nigeria. In Plateau State 4 different serotypes were observed in less than 3 years while in some cases outbreaks with 2 or 3 different serotypes were observed in a single State in as little as 2 or 3 months of time. Meanwhile, particular FMDV strains are widely distributed in Nigeria as shown by the presence of a strain of serotype A in 3 remote States in 2017. Topotype O/EA-3 seems to be the most prevalent through the years. More sampling and knowledge gain is necessary to support a vaccination-based control plan.

SEROTYPES IN NIGERIA

## FOOT-AND-MOUTH DISEASE IN SMALL RUMINANTS AND IN WILDLIFE IN NORTHERN NIGERIA

*Y.J. Atuman[1], D. Lefebvre[2,*], H.G. Ularamu[1], Y.S. Wungak[1], A. De Vleeschauwer[2], K. De Clercq[2]*

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### Introduction

Data on circulating FMD virus (FMDV) is scarce in Nigeria, particularly in species that generally present mild or sub-clinical disease.

### Materials and methods

Three-hundred serum samples from sheep and goats and 38 serum samples from wildlife (waterbuck, wildebeest and African eland) were collected until 2015 in Bauchi State. During an OIE Twinning laboratory project antibodies (Abs) against structural proteins (SP) of FMDV were detected with an in-house ELISA and Abs against non-structural proteins (NSP) with a commercial ELISA kit.

### Results

In sheep and goats 21.7% of the samples tested positive whereas 78.3% tested negative. Of the positive samples, 50.8% reacted with SP and NSP, 21.5% reacted with SP only and 27.7% with NSP only. Of the samples reacting with SP, 17.0% was mono-specific for serotype O, 23.4% mono-specific for A, 31.9% mono-specific for SAT2 and 27.7% reacted with 2 or 3 of these serotypes.
In wildlife, 44.7% of the samples tested positive whereas 55.3% tested negative. Of the positive samples, 76.5% reacted with SP and NSP, 11.8% reacted with SP only and 11.8% with NSP only. Of the samples reacting with SP, 6.7% was mono-specific for serotype O, 40.0% mono-specific for A and 53.3% reacted with 2 or 3 serotypes (O, A and SAT2).

### Discussion

Data confirms the concomitance of FMDV O, A and SAT2 in Bauchi State (Nigeria) and confirms the absence of SAT1 until late 2015. Data suggests a lower prevalence of FMDV in small ruminants compared to cattle and wildlife. 72.3% of the SP-positive small ruminants reacted with just 1 serotype and 41.2% of these animals tested NSP-negative. Interestingly, all animals (small ruminants and wildlife) reacting with 2 or 3 serotypes tested NSP-positive. It is currently not known if the animals that were NSP-positive but SP-negative were false positives for NSP or false negatives for SP.

## SEROTYPING SEROPREVALENCE OF FMD IN CATTLE FROM UGANDA SURVEILLANCE 2014-2018.

*K. Scott*[*,1], *F. Mwiine*[3,4], *O. Sylvester*[4], *J. Lutwaama*[5], *M. Chitray*[1], *K. van der Waal*[6], *E. Rieder*[7], *F. Maree*[1,2]

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### Introduction

FMD remains endemic in many regions of the world causing significant economic losses. The problem is heightened due to poor surveillance; limited resources for vaccination programmes; limited vaccine matching information; and failure of serological methods to differentiate infected and uninfected animals. The FAO defined FMD Progressive Control Pathway positions Uganda at stage 1, which aims to get a better understanding of the epidemiology of FMD in Uganda and develop a risk-based approach. Here we report on the serotyping seroprevalence and NSP results of a cross-sectional FMDV study in cattle conducted in Uganda 2014-2018.

### Material and methods

The study was designed to conduct FMDV surveillance in cattle from widespread regions of Uganda from 2014-2018. Serum samples were subjected to the FMDV antibody detection against non-structural proteins (NSP) using the Priocheck FMDV NS ELISA kit and for serotyping the Priocheck FMDV type A and O kits following manufacturer's instructions. For SAT serotyping the in-house SPCE test was performed (ARC, South Africa). Briefly, Rabbit anti-serum raised to SAT serotype specific FMDV was used to capture the SAT type-specific

antigen, thereafter the competition between guinea-pig antiserum and antibodies present in the test serum were measured. The percentage inhibition was calculated with >50 % classified as positive.

### Results

The results were classified into groups: (1) non-vaccinated, NSP negatives; (2) non-vaccinated NSP positive; (3) vaccinated NSP positive; and (4) vaccinated NSP negative animals. Serotyping results confirmed the antibody status of vaccinated animals and the seroprevalence of each serotype present in the vaccine/s. The seroprevalence of circulating serotype specific antibodies from infected animals was also analysed.

### Discussion

We demonstrated the active circulation of multiple FDMV serotypes and their seroprevalences in different Ugandan regions. Additionally, through serotyping of vaccinated animals, post-vaccination monitoring was able to evaluate the efficacy of the vaccine to aid efforts in identifying a risk-based approach to control of FMD in Uganda.

## FOOT-AND-MOUTH DISEASE IN BURUNDI

*A.I. Estevez Garcia[1], D. Lefebvre[1], L. Nyabongo[2], A. Haegeman[1], C. Nkundwanayo[2], A. De Vleeschauwer[1], D. Ntakirutimana[2], I. De Leeuw[1], D. Ngsanganiyumwami[2], T. van den Berg[1], A. Niyokwishimira[2], K. De Clercq[1,*]*

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### Introduction

In March 2016 clinical signs of FMD were reported in several provinces of Burundi, a small country with subsistence-oriented crop-livestock agriculture located between D.R. Congo and Tanzania. Several hundred of affected cattle were reported. The outbreak was contained by closing cattle markets and banning transhumance and free range grazing. There was no vaccination. A quarantine center was built near the Tanzanian border as FMD was suspected to be introduced by cattle imported from Tanzania.

### Materials and methods

Tissue samples or saliva were collected from 194 diseased cattle in 6 provinces and analyzed by virus isolation, antigen-ELISA, RT-qPCR, VP1 sequencing and phylogeny. Serum was analyzed by ELISA for the presence of antibodies against structural or non-structural proteins (NSP) of FMD virus (bilateral collaboration LNV-Sciensano).

### Results

Seroprevalence was 87.3% for NSP, 45.1% for serotype (st) O, 38.2% A, 31.8% SAT1, 51.4% SAT2, 20.2% C and 19.7% SAT3. Virus isolation was successful for samples from 3 remote provinces: FMD virus (FMDV) st SAT2 was found in Rutana (south-east), Mwaro (central) and Cibitoke (north-west) while FMDV st A was found in Cibitoke. Topotype was characterized as SAT2/IV and A/Africa/G-I, respectively. Combining serological and virological data indicates an older st O outbreak in Bubanza (mid-west).

### Discussion

Serological data suggests the presence of FMDV st O, A, SAT2 and perhaps SAT1 in Burundi. Seroprevalence for C and SAT3 was around 20%, presumably due to cross-reactivity. This % of cross-reactivity is comparable to previous data in Nigeria (Ehizibolo et al., 2017).

Data demonstrates that SAT2/IV, previously reported in Tanzania (EuFMD Global Monthly Reports), is widespread in Burundi, supporting the hypothesis of introduction by cattle imported from Tanzania. A/Africa/G-I, previously reported in Tanzania and D.R.C. (EuFMD Global Monthly Reports), was only isolated in the most north-western province, bordering D.R.C. and Rwanda, suggesting another source of FMD.

## DOES THE AFRICAN BUFFALO REALLY SPREAD FMD? A SERO-SURVEY OF FMD IN CATTLE AROUND MANA POOLS CONSERVATION PARK OF NORTHERN ZIMBABWE

*W. Chikurunhe[1], G. Matope[2], D. Pfukenyi[2], P. Tshabalala[3], M. de Garine Wichatitsky[2, 4, 5]*

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### Introduction

African buffalo (Syncerus caffer) has been demonstrated as the main reservoir of the FMD virus serotypes afflicting Southern Africa. Cattle are highly susceptible. In Zimbabwe, the southern provinces frequently report clinical cases of FMD but no clinical disease has been reported from the north despite observed buffalo/cattle contact. Our aim was to describe FMD virus circulation in cattle herds around Lower Zambezi-Mana Pools Transfrontier Conservation Area (LZ-MP TFCA) in order to design FMD management strategies for northern Zimbabwe.

### Materials and Methods

The study investigated whether the serological picture in cattle explains buffalo/cattle contact patterns observed at the periphery of LZ-MP TFCA. 1238 cattle sera were collected from 2 districts in a two-stage random sampling protocol. Samples were tested for antibodies to the

non-structural protein of FMD virus using the Enzyme Linked Immunosorbent Assay (NSP-ELISA). NSP positive sera were subjected to the Liquid Phase Blocking ELISA (LPBE). Risk factors perceived to influence FMD virus infection in cattle were interrogated using a questionnaire.

### Results

3.6% (45/1238) sera tested positive for antibodies to FMD. Positive cases were spread in all three seasons in both districts. SAT 2 and SAT 3 serotypes were confirmed by LPBE (13/45). Distance from game park, hot-dry season, and area of origin were associated with increased risk. The results complement the findings of Jori et al but are at variance with various publications on FMD sero-prevalence at the interface of wild buffalo and cattle in Zimbabwe.

### Conclusion

Results confirmed FMD infection in cattle in the periphery of the TFCA and suggest low-level FMD virus circulation which is inconsistent with various published data. There is need for a livestock movement control policy in the north. Factors responsible for low-level FMD virus circulation need further research.

## CONTROL METHODS OF FMD IN BENIN BY TRIAL VACCINATION AND MEDICINAL PLANTS

*E. Houndje[1], Y. Akpo[2], C. Ogni[1], N. Noudèkè, A. Youssao[1], G. Aplogan[2], S. Farougou[1], M. Kpodekon[1]*

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### Introduction

Foot and Mouth Disease (FMD) is endemic in Benin which is located in West Africa that is considered as a risk zone (Rweyemamu et al., 2008).

### Material and methods

Trial vaccination was made in two farms and included serotypes O and A which were isolated in this country. Sera were collected before vaccination (day 0), on the 30th and the 120th days post vaccination. Sampled sera were analysed for the detection of non-structural protein (NSP) antibodies. After, a survey using semi-structured questionnaires was undertaken to identify the recipes used by breeders to treat cases of FMD

### Result

The NSP rates from farm 1 were 54%, 62.5% and 48.97% on days 0, 30 and 120, respecti-

vely without a significant difference. However, in Farm 2, the NSP rate of day 30 (71.19%) was not differed significantly from that of day 120 (75%). 32 medicinal plants were listed by breeders. Vitellaria paradoxa is the most cited with three types of recipes. Thus medicinal plants such as Citrus limon L., Pterocarpus erinaceus, Acacia nilotica L, Lannea acida, Khaya senegalensis were each involved in two types of recipes. Barks are involved in 14 types of recipes and maceration is the method most used with 27% types of recipes followed by the powder with 24%.

### Discussion

The use of NSP alone cannot be a reliable method to conclude the effectiveness of the vaccine in cattle in an endemic country. The risk factors such as introduction of another virus strain (Serotype and topotype) can explain the result of trial vaccination. Others investigations could reveal endogenous recipes which are best.

## MOLECULAR CHARACTERISATION OF FMDV DETECTED DURING 2015 - 2018 IN TANZANIA: INSIGHTS FOR VIRUS DIVERSITY AND EVOLUTION IN AFRICA

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### Introduction

Foot-and-mouth disease (FMD) is endemic in most countries in Africa where it causes significant food security and economic losses. The control of FMD in Africa is mainly through vaccination, which depends on the knowledge of circulating FMD virus (FMDV) in specific geographic locations. This study was conducted to investigate the occurrence of FMD and determine the genetic characteristics of viruses detected in different geographic locations of Tanzania between 2015 and 2018.

### Methods

Tissue epithelia and fluids (n = 844) were collected from cattle and pigs exhibiting oral and foot vesicular lesions suggestive of FMD. The analysis of these samples was performed by serotype-specific antigen capture ELISA, RT-PCR and sequencing. VP1 nucleotide sequences were generated for RT-PCR amplicons, and phylogenetic reconstructions were determined by maximum likelihood and neighbour-joining methods.

### Results

The results of this study indicated that 432 out of 844 (51.2%) samples contained FMDV antigen. Of the 432 positive samples, 140(32.4%) were type A, 208 (48.1%) type O, 34(7.9%) SAT 2, and 50 (11.6%) serotype SAT 1. All four FMDV serotypes were found in the Southern, Northern, Coastal, Central and Eastern zones. Phylogenetic analysis of VP1 nucleotide sequences showed that Tanzanian type O viruses fell into the EAST AFRICA 2 (EA-2) topotype, type A viruses fell into the AFRICA topotype (genotype I), type SAT 1 viruses into topotype I and type SAT 2 viruses into topotype IV.

### Discussion

These findings reveal that serotypes O, A, SAT 1 and SAT 2 that caused FMD outbreaks in Tanzania were genetically related to lineages and topotypes occurring in the East African region, with minor genetic variations among strains recovered from different geographic locations with time and space. The presence of multiple serotypes and genotypes complicates FMD control in Tanzania and the region. Further studies are required to investigate the evolutionary characteristics, transmission dynamics and antigenicity of circulating strains so that rational FMD control method(s) in Tanzania and the neighbouring countries can be recommended.

### Acknowledgement

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## FMD SURVEILLANCE AND CONTROL IN MALI

*Dr. Abdoulaye Diaouré*

*VSF-international*

Mali is a vast country where transhumance is constant. The authorisation to practice as a private veterinarian has been possible since the late 80s but the animal health problems remain a major concern. A VSF-International database shows that only 21% of the country is covered by the private professionals and shows the necessity to get in addition other 845 veterinary doctors to ensure an optimal coverage (on the basis of 25 000 TLU per specialist). Moreover, the regal powers of the government (especially in the control of and regulation) is less or not assured. In 2018, the Directorate of veterinary Services observed a vacancy of 66 positions for the national territory. The global performances of veterinary services (public as well as private) is relatively weak and the veterinary actions, focus of health mandates present, are not in a way of increasing that performance since the veterinary surgeon can't live on their profession.

On behalf of EuFMD, VSF-International is conducting two simultaneous studies on foot and mouth disease to establish an efficient surveillance and control system for the disease. Collecting and shipping samples but also rapid field testing including the participation of para-veterinarian personnel, setting fitting offer and demand services for FMD and identifying necessary changes (institutional, judicial and/or regulations, etc.) in the case the demand services would be efficiently covered.

Considering the realities of the livestock system in Mali (extensive system with transhumance but also an intensive system with crossbreeding of local and imported breeds) has pushed to determining 3 areas for this study

Western and Eastern area to cover the herds' transboundary movements and
The peri-urban area of Bamako to cover crossbred dairy cows.

The initial training and subsequent discussions have allowed strengthening stakeholders' capacity in disease diagnostics and sample collection as well as their handling before sending them reference laboratories. Biosecurity measures as a way to stop the disease spread has attracted the stakeholders' attention. Samples were collected from five sick cows of the same farm and the test came out positive, further results of the survey on the perspectives of livestock owners for FMD control will be provided.

Key words

Foot-and-Mouth Disease, capacity building, Veterinary services performance, biosecurity, Serotyping, pastoral mobility, sample collection, rapid diagnostics, offer and demands in services, para-veterinarians.

POSTER

## FMD OUTBREAK IN LUKULU DISTRICT, EVIDENCE OF VIRAL SPREAD OUTSIDE THE KNOWN ENDEMIC AREAS

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FMD viruses are usually confined to specific geographical regions and spread to new areas may lead to significant epidemics. In Zambia, the disease is endemic around the three known high risk areas. However, in 2015 an outbreak outside the traditional endemic areas involving Western Zambia attributed to SAT 3 was reported. A quiescence of the disease followed from May 2016 until April 2017. Precipitating factors behind the epidemiology change of disease

in this region is unknown. Herein we describe investigations on latest disease foci in Lukulu District which historically has never recorded FMD.

In May 2017, reports were received of suspected FMD and clinical examination of six kraals for presence of FMD lesions was conducted. Five epithelial tissues and 22 blood samples were collected. Lab investigations involved Cell culture, RT-PCR, Antigen ELISA and NSP ELISA. 75 animals (76.5%) out 102 animals examined manifested clinical signs and lesions suggestive of FMD. All tissues showed 100% CPE and RT-PCR detected the Virus Genome with antigen ELISA classfing the virus as SAT 3. Twelve (54.5 %) out of 22 blood samples were positive on NSP ELISA with percentage inhibition between 60.8 % - 95.9 %.

This outbreak after a quiescence of 11 months indicates possibilities of undetected viral circulation in carrier animals. First ever FMD report in Lukulu thus factors facilitating epidemiology change require further investigation although preliminary investigations revealed uncontrolled movements. Earlier outbreak was controlled through strategic vaccination of cattle and even though spread was abated, this outbreak suggests circulating of virus despite vaccinations. Further studies to evaluate vaccine efficacy and vaccination strategies should be conducted and use the outcome to inform policy.

POSTER

## GENETIC CHARACTERIZATION OF FMDV RESPONSIBLE FOR OUTBREAKS IN NIGERIA DURING 2016: RESURGENCE OF THE NOVEL FMD- SAT1 TOPOTYPE

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### Introduction

It is critical to obtain and report up to date information on circulating foot-and-mouth disease virus (FMDV) strains and epidemiology to support future control strategies in West Africa and support risk assessment and legal international trade. These data are required to select appropriate vaccine strains and prioritize vaccine deployment.

### Materials and methods

Epithelial tissue samples (45) collected from suspected FMD-infected cattle during 2016 outbreaks in Nigeria, and an additional three samples (epithelial) retrieved from archival samples from 2014 outbreaks yet to be sequenced were shipped to PIADC, USA for analyses. Consensus sequences were obtained by Illuminaplatform NGS.

### Results

Using rRT-PCR, FMDV genome was detected in 93% (42/45) of epithelial tissue samples tested, and 40% (20/45) of these samples produced cytopathic effect (CPE) in cell culture after 48h in one or two passages. Four FMDV serotypes (O, A, SAT1 and SAT2) were identified. Phylogenetic evaluation showed that FMDV serotypes O/East Africa-3 and West Africa; A/AFRICA genotype IV (G-IV); SAT1 topotype X and SAT2 lineage VII were recorded to be in circulation during the study period. Regarding recently identified SAT1 viruses in Nigeria, two distinct groups within a cluster circulating in Nigeria and Cameroon were identified which have a common ancestor in 2007. The two Nigerian SAT1 topotypes from 1970's and 1980's were not identified and are apparently extinct. Divergence was identified within the serotype A viruses suggesting that there may have been more than one introduction in recent years.

### Discussion

The study provides an update on the FMD situation in Nigeria considering samples from outbreaks during 2014 and 2016. Highlights include serotypes/topotypes continuity, resurgence of the novel FMD-SAT1 topotype X in Nigeria and evidence of strong association between FMDV serotypes/topotypes in Nigeria and North Africa. Continuous molecular epidemiological studies like this are important to create awareness and understanding of the trans-border movement of FMDV.

POSTER

## CHARACTERIZATION OF FMDV ISOLATES CANDIDATE STRAINS FOR POLYVALENT VACCINE DEVELOPMENT IN NIGERIA

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### Introduction

Foot-and-mouth disease (FMD) is an endemic transboundary animal disease that affects livestock health across sub-Saharan Africa. Since the first official report of FMD in Nigeria in 1924, serotypes O, A, SAT 1 and SAT 2 have been documented in Nigeria (Fasina et al, 2013;

Lazarus et al., 2012; WRLFMD 2010; WRLFMD 2014)).These studies was to characterize the FMD viruses circulating in Nigeria from 2007-2014 and to antigenically match the isolates for the development of polyvalent indigenous vaccine, and also to assist policy makers with decisions for effective disease control.

## Materials and methods

104 suspected samples between 2007 and 2014, used for virus isolation (ZZ-R 127). The VP1 region of the FMDV genome was amplified using a one-step RT-PCR kit (Qiagen), as described previously (Knowles et al., 2009). Candidates vaccine isolates were selected by antigenic vaccine matching.

## Results

FMDV genome was detected and serotypes determined from 45 epithelium samples (yielding 47 unique sequences when accounting for mixed infections) from eight different states. Eight (8) vaccines candidates were selected based on the antigenic vaccine matching results.

## Discussion

VP1 sequences were used to establish the relationships among the virus serotypes isolated in Nigeria. However, the 2009 and 2011 isolates were more closely related to each other, than the 2007 isolates. These data support two separate introductions of serotype O/EA-3 viruses into Nigeria, as well as the persistence of this topotype in country from 2009-2011. The vaccine candidates were selected based on the antigenic vaccine matching results, ease of growth on the BHK-21 cell line and its topotypes.

# A GVII-2015, A NEW HIGH POTENCY VACCINE WITH BROAD PROTECTION AGAINST A/ASIA/G-VII THREAT

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## Introduction

In September 2015, a new FMDV serotype A lineage A/ASIA/G-VII emerged from the Indian sub-continent to cause outbreaks in Saudi Arabia, Iran, Turkey and Armenia. This strain was expanding westwards, and was added by WRLFMD to the list of priority strains for European banks. A commercially available vaccine strain (A Saudi95) provided partial protection against A/ASIA/G-VII, but a fully effective vaccine was missing. Therefore, a new A GVII-2015 vaccine strain was developed up to industrial scale and its suitability for use in at-risk regions was demonstrated.

## Materials and methods

A/ASIA/G-VII isolates were adapted to BHK cell culture and selected based on epidemiological relevance and manufacturability. An A GVII-2015 Master Seed Virus was produced under Good Manufacturing Practices and its purity was controlled according to the Eur. Pharmacopeia. Antigens produced at industrial scale were formulated as aqueous aluminium hydroxide saponin or oil emulsion vaccines. These pilot vaccines were evaluated in terms of safety, immunogenicity, cross-neutralization spectrum and efficacy against homologous challenge.

## Results

The new A GVII-2015 vaccines showed no abnormal local or general reactions in the target species. High homologous neutralization titers obtained after vaccination demonstrated an excellent immunogenicity of the new strain. Furthermore, high heterologous neutralization titers and high r1 values indicated a wide protection amongst the A/ASIA/G-VII lineage. Finally, the in vivo potency of the vaccine, formulated at low payload, was tested by challenge in cattle and established at 18PD50/dose.

## Discussion

The continuous development of new vaccine strains in high quality and highly potent vaccines, to target emerging strains that are not properly covered by existing vaccines, is a key factor in the success of FMDV control. The development of the new A GVII-2015 strain provides a well adapted solution to fight the A/ASIA/G-VII threat, and suits the needs of endemic and FMD-free countries. The registration and commercialization of this vaccine have started in Europe and Middle East countries and will contribute to increase vaccine security in the concerned regions.

# INTRADERMAL APPLICATION OF FMD VACCINES FOR PIGS

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## Introduction

Intradermal (ID) vaccination for FMD is a promising approach offering many advantages over conventional intramuscular (IM) vaccines. The aim of this experiment was to determine the optimal dose for a FMD vaccine that is administered intradermally with the IDAL® device (MSD Animal Health) and to select a compatible adjuvant.

## Materials and methods

Groups of five pigs were vaccinated either with conventional IM vaccine (strain O/TUR/05/2009) or intradermally with one of three different adjuvants (A1, A2, or A3) and with antigen at a full or 1/10 dose. The animals received homologous challenge 21 days post vaccination and were assessed for clinical signs, immune response and shedding of virus up to 8 days post challenge.

## Results

The challenge virus was highly pathogenic, however, all vaccinated pigs showed reduced clinical signs compared to the unvaccinated controls. Clinical protection (defined as the absence of lesions on the three non-inoculated feet) and sterile immunity were most notable in the IM vaccine and the ID A3 full dose vaccine (both 80% protection). These groups also had the greatest neutralising antibody response, and reduced viraemia and virus shedding.

## Discussion

ID vaccination using adjuvant A3 in this study provided protection comparable to IM vaccination and further studies to optimise antigen dose and investigate onset and duration of immunity are planned.

# EFFICACY OF A/MAY/97 FMDV VACCINE AGAINST HETEROLOGOUS CHALLENGE WITH A FIELD VIRUS FROM THE EMERGING A/ASIA/G-VII LINEAGE IN CATTLE

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## Introduction

Since 2015, outbreaks of FMD in the Middle East were increasingly caused by a new emerging viral lineage, A/ASIA/G-VII. In-vitro vaccine matching data indicated that this virus poorly matched with vaccine strains. Previous studies have shown that regardless of a poor antigenic match, high-potency vaccines can protect against heterologous challenge. We investigated whether vaccines available in our vaccine banks could protect against A/ASIA/G-VII. Because A/MAY/97 vaccine gave the best result in pilot-studies, this vaccine was tested in a potency test.

## Materials and methods

Groups of 5 cattle were vaccinated with a full, a 1/3 and a 1/9 dose vaccine. All vaccinated and 3 control cattle were challenged intradermolingually at 21 days post vaccination with A/ASIA/G-VII. After challenge, cattle were monitored for clinical signs and those with FMD lesions on their feet were considered non-protected. The potency of the vaccine was calculated (Spearman Kärber method). Blood samples and swabs (nose, saliva) were collected and tested for presence of virus (RT-PCR, VI). Serum was tested for antibodies to structural (VNT) and non-structural proteins (NSP-ELISA) of FMDV.

## Results

At time of challenge, the VNT-titre of the full dose group was 2.22 (log10) against A/MAY/97, and 1.38 against A/ASIA/G-VII. All cattle from the full, 4 from the 1/3 and 2 from the 1/9 dose groups were clinically protected, resulting in a potency of 6.5 PD50/dose. No viraemia was detected in protected cattle. The amounts of virus detected in swabs of the full and 1/3 dose groups were significantly lower as compared to the controls.

## Discussion

These data provide evidence that a high potency A/MAY/97 vaccine can protect against clinical disease when challenged with a heterologous A/ASIA/G-VII virus, even though in-vitro results predict a poor antigenic match. The extent of cross-protection is probably highly dependent on the quality and antigen load of the vaccine.

# A SIMPLE UNIVERSAL TEST TO QUANTITATE 146S ANTIGEN DURING PRODUCTION OF FMD VACCINES

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## Introduction

Conventional foot-and-mouth disease (FMD) vaccines are produced by the chemical inactivation of virus preparations grown in cell culture. The efficacy of inactivated vaccines is dependent on the presence of intact virus particles, distinguished by their sedimentation of 146S in sucrose gradient centrifugation. Such intact 146S antigen is unstable and can dissociate into capsid subunits (pentamers, 12S) during preparation or storage, resulting in a marked reduction in immunogenicity. Yield and stability of 146S antigen is therefore a crucial parameter for vaccine development and must be optimised for each new vaccine strain. The 'gold standard' in vitro test for assessing 146S particles involves analysis of particle sedimentation in sucrose density gradients. This is a laborious and low throughput method. Immunological reagents that specifically recognise 146S antigen have previously been reported but such reagents are specific for a single serotype of FMDV. Here, we describe the characterization of a 146S-specific monoclonal antibody (5B6) that recognizes all FMDV serotypes and its use in a simple, universal test to quantitate 146S antigen.

## Materials and Methods

The reactivity of monoclonal antibody 5B6 was characterised using ELISA, confocal microscopy and western blot. The 146S test was developed as a sandwich ELISA using recombinant bovine integrin to capture FMDV particles and 5B6 as a 146S specific detector. Preparations of FMDV antigens of various serotypes were used to evaluate the test, including parallel testing of samples before and after heating to induce complete dissociation of antigen.

## Results

The monoclonal antibody reacted with virus of all serotypes. The sandwich ELISA has specificity for 146S of all serotypes tested.

## Discussion

In summary, we have developed  a pan serotypic detection system specific for 146S particles. This has potential to be further validated as a high throughput test for quantitation of FMDV 146S particles during vaccine manufacture and quality control.

# IMPROVING THE DURATION OF IMMUNITY FOR FMD VACCINES

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## Introduction

Chemically inactivated, oil adjuvanted FMD vaccines are a critical element in FMD control in developing countries. Although these vaccines are effective in pigs and ruminants, protective immunity is not reached quickly, is short-lived (~3 months) and is serotype- and sometimes strain-specific. More appropriate vaccine strains that induce broader protection, together with identification of novel adjuvants that provide a greater duration of immunity and simplified methods to measure vaccine quality would make a significant contribution to FMD control and to livestock development in developing countries.Oil adjuvant vaccines induce variable T cell responses, whilst novel adjuvants can prime greater and more consistent T cell and humoral responses that may give longer duration of protection.

## Materials and methods

In our CIDLID funded grant, we had selected 8 new adjuvants as potent immune enhancers, including ligands for TLR receptors that enhanced Th1 priming in various human or animal vaccines. The aim was to supplement the oil component of the adjuvant with a novel immunostimulant that impacts on TLR or related signaling pathways. These eight new adjuvanted vaccines were tested in a pilot study in cattle at IIL, India. The four most efficacious ones (MPLA, Poly I:C, Abisco 300 and R848) were retested for Serotype A in a larger number of cattle at Pirbright, UK. The vaccinated cattle were challenged on 21 days post-vaccination. The most efficacious adjuvant, poly: I:C, tested further in cattle for serotype O FMD vaccine for 7.5 months to assess its impact on the duration of immunity.

## Results and Discussion

The enhanced humoral and cellular responses were observed by incorporating poly I:C in FMD vaccine that increased the duration of immunity in comparison to the conventional oil adjuvant vaccine . Therefore we conclude that there is a measurable T cell component to vaccine-induced protection in addition to humoral antibody component and strengthening this would improve efficacy and duration of immunity.

# THE USE OF REVERSE GENETICS TO FACILITATE THE GROWTH OF FMDV FOR THE PRODUCTION OF VACCINES

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## Introduction

FMD vaccines are chemically-inactivated virus preparations produced in BHK-21 mammalian cell culture. Since FMDV exists as a number of constantly evolving serotypes, there is a periodic need to produce new vaccines against emerging strains. However, field viruses typically show poor growth in cell culture, and require serial passage to adapt. Adaptation to cell culture often involves selection of variants with altered receptor specificity that are no longer dependent upon integrin receptors for infection.

## Materials and Methods

We have used reverse genetics to produce recombinant FMDVs from the four most prevalent FMDV serotypes (Type O, A, Asia-1 and SAT2), carrying defined mutations in the capsid designed to improve growth in cell culture without the need for cell culture adaptation. The capsid encoding sequence, containing targeted mutations to confer cell culture adaptation, was introduced in to an existing reverse genetics system. Recombinant viruses were rescued by transfection of plasmid derived RNA into cell cultures, and deep sequenced. Growth of viruses carrying wild type and mutated capsids was compared, both in adherent and suspension BHK cells.

## Results

In all four serotypes we were able to identify virus variants with targeted capsid mutations which showed improved growth relative to wild type, either in terms of increased speed of growth, improved titre/yield, or both. We have also used antisera in virus neutralisation assays to compare the antigenicity of the viruses.

## Discussion

In summary, we have used a reverse genetics approach to introduce targeted capsid changes to FMDV field viruses to improve growth in cell culture, without the need for adaptation by cell culture passage. This approach could greatly speed up the production of FMDV vaccine viruses from new field strains, by reducing the need for cell culture adaptation by passage and reducing the testing required for extraneous agents, since virus is produced recombinantly.

# MULTIPLEX REAL-TIME RT-PCR FOR DETECTION OF FMDV, RIFT VALLEY FEVER VIRUS AND BOVINE VIRAL DIARRHEA VIRUS IN BULK TANK MILK

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## Introduction

Dairy cows shed large amounts of FMDV in milk, even before clinical signs appear. This constitutes a considerable risk of transmission within and between farms, but also creates an opportunity for early detection of virus introduction using an easily available sample. The first goal of the project was to show that a previously published multiplex RT-qPCR assay (Wernike et al., 2015, J Virol Methods 217:28-35) can reliably detect FMDV, RVFV and BVDV in milk.

## Materials and Methods

RNA extraction from spiked milk by silica membrane spin columns alone or by a combination of TRIzol LS and columns was evaluated. Using RNA of BVDV-1d strain 2017BVD02027, RVFV MP-12 and FMDV A IRN/22/2015, plates with a constant high concentration of RNA of one virus overlaid with orthogonal serial dilutions of RNA of the other two viruses were set up to test for competition between assays. The performance of each assay individually and in combination was compared as well.

## Results

Combined TRIzol/RNeasy extraction gave the most reproducible results. The RT-qPCR assays were not significantly inhibited by concurrent amplification of other viral targets. There was minimal inhibition of the BVDV assay in the presence of high amounts of FMDV or RVFV, but this did not affect the qualitative read-out. Concordance between the Cq values obtained with individual assays and those obtained with the multiplexed assay was very good across several dilutions.

## Discussion

The multiplex RT-qPCR can reliably detect FMDV, RVFV and BVDV in milk, with no cross-reactions, minimal competition between the targets and only marginally reduced sensitivity compared to individual assays. The assay is now fully validated and ready to be used with field samples. A large panel of milk samples from Kenya is currently being tested and the results will be presented at the Open Session.

# EMERGENCY SUPPLY OF FMD DIAGNOSTIC KITS: REAGENT BANKS AND IDVET SOLUTIONS

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*IDvet, France*

## Introduction

Foot-and-mouth disease (FMD) is an economically important disease of livestock.
As FMD significantly constrains trade in animals and animal products, most FMD-free countries invest important resources to prevent and prepare for possible incursions. Vaccine banks have been established either at multinational or national level to enable rapid implementation of emergency vaccination in the event of an outbreak
Availability of diagnostics is also crucial to the management of an outbreak, both to monitor for virus spread with non-structural (NSP) tests, or to perform post-vaccination monitoring with structural protein (SP) ELISAs.
Due to financial constraints, however, some countries have reduced their contingency plans, increasing the potential impact of an FMD outbreak, or would be willing to reduce its cost.

## M&M

IDvet offers a range of FMD NDP and SP ELISA kits.
Kits and reagent banks are mainly of two types. The first type consists of reserves of quality controlled ELISA kits, ready for immediate use but with limited shelf life. The second type consists of reserves of master bank solutions with long shelf life, which may be assembled into ELISA kits as required.
IDvet will present additional options for kit or reagents banks, and advantages and limits of each option will be discussed.
IDvet offers up to 5 different solutions for personalized storage of kits/reagents to be shipped worldwide as kits or in a bulk format.

## Conclusion

The rapid supply of diagnostic kits in the event of an FMD outbreak is essential to limiting the sanitary and financial impact of an outbreak. IDvet offers flexible and customized solutions to meet contingency requirements and to ensure that reagent supply is sufficient, economical, and on-time.

# RESULTS FROM A INTER-LABORATORY EXERCISE TO EVALUATE NON-STRUCTURAL PROTEIN ELISA KITS

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## Introduction

Serological assays used to detect the presence of antibodies to Non-Structural Proteins (NSP) of foot-and-mouth disease virus (FMDV) are used for disease surveillance in endemic countries, and are essential to determine the 'status' of a country after an FMD outbreak, proving freedom of the disease with or without vaccination. The purpose of this inter-laboratory concordance study was to assess whether two commercially available NSP ELISA's have broadly equivalent performance; focussing on the ID.Vet NSP ELISA (single day and overnight protocols) and the PrioCHECK NSP ELISA.

## Method and Results

The serum panel consisted of 90 negative sera (from an FMDV free country) and 90 experimentally infected sera (a minimum of 8 dpi) from cattle, sheep and pigs. Two blind-coded sample panels were dispatched to 5 ISO17025 accredited European laboratories, where they were tested in duplicate by separate operators (using ID.Vet single day, overnight and PrioCHECK NSP ELISA's protocols). There was a 95.5% concordance among the 10 operators for both kits. However, six samples generated false positive results on all assays by all operators. These were unexpected results and further studies indicated that heat inactivation (56oC for 30 minutes) after long term storage at ambient temperature is necessary to prevent these type of false positive results.

## Discussion

These results support the idea that these two commercial assays have equivalent performance for the detection of FMDV NSP-specific antibodies, and provide laboratories with validation data to accredit alternative assays for routine diagnostic purposes; hopefully mitigating any potential supply difficulties that may arise during an outbreak. The authors take this opportunity to thank all the scientists who contributed to this study.

# RESULTS OF THE 2016 AND 2017 PROFICIENCY TESTING SCHEMES FOR FMD DIAGNOSTIC METHODS

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## Introduction

The Pirbright Institute as the OIE, FAO and European Union Reference Laboratory for Foot-and-Mouth Disease (FMD) carries out an annual proficiency testing scheme (PTS) for laboratories. This exercise is used to demonstrate equivalent performance of diagnostic tests used by FMD International and National Reference laboratories. This presentation summarises the results for the 2016 (Phase XXIX) and 2017 (Phase XXX) exercises and highlights some of the common difficulties that laboratories face in diagnosing FMD.

## Materials and Methods

During 2016 and 2017, FMD virological ("live" and inactivated FMDV) and serological panels were made available to laboratories. The particular diagnostic methods that the laboratories utilise were not specified; rather, it was up to each laboratory to select the most appropriate tests using outbreak scenarios that accompanied that samples. For each year, an additional panel was provided to assess whether diagnostic methods can identify the FMDV lineages that are currently circulating. Overall status of each sample was required as well as overall "case" interpretations.

## Results

PTS sample panels were sent to 70 laboratories in 2016 and 72 laboratories in 2017. Laboratory performance continues to improve particularly with virological panels; however, technicians continue to encounter serotype cross-reactivity on serological assays and this often leads to mistyping of serum samples.

## Discussion

A further PTS for 2018 (Phase XXXI) supported by funding from the EU and FAO/EuFMD is being planned. Please note that we propose to divide the serological panel (Panel 3) into two separate panels: to test (i) outbreak scenarios and (ii) FMDV serotype specificity.

POSTER

# FMDV ADSORBED TO GENOTUBE SWABS REMAINS INFECTIOUS AT HIGH TEMPERATURE

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## Introduction

Self-drying foam swabs (GenoTube, Thermo Fisher Scientific) have been successfully used to collect classical and African swine fever samples (Petrov et al., 2014, Vet Microbiol 173[3-4]:360-5). We considered them as an alternative method for the safe transport of FMDV samples from endemic areas to diagnostic laboratories in free regions. The first part of the project studied the heat inactivation of FMDV in these swabs.

## Materials and Methods

GenoTubes were dipped in FMDV A IRN/8/2015, dried and then incubated for 2 hours in a forced-air oven preheated to 100°C. A second set was kept at room temperature. To assess the time required for heat transfer to the swab, a thermocouple was placed inside a sealed swab tube that was also placed in the oven. After heating, the foam tip of each swab was washed with culture media. The eluate was titrated on LFBK-αVα6 cells. Cytopathic effect on the titration plates was evaluated microscopically and confirmed by antigen ELISA.

## Results

It took under 20 minutes for the interior of the swab tube to reach 100°C. On average, virus titers in eluate from non-heated GenoTubes were reduced by 1.2 log10 relative to the original suspension. Virus eluted from heated swabs had a titer of 2.7 log10 TCID50/100 µl, a 2.9 log10 reduction compared to the non-heated swabs.

## Discussion

High-heat treatment of GenoTube swabs does not inactivate FMDV. Samples from suspect cases must be shipped as infectious substances (UN 3373). However, the high resilience of FMDV adsorbed to the swabs suggests that they can be useful for the shipment of infectious virus. Sending infectious FMDV at room temperature avoids the effort and costs of dry ice shipping. It is now being investigated how long FMDV adsorbed to GenoTube swabs remains infectious at room temperature. The results will be presented at the Open Session.

POSTER

# COMPARATIVE PERFORMANCE OF MONOCLONAL AND POLYCLONAL-BASED ANTIGEN ELISAS FOR FMDV DETECTION

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## Introduction

Serotyping assays are integral for the detection and characterisation of foot-and-mouth disease virus (FMDV). The WRLFMD currently uses a rabbit/guinea-pig polyclonal antibody-based indirect sandwich ELISA (PAb ELISA) to serotype diagnostic submissions. In recent years, the number of samples that could not be typed with this assay has increased. Thus, the IZSLER/Pirbright kit based on monoclonal antibodies (MAb ELISA) was validated with the view to include in the WRLFMD portfolio of ISO/IEC 17025 tests.

## Methods

The serotypes common between both assays and therefore selected for testing were O, A, C, Asia1, SAT1 and SAT2. The MAb ELISA sensitivity was evaluated with FMDV isolates that represent serotypes, topotypes and lineages circulating between 1964 and 2018, representing all seven serotypes O (n=99), A (n=52), C (n=5), Asia1 (n=22), SAT1 (n=23) and SAT2 (n=52). The MAb ELISA limit of detection was evaluated using isolates and original epithelium suspension. The results obtained were compared with those reported by the WRLFMD using the PAb ELISA. The MAb ELISA was also evaluated with isolates that gave borderline or negative results on the PAb ELISA O (n=60), A (n=18), Asia1 (n=1) and SAT2 (n=1). Lastly, the MAb ELISA specificity was assessed with viruses that cause similar clinical signs to FMD.

## Results

Overall, there was good concordance between the assays; however, the MAb ELISA demonstrated an improved sensitivity with both isolates and original suspension. The MAb assay detected all isolates missed by the PAb ELISA, apart from two recent O/CATHAY samples. The pan-FMD test included in the MAb ELISA detected all type O, A, C and Asia1 isolates, but demonstrated reduced sensitivity for the SATs. No cross-reactivity was observed with other vesicular disease viruses.

## Discussion

The MAb ELISA is a simple and robust kit for serotyping with an overall sensitivity of 90% compared to 83% for the PAb ELISA.

POSTER

# INACTIVATION OF FOMDV IN TISSUE SAMPLES TO ENSURE SAFE TRANSPORT FROM INFECTED PREMISES TO DIAGNOSTIC LABORATORIES

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## Introduction

During an outbreak in an FMD-free country, provincial/state labs will handle samples, including epithelium from suspect lesions. Such samples present a biosafety risk. The objectives of this project were to test the ability of RNA preservation reagents to inactivate FMDV in epithelium samples and ensure suitability of the FMDV RNA for RT-qPCR, sequencing, and recovery of infectious virus by transfection.

## Materials and methods

Lesion material was recovered from cattle infected with FMDV A IRN/22/2015 or O ALG/3/2014. Pieces of lesion epithelium were placed in 3 ml of either RNAlater, RNAShield or phosphate-buffered saline and incubated at RT for 2, 6, 24 or 48 h. After incubation, tissues were homogenised and used for virus isolation (VI) and RNA extraction. VI-positive samples were titrated, and extracted viral RNA was quantified by RT-qPCR, used for sequencing and transfected into $LFBK_{\alpha V\beta 6}$ cells to recover virus.

## Results

RNAlater did not reduce serotype A virus titres after 2 or 6 h, however a 4 log10 reduction was seen after 24 h, and no infectious virus was recovered after 48 h incubation. While serotype O virus was detected following VI after 2, 6 and 24 h, titration yielded no infectious virus. RNA loads were slightly reduced, particularly after 24 and 48 h. RNAshield was toxic to cells at high concentrations but was effective at inactivating both serotypes. A significant reduction of detectable viral RNA was observed in samples after 2 or 6 h incubation, but not following longer incubation periods. Sequencing and transfection of FMDV RNA and recovery of infectious virus were possible for both serotypes, regardless of reagent used or inactivation period.

## Discussion

Of the two reagents tested, RNAShield appears a better choice for inactivation of FMDV in tissue samples, however at least 24 h incubation is recommended before processing to ensure virus inactivation and preservation of the majority of viral RNA.

## DETECTION OF EARLY FMD VIRUS INFECTION IN PIGS USING IgA AND IgM ASAAYS

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### Introduction

The 2001 outbreak in the UK showed that strict movement controls combined with the stamping out of infected and contact animals are not always sufficient to eradicate FMD quickly, have high economic costs and cause great public alarm. In future, a policy of vaccinate-to-live may be included for pigs as in cattle. Pigs are included for vaccination in China and Southeast Asia in the repertoire of control measures and in support of this approach, we have investigated the early detection of IgM and IgA antibodies in the experimental and field outbreak pigs.

### Materials and Methods

As presented in past EU FMD open sessions we have developed FMD virus (FMDV) specific IgM and IgA assays for pigs. Further the assay has been evaluated with the replacement of FMD empty capsids with inactivated antigen of serotype O, A and Asia1 for 300 vaccinated infected sera and, nasal/mouth swabs and 200 naïve sera/swab samples from pigs.

### Results and discussion

The Infection was detected within two to three days of infection by both the assays. The assay has more than 90 and 99% sensitivity and specificity respectively. The assays are comparable to the other serological tests including NSP and PCR. Further details of FMDV detection will be presented in full.

POSTER

## PRODUCTION OF SWINE SEROLOGICAL PANEL FOR THE VALIDATION OF FMD ANTIBODY TESTS

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### Introduction

South Korea has been suffered foot-and-mouth disease (FMD) epidemics since 2000. One of the major changes in FMD control policy in last decade was introduction of FMD vaccines.

All susceptible animals are subjected to FMD vaccination. Thus, both SP and NSP antibody detecting assays are performed as a part of National FMD sero-surveillance program. Here we described the production of swine sera panel to validate FMD serological assays and characterized its reactivity in NSP Ab assays.

## Materials and methods

Thirty-seven conventional pigs at various status of vaccine-induced antibody level were challenged with serotype O FMD virus. To maximize the blood collection, number of pigs was sacrificed on scheduled date as early as 2 days post challenge (dpc) to 30 dpc. Final swine sera panel was consisted of total 35 heat-inactivated swine sera, each in 450~600ml. Two commercial NSP ELISAs and one lateral flow device (LFD) test were used to define the status of NSP antibodies.

## Results

Out of 35 sera, 24 sera were consistently negative or positive in all three NSP assays. Among the 12 positive sera, 10 of them were SP O antibody positive before FMD infection. All three tests detected first positive in 10 dpc. Rest 11 sera at various dpc showed conflicted results between assays; 5 out of 11 were positive in LFD but negative in both ELISAs while 6 did not coincide between the ELISAs.

## Conclusion

As the importance of serological diagnosis grew more in FMD control, application of validated serological assay is critical and well-defined serological panels, originated in various species, are required for the validation. However, FMD infected field serological samples are scarce, especially the ones from swine that we have developed panel of swine sera for the purpose of validation of NSP ELISAs.

# THE INTERDEPENDENCE OF FMDV PATHOGENESIS, CHALLENGE SYSTEM, AND OUTCOME OF VACCINE STUDIES

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## Introduction

The standard procedure for FMDV vaccine testing in cattle involves challenge through virus injection into the tongue epithelium. This approach provides a stringent test of the ability of vaccine-induced immunity to prevent generalized infection. However, accumulated scientific evidence have demonstrated that the outcome of vaccine trials may vary based upon experimental design, including route and timing of challenge. Specifically, the use of natural challenge systems may substantially affect the occurrence of subclinical and persistent infection in vaccinated cattle. This suggests that it is critical to consider experimental design, when interpreting the outcome of FMDV vaccine trials.

## Materials and Methods

Accumulated data from studies of FMDV pathogenesis in vaccinated and naïve cattle were analyzed in combination with previously published works, with specific attention to the occurrence of subclinical and persistent infection in relation to use of different challenge systems.

## Results

Studies based on natural and simulated-natural FMDV exposure systems demonstrated a high prevalence of subclinical and persistent FMDV infection in vaccinated cattle, despite complete protection against clinical FMD. However, tongue-inoculation of vaccinated cattle substantially reduced the occurrence of persistent infection.

## Discussion

Studies of FMDV pathogenesis in cattle have demonstrated that the bovine nasopharynx is a unique anatomic site for both primary and persistent infection. Virus exposure of the bovine upper respiratory tract is thus a critical component of FMDV pathogenesis under natural conditions. It is likely that strong vaccine-induced immunity may prevent exposure of the nasopharynx when virus challenge is performed by tongue inoculation. In contrast, natural exposure conditions may facilitate subclinical infection of the upper respiratory tract leading to higher prevalence of persistent infection, despite clinical protection. Thus, although tongue inoculation is useful as a standardized approach to FMDV vaccine testing, it is important that intrinsic aspects of FMDV pathogenesis are considered in the interpretation of experimental outcomes.

# GOOD CORRELATION BETWEEN VACCINE MATCH IN POTENCY TESTS AND r1-VALUE

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## Introduction

The choice of FMDV vaccine is often based on r1-value. This is based on the fact that antibody titres correlate strongly with protection. However the optimal measure for vaccine match would be the heterologous potency of a vaccine divided by the homologous potency of a vaccine.

*Heterologous potency = match x homologous potency.*

If the homologous potency is high, e.g. 24 PD50/dose the vaccine can be used for strains with a match (potency ratio) of 0.13 to obtain 3 PD50/dose in the field. The objective of the current study is to review published cross-protection studies and to evaluate if the observed vaccine match correlates with the observed r1-value

## Materials and Methods

Using Scopus and known references to (conference) papers that describe quantitatively cross-protection studies were selected and included. The r1-value was either calculated from reported VNT titres, or based on results in the WRL in Pirbright. The potency ratio was based on the homologous and heterologous potency reported in the paper, or when not present in the paper it was recalculated.

## Results

A total of 15 studies were found, but in some studies the homologous potency or r1-value was not reported, so in total 12 studies could be included. In 8 studies the homologous potency was > 32 PD50/dose, in that case this upper value was used. In 11 of the 12 included studies there was a significant correlation (R-squared: 0.57) between potency ration and r1-value. In one study the r1-value was much higher (0.63) than the potency ratio (0.04).

## Discussion

The review of the literature produced limited number of cross-protection studies with FMDV vaccine. But there might be more studies performed than reported, and probably there is publication bias. In many studies estimates of the relation between heterologous potency and homologous potency correlate well, but there are also exceptions. It is not always clear if this is due to the variability of the potency test or differences between FMDV strain.

# POTENCY ASSESSMENT OF FMD VACCINES USING STANDARDISED SEROLOGICAL ASSAYS

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## Introduction

Many studies have been performed on the correlation between vaccine-induced antibodies and protection after FMD vaccination. Methodology used in these studies was not the same. This study aimed to standardise antibody titre against O/Manisa for determination of vaccine potency in two different ways; by using a standardised commercial type PrioCHECK® FMDV Type O ELISA and by inclusion of a standard 4 week post-vaccination serum from a cow vaccinated with Cedivac® O Manisa FMD vaccine using ELISA and VNT.

## Materials and methods

Sera were available from O/Manisa potency tests performed in the FMD laboratories in Lelystad, Brussels and Pirbright. Sera were titrated in the respective laboratories using PrioCHECK® FMDV Type O ELISA and VNT. In each test, standard serum was included. Standardised antibody titres were calculated. Titres of the control serum were compared. Serological responses were fitted by logistic regression. In each analysis, the contribution of the laboratory performing the tests was included if this resulted in a better fitting model.

## Results and discussions

Significant differences ($p<0.05$) were found in the titre of the control serum tested in the three laboratories, in both the ELISA and VNT. Only a small difference was found in the mean titre in protected and non-protected cattle.

In both the ELISA and VNT, a very significant ($p<0.01$) influence of the laboratory was found on the correlation between antibodies titres, but also between standardised titres, and protection. In the ELISA, slope the correlation between antibodies and protection was lower when titres of the sera collected at day 21 post-challenge were analysed in comparison with the titres found on the day of challenge (for experiments performed in Brussels and Pirbright, these were the same). The slope was steeper when analysing the results obtained in VNT compared to ELISA.

Inclusion of the standard serum reduced variation between the laboratories. Still significant differences were present. Inclusion of a standard serum is a good way to make results between laboratories more comparable.

POSTER

# INSIGHTS AND OPTIMISATION OF THE FMD VIRUS NEUTRALISATION TEST FOR R1 ANTIGENIC MATCHING

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## Introduction

Vaccination is one of the most important interventions in foot-and-mouth disease virus (FMDV) outbreak prevention and control. Antigenic matching between vaccine and outbreak virus is critical for vaccination effectiveness and is usually determined using serological assays, such as virus neutralisation tests (VNTs). The ratio between the titer of vaccine serum against a field virus and the titer of vaccine serum against the vaccine virus (r1) is calculated. An r1 value <0.3 is supposed to be predictive of a vaccine mismatch. However, while the r1 value is generally used as indicator for protection, its reliability is limited, it has high variance and substantial variation between laboratories exists. Currently, there is no reliable in vitro alternative for r1. Therefore, there is a need to optimize the current assay as well as develop novel assays.

## Materials and methods

At Boehringer Ingelheim Animal Health an r1 VNT protocol had been developed using publicly available IBRS-2 cells (FLI), and reagents and disposables which are commercially available to enable assay harmonisation between laboratories. This assay has been validated and the intrinsic test variance was determined. Thereby the effect of repeating the assay and performing the assay in 1D or 2D was examined.

## Results

This VNT has a low variance, but r1 variance is still high. Repeating the assay increased confidence and use of strict cell culture protocols did reduce the assay variance. Performing the assay in 1D instead of 2D gives similar variance, while enabling the performance of repeats.

## Discussion

These studies have led to more insight in r1 variability and to what extend it can be reduced. A VNT protocol for FMDV antigenic matching has been created, enabling assay harmonisation between laboratories. This assay could contribute to more reliable vaccine matching, however, research on novel assays enabling more predictable vaccine matching is still required.

POSTER

# USE OF SEROLOGICAL TESTS FOR CHECKING NSP PURITY OF FMD VACCINES

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## Introduction

Serosurveillance of FMD vaccinated livestock can help determine rates or absence of infection but viral non-structural proteins (NSP) must be largely removed from the vaccine during manufacture, so that only infection and not vaccination induces NSP antibodies. The specificity of NSP antibody tests for detecting infected animals can be >99%, but reduced after vaccination depending upon vaccine purity and number of doses. Even small proportions of vaccine-induced NSP reactors hamper interpretation of large-scale serosurveys, especially for substantiating freedom from infection.

The OIE Manual requires manufacturers to support vaccine purity claims by demonstrating lack of immunogenicity against NSPs, with a proposed schedule for booster vaccinating and testing 8 cattle, with batch rejection if >2 animals become seroreactive. The OIE methodology has been adapted for use in vaccine quality control in Brazil and Argentina.

## Materials and Methods

An online binomial calculator was used to estimate probabilities of rejecting/accepting vaccine batches inducing NSP seroreactor rates of 1%, 5% and 10%, after purity testing according to the OIE, Brazil and Argentina methods.

## Results

Using the OIE method, after testing 9 batches of vaccine, the probability of rejection for a vaccine inducing a 5% rate of NSP seroreactors is <20%. For a single batch inducing 10% NSP seroreactors, the rejection probability remains <15%. The Brazilian and Argentinian methods are more stringent, but have low chances (<15% and <60%) of rejecting a batch inducing 5% NSP seroreaction.

## Discussion

The sensitivity of the analysed purity testing approaches are variable but inadequate to detect small NSP seroreactor rates induced by vaccination prior to vaccine supply. Larger sample sizes are needed to estimate vaccine-induced seroreator rates reliably. This may be achieved after vaccinating whole populations by careful stratification of serosurveillance findings.

POSTER

# WHAT CAN WE SAY? HARMONY OR DISHARMONY BETWEEN VACCINE MATCHING AND CHALLENGE STUDY

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## Introduction

The struggle and control policies of countries against FMD vary according to their geographical location, economies, disease prevalence and community awareness levels. In Turkey the program of FMD control mainly based on vaccination. Thrace Region was accepted as FMD free zone with vaccination by World Organization for Animal Health (OIE), in 2010. However in Anatolia, the endemic situation continues. Due to incursion of exotic strains and high mutation rate, FMDV continues to evolve. So, it affects the vaccine effectiveness and costs.

## Materials and methods

In this study, the protection levels (r1 value) of four vaccine strains of the serotype A produced by FMD Institute were investigated. The vaccine strains were belonging to A/Asia/GVII lineage (ANep84, ATUR16, ATUR17) and A/Asia/IRN05 (ATUR06). Results were obtained by using in vitro and in vivo methods; VNT for r1 value and challenge studies, respectively. In challenge studies 42 cattle were used.

## Results

There were poor antigenic relationship detected between A/Asia/IRN05ATUR06-A/Asia/GVIIANep84 and A/Asia/GVIIATUR17-A/Asia/GVIIATUR16 in vitro. A/Asia/GVIIATUR16-A/Asia/GVIIATUR17 r1 value was detected as almost 0,3 (0,27) and the rest of four tests were showed protection (r1-values ≥0,3). After challenge, lesions were occurred on tongue (inoculation site) in all animals. Most severe clinical signs including back hoof were seen in the challenge study with A/Asia/IRN05ATUR06. Lesions on the palate were seen on the A/Asia/GVIIATUR17 challenge study, finally there were no lesions occurred except inoculation sites on the other three challenge studies which A/Asia/GVIIANep84 and A/Asia/GVIIATUR16 were used.

## Discussion

The findings of A/Asia/GVIIATUR17-A/Asia/GVIIATUR16 challenge study which has got less antigenic difference between two strains showed that in vitro and in vivo studies are not always overlapped while considering the vaccine effectiveness. It can be concluded that in accordance with the other studies, there is a need for more effective method concordant with the challenge study results.

## EuFMDiS

## Demonstration

*K. Mintiens*

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The EuFMDiS model is a sophisticated decision support tool.
It simulates spread and control of FMD in Europe and can estimate the cost of an FMD outbreak.

EuFMDiS will help countries to evaluate control policies, improve contingency plans and design simulation exercises.

*The pilot project was funded by EuFMD FAR (Funds for Applied Research) and involved seven pilot countries (Austria, Bulgaria, Croatia, Hungary, Italy, Romania, Slovenia).*

## Debate

The value of the EUFMDis model is debated. Upgrades and extensions and of the models is discussed amongst public and private stakeholders representatives and academia. Also the audience is invited to share ideas.

# DETECTION OF FMFV O/ME-SA/IND-2001E IN JORDAN

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## Introduction

Foot-and-mouth disease (FMD) is a highly contagious vesicular disease that is caused by FMD virus (FMDV). This disease affects both wild and domestic cloven-hoofed animals. FMD is endemic to Jordan and has a severe impact on the productivity of domestic livestock. In January of 2017, FMD outbreaks were detected in different animal species across Jordan, resulting in high mortality rates among young lamb and goat populations and in the classic FMD symptoms in cattle.

## Materials and methods

Jordanian veterinary authorities were notified through their field-based veterinarians about FMD outbreaks across Jordan. Tissue samples from recently deceased lambs were obtained for both molecular and histopathological examinations. Viral RNA extraction was performed followed by nested RT-PCR for the VP1 gene. The PCR products were sequenced by Sanger sequencing and sequences were aligned using BioEdit and subjected to evolutionary analyses using MEGA7.

## Results

The FMD outbreak started on January 25th, 2017 and ended on March 18th, 2017. The total number of affected farms was 55 in total and included 19 cattle, 26 sheep, 4 goat, and 6 mixed (sheep and goat) farms. The results obtained from sequencing the VP1 gene place this FMDV strain within the newly established FMDV/O/ME-SA/Ind-2001e sublineage. The FMDV/O/ME-SA/Ind-2001e sublineage comprises sublineage Ind-2001d viruses that were isolated between 2015 and 2017 and clustered into a separate sublineage known as Ind-2001e.

## Discussion

During this period, viruses of the FMDV/O/ME-SA/Ind-2001e sublineage have spread to the Middle East, North Africa and Southeast Asia. The nucleotide sequence of the V1 gene of the O/JOR/1/2017 sublineage is very similar to that of viruses isolated from Saudi Arabia in 2016, indicating possible introduction of this strain to Jordan through animal movement or other

transmission routes from Saudi Arabia.

## THE ASSOCIATION BETWEEN BORDER PROVINCES OF TURKEY AND FMD OUTBREAKS

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### Introduction

Foot-and-Mouth Disease (FMD) which is endemic in the Anatolian region of Turkey, affects susceptible livestock like cattle, sheep and pigs, with a socio-economic importance, is an important viral contagious disease. Globalization-related factors such as movements of animal, animal products and human, and interaction between domestic and wildlife populations, increase the risk of FMD virus spread. Trading of animal and animal products varies depending on environmental and ecological factors such as drought and pasture availability, and changes in export and meat demand, including religious festivals and national celebrations. In this sense, illegal animal movements and animal trade between neighboring countries, as well as local animal movements and common pasture are important in spreading of FMD.

The objectives of this study are to (1) statistically identify the association between 23 border provinces of Turkey which are categorized as "North East", "East" and "South" and located next to at least one of the six eastern neighbour countries and FMD outbreaks, (2) determine the difference between the risk of FMD from the western part of the country and the east, excluding the Thrace region, and (3) determine the effect of the geographical region on FMD outbreaks.

### Materials and methods

This will be a retrospective register-based observational study. The data of the World Organization for Animal Health (OIE)-World Animal Health Information System (WAHIS) database between 2008 and 2017 will be used and the data will be analysed by using logistic regression with R software version 3.2.1.

### Results

The results of this study will be presented in the OS18.

### Discussion

The discussion will be presented in the OS18.

## INTEGRATED RISK-BASED STRATEGIC PLANS FOR FIVE PRIORITY DISEASES IN THE PALESTINIAN AUTHORITY

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### Introduction

Risk-based control strategies for five priority animal diseases (PDs) were developed at the request of the Palestinian Authority veterinary services and with the support of the Food and Agriculture Organization of the United Nations.

### Materials and methods

Over two years (2015-2017), with seven in-country workshops and online support, risk-based strategic plans (RBSPs) were developed following the approach advocated under the Progressive Control Pathway for FMD control (PCP-FMD). A prioritization exercise was undertaken to identify the PDs. A taskforce was established for each PD. Each taskforce analysed the current situation, identifying risk hotspots and gaps. Subsequently, using a framework of considering a) surveillance, b) outbreak response and c) prevention, they defined strategic objectives, component objectives, tactics and activities. Finally, monitoring and evaluation (M&E) indicators, targets and means of verification were established.

### Results

Bluetongue, lumpy skin disease, scrapie, peste des petits ruminants and salmonellosis in poultry were identified as the PDs. Despite the different species and modes of transmission involved, substantial aspects of each control plan overlapped with the others. Thus, the disease-specific RBSPs were complemented by an overarching chapter addressing issues related to capacity building of the veterinary services, private and public stakeholder engagement and M&E.

### Discussion

The development and implementation of RBSPs are intended to ensure effective use of limited resources for animal disease control. As substantial aspects of disease control relate to overarching issues, an integrated approach including multiple priority diseases is useful and efficient.

### Key words

Progressive Control Pathway for FMD control (PCP-FMD), risk-based, integrated disease control.

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## EMBEDDING PROGRESSIVE CONTROL FOR FMD IN THE POLICY AGENDA FOR LIVESTOCK PRODUCTION IN THREE COUNTRIES IN SOUTHEAST ASIA

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### Introduction

The occurrence and impact of endemic Foot-and-Mouth Disease (FMD) in Southeast Asia (SEA) is impairing income of livestock owners and trade opportunities with China.
As part of the South-East Asia and China Food and Mouth Disease (SEACFMD) Roadmap 2016-2020, the veterinary services of Cambodia, Lao PDR and Myanmar are following the progressive control pathway for FMD control (PCP-FMD). According to PCP-FMD principles, FMD control should be evidence-based, measures are feasible and targeted according to risk, and both the implementation and impact of the control strategy are continuously monitored and evaluated. Such an approach to disease control is often different from that traditionally taken by the veterinary services.

### Material and methods

To ensure that decision makers support the risk-based approach inherent to the PCP-FMD, a policy document was developed that outlined the vision, goals and strategic objective of FMD control over a relatively long-term (10-15 years). Called the 'National Strategy Framework for FMD', it outlined how FMD control would support livestock development and open international trade opportunities. Subsequently, each of the national FMD committees developed a technical risk-based strategy plan. This work was facilitated by the OIE-SRR-SEA, with the help of Animal Health Works, through online webinars, in-country workshops and regional meetings.

### Results

The strategy frameworks have been endorsed by the authorities in each country. FMD control has been recognized as an integral part of the livestock production sector development.

### Discussion

Developing a strategy framework has secured long-term commitment from the respective ministries of agriculture. Concurrently, it is supporting a mind-shift for veterinary services: Progressive FMD control as part of a nationwide policy to improve of livelihoods and livestock production and requiring intense private and public stakeholder engagement.

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## TRANS-POOL MOVMENT OF TWO FMD VIRUS SEROTYPE A LINEAGES: A/ASIA/G-VII AND A/AFRICA/G-IV

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### Introduction

The distribution of FMDV lineages tends to be contained within geographical areas loosely defined as seven regional virus pools. Recently, however, long- distance "trans-pool" FMDV movements have led to the introduction of viruses into new areas both in Asia and Africa. Thus, the A/ASIA/G-VII lineage emerged from the Indian subcontinent in 2015 and entered the Middle East and continues to circulate and spread. Moreover, in Africa, the A/AFRICA/G-IV lineage was reported to cause outbreaks in the Maghreb region in 2017 for the first time for over 35 years.

### Materials and methods

Whole genome sequences (WGS) were obtained from representative samples from recent and historical outbreaks caused by the A/ASIA/G-VII and A/AFRICA/G-IV lineages. These were analysed alongside WRLFMD VP1-coding sequences and publically available sequences belonging to these two lineages.

### Results

A/ASIA/G-VII: Phylogenetic analyses of the VP1-coding and WGS data confirm expansion of the lineage circulation within the Indian subcontinent into countries were serotype A viruses are rarely reported (Bhutan and Nepal). Furthermore, the analysis shows continuation of circulation in the Middle East in areas affected since 2015, and its further spread into new countries in the region (Israel and Jordan).
A/AFRICA/G-IV: Sequence data analyses show grouping of sequences based on geographical

origin into East and West African clusters. These analyses also indicate the origin of the 2017 Algeria and Tunisia outbreaks in West Africa.

### Discussion

The globalisation in livestock trade and the increased access to the global export markets increase the risk of introduction of emerging FMDV lineages into previously unaffected regions. Here, two different lineages within the A serotype are shown to spread outside of their normal distribution adversely affecting and/or becoming established in new geographical areas. This stresses the importance of close and careful surveillance as well as highlights the complexity of application of robust FMDV control measures.

## SOCIO-ECONOMIC IMPACT OF FMD OUTBREAKS AND CONTROL MEASURES AT DIFFERENT SCALES IN MONGOLIA: FROM NATIONAL LEVEL GROSS LOSSES TO HERDERS' FOOD SECURITY

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### Introduction

Mongolia is a large landlocked country in central Asia. Livestock is mainly kept by nomadic herders which is their main source of food and income. Since January 2017, reported FMD outbreaks have considerably increased compared with previous years. The current control policy consists of vaccination, modify stamping out and movement control.

This study aims to estimate (i) the socio-economic impact of FMD and the control measures on herders and (ii) the national gross economic losses due to reaction and expenditure during 2017.

### Material and Methods

From each FMD affected Province, 10 herders affected by FMD and 5 herders not affected but within quarantine areas were randomly selected and data were collected using a standardised questionnaire. National level gross losses due to reaction and expenditure in 2017 were based on governmental data in a deterministic model.

## Results

Data were collected from 112 herders (70 affected and 42 within the control zone). The average attack rate was 45.4%, 16.4% and 4.6 in cattle, sheep and goats respectively. There was incongruity between number of animals affected and culled in 14 (12%) herds consistent with government surveillance data. Overall, 86 (76.8%) herders reported that did not drink milk for a period of time (average 46 days) and 19 (17.0%) did not eat meat for a period of time (average 10 days). Furthermore, 55 herders (49.1%) had to borrow money to buy food, buy medicines and/or pay bills or bank loans. The current estimate of gross economic loss at national level was US$10 million USD although analysis is ongoing.

## Discussion

Further economic analysis is needed to estimate the full impact and evaluate the benefits of interventions. However, the current control policy has negatively impacted herders' livelihoods by generating extra expenses, increasing debt and compromising food security with implications for stakeholder advocacy.

POSTER

# REGIONAL COOPERATION BETWEEN TRANSCAUCASIA AND NEIGHBOURING COUNTRIES ON PREVENTION AND CONTROL OF FMD

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## Introduction

The Transcaucasia region is constantly exposed to the risk of incursion of new FMD strains which can represent a high risk of epizootic development with significant economic impact. The veterinary services of Armenia, Azerbaijan, Georgia, Iran, Turkey and the Russian Federation agreed on a common vision for the intensified collaboration in the prevention and control of FMD and other TADs in the Transcaucasus and neighbouring territories.

## Material and methods

The collaboration is aimed to facilitate the sharing of risk information between neighbou-

ring countries and enhance collaboration and cooperation for improving the FMD control in the area. The agreement between the countries is focused on specific aspects: -Sharing of information on vaccination programmes and outbreaks of disease; - Co-operation in activities aimed to build confidence in the effectiveness of control programmes in the region; - Reduce the risk of epidemics of TADs in the region through planning and implementation of programmes aimed to progressively reduce the circulation of infection and the impact of new incursions.

### Results

An improved system for immediate as well as monthly reporting of the FMD outbreaks in the Trans Caucasus and neighbouring territories has been developed through an improved on-line system and mapping tool. A new online system for collection and sharing the monthly reporting of the level of implementation of the vaccination programmes, with improved visualisation mapping tool has been developed;
Countries have agreed to collaborate for the development of a risk mapping system that can utilise national data on live animals values, market activities and known animal movement patterns. Countries have participated in a simulation exercises for testing the emergency preparedness, improved their capacity to monitor effectiveness of vaccine and vaccination programmes (through workshops, e-learning) and agreed to share results on immunogenicity studies and post vaccination serosurvey.

### Discussion

The activities implemented contribute at improving the confidence in effectiveness of control programmes implemented and at assessing and mitigating the risk of epidemics in the region.

POSTER

## FMD OUTBREAKS DUE TO AN EXOTIC VIRUS SEROTYPE A LINEAGE (A/AFRICA/G-IV) IN ALGERIA IN 2017

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### Introduction

In the spring of 2017, a new introduction of FMDV serotype A occurred in the Maghreb region (Algeria and Tunisia). These were the first reports of field outbreaks of FMD due to serotype A in these countries after >25 years. Here, we report the whole genome sequence of viruses recovered during the outbreaks in Algeria and phylogenetic analysis, based on the

VP1 coding region, highlighting connections with countries in sub-Saharan Africa.

### Methods and Results

A total of six samples originated from three different outbreaks in Algeria were analyzed, FMDV serotype A was detected by Ag-ELISA. Virus isolates from all three outbreaks were obtained after the first passage in LFBK-αVα6 cells, which showed a better performance for virus isolation compared to BHK-21 and IB-RS2 cells. The phylogenetic analysis of the VP1 coding sequences grouped them within the A/AFRICA/G-IV lineage, most closely related to sequences originating from Nigeria in 2015, sharing more than 98% nt identity; older FMDV sequences (2009-2013) from Nigeria had lower nt identities (86.3-94.2%). FMDV sequences from countries in West Africa (Cameroon, Togo, Mali) and North-East Africa (Egypt, Eritrea, Sudan) were found to be more distantly related with nt identities ranging between 84.2-90.2% and 82.8-87.8%, respectively. One complete full genome sequence (8119 nt) and two near-complete sequences (7616 and 7627 nt) were obtained from the three isolates by Miseq Illumina platform. Sequences differed at only 27 nt sites, 19/24 located within the polyprotein-coding region were synonymous and five non-synonymous.

### Discussion

This study provides evidence for the transmission of the A/AFRICA/G-IV lineage outside its endemic areas in West Africa into the Maghreb region. Together with FMD cases due to serotype O that have also been previously reported in the Maghreb, these serotype A outbreaks represent the second independent introduction of FMD into region since 2013. These unpredictable dynamic FMDV movements may lead to new viral lineages becoming endemic in the region, which will inevitably heighten the risk of FMD introduction to Europe.

POSTER

## SERO-SURVEILLANCE FOR FMD IN SMALLHOLDER GOAT PRODUCTION IN LAO PDR, 2017–2018

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### Introduction

Foot and Mouth Disease (FMD) causes significant economic loss in Lao PDR and perpetuates the cycle of smallholder poverty through reduction in animal production and limitations to

market access for trading in livestock and their products. To determine the role of goats in the epidemiology of FMD in Lao PDR, we used a cross-sectional sero-prevalence study that identified antibodies to the non-structural proteins (NSP), an indication of previous infection, and structural proteins (SP) that could be due to vaccination or infection.

## Materials and methods

The study commenced in late 2017 when clotted blood samples were collected for serology from 26 randomly selected villages (6 districts in 5 provinces in Northern Lao PDR and 4 districts in 3 provinces in Southern Lao PDR). Paired sera and salivary swab samples (n=124) were collected by a simple random sampling method. Serological assays were performed using Prionics kits (supplied in kind by M/s Thermofisher Scientific, Australia). Real-time RT-PCR targeting the IRES region was used to detect presence of FMDV genome in the saliva swab samples.

## Results

Only Borkeo and Xayabouli in the north and Khammoaun in the south showed a significant sero-prevalence to both NSP and serotype O (42%, 8% and 20% respectively), indicating possible recent outbreaks. In the other provinces the sero-prevalence was close to zero, and analysis of the results showed that the sera that tested positive were close to the cut-off value.

## Discussion

Goats seem to become infected, but at a lower rate than cattle and buffalo. It is recommended that sero-surveillance for FMD in goats continue to improve our understanding of their role in the epidemiology of FMD in the region and to extend support to FMD control decisions, particularly regarding vaccination.

# RETROSPECTIVE FMD OUTBREAK REPORTS FROM UGANDA AND TANZANIA BORDER DISTRICTS (2011-2016): IMPLICATIONS FOR FMD CONTROL BY VACCINATION

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## Introduction

Foot-and-mouth disease in endemic in East Africa with annual outbreaks affecting farmers, individuals along the livestock value chain and individual governments. Uganda and Tanzania share an international border which was recently identified as one of the border areas in Eastern Africa important for FMD circulation. Both Uganda and Tanzania are in the initial stages of the progressive control pathway for FMD control. For the programme to be successful, baseline information on circulating serotypes and risk areas is important in strategizing control methods.

## Materials and methods

In this study, retrospective data on outbreaks between 2011 and 2016 was compiled for the four border districts of Isingiro and Rakai in Uganda and Missenyi and Kyerwa in Tanzania. Outbreak reports between 2011 and 2016 were compiled and analysed in R using regression models. Maps were drawn using QGIS to show the spatial distribution of the reported outbreaks.

## Results

The results showed that most outbreaks were not confirmed by laboratory analysis and lacked information on circulating serotypes, GPS location and number of animals affected. However, the data showed that most reported outbreaks occurred in sub counties/wards adjacent to the international border. Additionally sub-counties/wards with major cattle markets had recurrent outbreaks for the six years. Sub-counties/wards near game reserves were not affected during the six years save for two reported outbreaks.

## Discussion

The limited information on circulating serotypes for reported cases has implications on vaccination particularly when choosing the right vaccines. This study delineated FMD hot spot areas that can be strategically targeted for control by vaccination. The different policies on FMD control in the two countries has implications on the sustainability of using vaccines for FMD control in the light of the PCP-FMD. The study recommends better FMD surveillance and regional collaboration for strategic FMD control.

*Key Words: Retrospective, Foot-and-mouth disease, Control, Vaccination.*

# UPDATE OF FMD IN THE MAGHREB REGION: VACCINATION ISSUES

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## Introduction

Lately, North Africa suffered from several outbreaks of FMD caused by new viral lineages. This review aims to present the problematic of the choice of vaccine strains in North Africa.

## Materials and methods

This study is based on the results of world, European and national FMD reports, as well as the synthesis of different related works.
Also, a contribution to the evaluation of the prophylactic strategy adapted by the countries of the Maghreb based on the risk analysis is realized.

## Results

The viral lineage of serotype O that exist in North Africa is O/ME-SA/Ind-2001, which is normally present in the Indian Subcontinent, which proves the long distance movements of genotype
The most used method to choose the vaccine strain is in vitro vaccine matching. This test, gives different results of 2 isolates (Morocco, Algeria) belonging to the same topotype, and even more, a difference in the results for 2 viruses of the same topotype isolated in Algeria has been shown.
The virus prevailing in Morocco and Algeria, have a poor in vitro vaccine matching of O1Manisa vaccine strain, while a study using the in vivo efficacy, support the use of the O1 Manisa vaccine to control the same topotype.
The A/AFRICA G-IV was isolated in Algeria in 2017. The vaccine matching shows that the isolates correspond to two available vaccine strains, with r1 limit values.

## Discussion

Even if antigenic diversity of serotype O is low, discordances were found between vaccine strain results based on vaccine matching and potency cross protection techniques.
For the control of serotype A, North African countries use the old reference vaccine strains. However, Middle East study conduct in 2015, showed a quick spread of a serotype A lineage because the poor in vitro vaccine-match of field isolates to vaccine strains used.
There are threats for the potential spread of FMD into Europe from North Africa. Investigation of new vaccine strain adapted to these countries and setting up a regional vaccine bank, will have an important benefit.

# ASSESSMENT OF FMD VACCINES IN MONGOLIA AND THE ROLE OF BACTRIAN CAMELS

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## Introduction

Since early 2017, an upsurge of FMD cases were seen in Mongolia with both O-Ind-2001 and O PanAsia lineages having been detected. Vaccination and a modified stamping out policy has been applied in addition to other control measures. This study presents data from these outbreaks including incidence data in different species and the results of a small-scale post vaccination immunogenicity study.

## Material and methods

Government surveillance data was used to estimating the disease incidence in different species. Sampling protocols for evaluating immunogenicity were as described in the FAO-OIE Post Vaccination Monitoring (PVM) guidelines. Comparisons were made between cattle, sheep and camels, with oil and aqueous adjuvant vaccines and one or two dose primary courses. Virus neutralisation tests were performed using priority field strains from the region including O Mya-98, O Ind-2001, O PanAsia and A Sea-97. Data were analysed using multivariate interval regression.

## Results

Between January 2017 and April 2018, outbreaks were reported in cattle, sheep, goats and camels in 9 different provinces affecting 1,277 herders. During this time, the province level incidence in different species ranged from 0.02-2.3% in cattle, 0-0.17% in sheep, 0-0.07% in goats and 0-1.7% in Bactrian camels. From the latter, virus has been successfully isolated and is awaiting sequencing.
The PVM studies revealed higher titres in response to oil based vaccines for all strains. Although similar in cattle and sheep, in camels titres were significantly lower. Significantly higher titres were seen with a two dose primary course.

## Discussion

This study gives an overview of the FMD outbreaks in Mongolia and highlights the role of Bactrian camels. This PVM study indicates an oil-based vaccine with a two dose primary course gives maximum titres although further studies are needed to optimise the dose in Bactrian camels.

## A NOVEL VP2 PEPTIDE ELISA FOR UNIVERSAL DETECTION OF ANTIBODIES FOR FMD SERO-SURVEILLANCE

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### Introduction

FMD diagnostics include the use of serological tests to detect FMDV specific antibodies. Conventional serology tests are reliable and rapid but do not detect antibodies against all virus serotypes. The aim of this study was to assess the potential of conserved sequences at the N-terminus of capsid protein VP2 as universal epitopes for the detection of FMDV specific antibodies against multiple FMDV serotypes.

### Materials and Methods

An ELISA was developed using synthetic peptides corresponding to the N-terminus of VP2 as the capture antigen. The ELISA was evaluated using experimental and reference antisera (n=170) from the world reference laboratory for FMDV (WRLFMD, The Pirbright Institute).

### Results

The peptide ELISA based on the highly conserved VP2 peptide detected antibodies to all seven serotypes of FMDV in sera from immunized and convalescent animals. The peptide-ELISA provides sensitive and specific detection of antibodies to all FMDV viruses used in this study.

### Discussion

In summary, this study highlighted the potential of synthetic peptide as a capture antigen in rapid detection of antibodies to all serotypes of FMDV in animal sera. The test is robust, simple and cost effective and may be beneficial for endemic areas as well as for FMDV free countries which do not vaccinate to maintain status of free from FMD. .

## CLINICAL SENSITIVITY OF CATTLE, SHEEP AND GOATS TO DIFFERENT SEROTYPES OF FMDV IN CENTRAL REGION OF IRAN

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Iran is an FMD endemic country in FMD Pool 3 of West Eurasia assessed in Stage 2 of the Progressive Control Pathway.

In this study, we analyzed the reported FMD outbreaks in Qom province in central region of Iran between 1997 and 2016 that has 376 holdings/epi-units: beef fattening complex (3), villages (371) and dairy complex (2). Due to its proximity to the Iranian capital Tehran, Qom serves has high ruminant density and high rate of animal movements.

The objective of this study was to investigate the relative occurrence of different FMD serotypes in large and small ruminants.

In this period, 3993 outbreaks (3462 bovine and 531 ovine) were reported. There were reported 7 major surges in FMD outbreak reporting due to new strains of FMD virus (A Iran 05/AG-VII and O PanAsia2 Qom-15). 964 outbreaks randomly sampled and there were reported 475 O serotypes, 362 A serotypes and 127 Asia1 serotypes.

There were 3 reported surges in FMD outbreaks due to Asia1 serotype (2009, 2011, 2014) only in cattle, 5 due to A serotype (1998, 2005, 2010, 2013, 2015) in cattle herds and only one sheep flocks and 7 due to O serotype (1998, 2004, 2005, 2006, 2010, 2013, 2015) in cattle herds and sheep flocks.

During 1997 to 2016, out of 63 sheep outbreaks only 21 were serotyped. From the serotyping, 20 were serotype O and one was serotype as A.

Our data showed that FMD outbreaks due to O serotype occurred in cattle and sheep at the same time in one epidemiological unit but outbreaks due to A and Asia 1 serotypes occurred only in cattle.

This study underpins that sheep and goats have a different clinical sensitivity to various FMD serotypes compared with cattle. This finding is important in prevention and control strategy of FMD.

## DEVELOPMENT AND EVALUATION OF A MULTIPLEX CLASSICAL RT-PCR FOR SIMULTANEOUS DETECTION AND TYPING OF FMDV IN WEST AFRICA

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### Introduction

The West African territories are considered as regions with continuous FMDV circulation whe-

re outbreaks of FMDV serotypes O, A, SAT1 and SAT2 have been reported. An early diagnosis of FMD is crucial to implement adequate outbreak management. This study describes the development of a multiplex conventional RT-PCR for both detection and typing of FMD virus circulating in this region, and its evaluation on panels of field samples.

### Materials-methods

The RT-PCR reactions were developed by using primer sets targeting the 3D coding region, the VP1 coding region (O/A/SAT1/SAT2-specific) and the α-actin gene in order to produce amplicons of different sizes, easily distinguishable on agarose gel electrophoresis. Two FMDV strains of each targeted serotype as well as two negative samples were used to evaluate intermediate and final RT-PCR protocols. A 6-plex prototype (O/A/SAT1/SAT2/3D/α-actin) was finally developed and additionally tested with a panel of reference strains including all serotypes of FMDV. The sensitivity of RT-PCR was evaluated on 24 negative field samples and 37 positive field samples from Benin together with the corresponding virus isolates. This test is currently evaluated on a larger panel of field samples collected in Nigeria and Senegal.

### Results

The 6-plex prototype detected all FMDV strains tested and identified the four serotypes of interest (O/A/SA1/SAT2) without any improper amplification. Using this multiplex protocol, 37 samples from Benin were positive for the 3D target and were correctly serotyped by 6-plex (33) or by 3-plex and simplex RT-PCR (4). 39/40 isolates from Nigeria were properly serotyped using 6-plex (30) or simplex (9). The corresponding field samples as well 39 clinical positive samples from Senegal are under investigation.

### Discussion

We have developed and evaluated a 6-plex RT-PCR that could be easily implemented in diagnostic laboratories in endemic countries, providing thus an improvement for rapid detection and typing of FMDV strains.

## A MODIFIED DENDRIMER-RNA VACCINE PLATFORM AGAINST FMD

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### Abstract

Tiba Biotech has developed a synthetic replicon mRNA vaccine platform composed of engineered antigen-expressing mRNA replicons and a chemically-defined modified dendrimer delivery material. This allows for the rapid design and scalable manufacturing of synthetic

vaccines that generate cellular and humoral immune responses against a range of diseases. This technology has conferred protective immunity in multiple animal lethal challenge models, including Ebola virus, H1N1 influenza, Toxoplasma gondii, and HPV-induced cancer. Moreover, immunogenicity has been proven across a broad range of species, including mice, alpacas, and nonhuman primates. The synthetic encapsulation technology allows for large heterogeneous nucleic acid payloads, making it possible to simultaneously and cost-effectively immunize against multiple strains, and incorporate controlled copy numbers of other genetic factors necessary to ensure antigen processing and immunogenicity. In order to create a prototype FMDV vaccine to test in South Africa, we have generated and in vitro-tested RNA payloads encoding SAT2 P1 antigens in combination with different protease coding strategies to ensure correct processing of the FMDV structural proteins and minimize cytotoxicity. With our collaborators at the Moredun Research Institute in Scotland and The Agricultural Research Council in South Africa, we are evaluating T cell and antibody responses to the validated prototypes to determine optimal dosing strategies and durability of immunity. The ultimate goal of this ongoing work will be to test a multivalent formulation against multiple endemic FMDV strains. If successful, this will provide a platform suitable for rapid (<2 week) vaccine production in response to new strains, and the custom formulation of tailored, region-specific FMDV vaccines.

## THE USE OF NOVEL SINGLE-CHAIN ANTIBODY FRAGMENTS AGAINST SAT SEROTYPE FMD VIRUSES IN DIAGNOSTICS

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### Introduction

The key focus in the control of FMD in endemic regions is reliable diagnosis and good quality vaccines. Monoclonal antibodies are an essential requirement for the production of sensitive and specific reagents in the ELISA. Here we report on the use of single chain variable fragments (scFvs) selected from a naïve semi-synthetic chicken IgY phage display library, known as the Nkuku® library in the detection of SAT1, SAT2 and SAT3 viruses. Serotype-specific, soluble scFv's react with different binding profiles to intra-serotype viruses, which is information that may aid in the selection of antigenically appropriate vaccines for an outbreak situation. Alternatively, the knowledge concerning the antigenic composition of SAT viruses

may be used in the production of engineered vaccines with broad cross-reactivity.

Material and methods

Biopanning of the Nkuku® library with SAT1, SAT2 and SAT3 viruses resulted in six novel serotype-specific scFvs. Selected scFvs were tested as FMDV diagnostic reagents as well as to identify scFv binding footprints on the capsid.

Results

One SAT1, three SAT2 and two SAT3 FMDV serotype-specific scFvs were obtained. ScFvs were tested in an indirect and a sandwich ELISA format and its analytical sensitivity and specificity measured. Additionally, scFv binding footprints were mapped and one confirmed to include residue 159 of the VP1 capsid protein.

Discussion

ELISA and structural data was utilised to predict potential SAT1 and SAT3 epitopes and using a synthetic peptide, a SAT2 antigenic site was confirmed. Epitopes predicted corresponded to previously identified antigenic sites. Such knowledge can be used in the design of chimeric FMDV vaccines to afford better immunological protection. The use of the scFvs as diagnostic reagents in an ELISA format has proven beneficial for potential use in improved FMD diagnostic assays.

## GENETIC CHARACTERIZATION OF THE 2018 FMD VIRUSES IN SOUTH KOREA

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Introduction

In March 2018, an outbreak of foot-and-mouth disease A serotypes (A/GP/SKR/2018) occurred in South Korea. The A- type virus isolated belonged to the topotype ASIA, genotype Sea97 that had been occurred twice in Korea between 2010 and 2017. Unlike the previous strains which affected only cattles, the strain A/GP/SKR/2018 affected two pig farms in six days. To establish the relationship with the virus causing the 2017 serotype A (A/YC/SKR/2017) epizootic in Cattle, a genetic characterization was performed.

Materials and methods

Viral RNAs were extracted from nasal epithelium and vesicular fluid samples using a MagnaPure96 system (Roche). The VP1 region was amplified using a one-step RT-PCR kit (Qiagen) and then purified with ExoSAP-IT (USB) and directly sequenced on an ABI3130 genetic analyzer (Applied Biosystems). Phylogenetic tree of the VP1 (639bp) estimated using the neighbor-joining method in MEGA-6. For the complete genomes sequence, we designed pairs of primers to produce 20 overlapping amplicons spanning the entire viral genome. Sequence analyses were performed using SeqMan Pro (DNAStar Lasergene, USA).

### Results

The complete genome of strain A/GP/SKR/2018 was 8,193 nucleotides (nt) in length, including a 1011-nt 5'untranslated region (5'UTR) and a 122-nt 3'UTR. The sequence data of the complete genomes exhibited low homology to the virus (A/YC/SKR/2017) causing the 2017 serotype A epizootic in cattle with percentage nucleotide and amino acid identities of 95.6% and 97.7% respectively. And also, the strain (A/GP/SKR/2018) showed a partial deletion (69 nt) in 5'UTR. This genetic feature has not been found in the 2017 serotype A (A/YC/SKR/2017) epizootic in cattle.

### Discussion

This low degree of homology and genetic feature indicated that the outbreak of FMD A serotype in Korea in 2018 is considered to have not originated from the previously isolated strain A/YC/SKR/2017. These findings can conclude that this outbreak has been newly introduced from FMDV outbreak area.

## INVESTIGATING CROSS REACTIVITY OF SEROLOGICAL ENZYME LINKED IMMUNOSORBENT ASSAYS

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### Introduction

Serological assessments are vital in supporting official programmes aimed at monitoring, controlling and assessing the prevalence of foot-and-mouth disease virus (FMDV). The World Organisation for Animal Health (OIE) details the virus neutralisation test (VNT) as the 'gold standard' for the detection of antibodies reactive to FMDV structural proteins. However, a number of in-house and commercial serological Enzyme Linked Immunosorbent Assays (ELISAs) are widely employed to indirectly assess the immune status of an animal.

The purpose of this study is to determine the extent of cross reactivity that exists for three routinely used serological ELISAs: polyclonal Liquid Phase Blocking ELISA (LPBE), polyclonal Solid Phase Competition ELISA (SPCE) and commercially available kits based on SPCE principle and monoclonal antibodies (IZSLER kits).

### Method and Results

Three routinely used serological ELISAs for detection of antibodies against five FMDV serotypes (O, A, Asia 1, Southern African Territories (SAT) 1 and SAT 2) were employed for comparison: LPBE, SPCE and IZSLER SPCE kits. A selection of 365 monovalent experimental sera, representing all seven serotypes: O (n=116), A (n=120), C (n=18), Asia 1 (n=61), SAT 1 (n=14), SAT 2 (n=30) and SAT 3 (n=6) were assayed and analysed according to the validated protocol for each ELISA. Thus far, the presence of cross reactivity is evident in all three ELISAs for the seven serotypes, although higher sensitivity was observed for the sera specific to the serotype of the ELISA.

### Discussion

The presence of cross reactivity using ELISAs prevents serotyping of an individual serum, therefore interpretation should be considered at population level. In the context of a known outbreak scenario the assays are sensitive for that specific serotype.

## CONSTRUCTION OF A RECOMBINANT ANTIBODY PHAGE DISPLAY LIBRARY DERIVED FROM THE IMMUNE REPERTOIRE OF FMD–SAT IMMUNE BUFFALO. POTENTIALLY NEW DIAGNOSTIC REAGENTS?

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### Introduction

Foot-and-mouth disease (FMD) is one of the most economically important and socially devastating livestock diseases. To ensure proper control of the disease, vaccination programs and rapid and precise laboratory diagnosis is critical. The current recommended OIE diagnostic assay for diagnosis and screening of FMDV samples is the liquid phase blocking ELISA (LPBE). Although LPBEs for detecting the SAT serotypes are well established, there is still a need to improve the sensitivity and specificity. Antibodies have been harnessed as diagnostic and research reagents but are plagued with limitations. We aim to select SAT serotype-specific single chain variable fragments (scFvs) from an immune phage display library with the intentions to

achieve improved FMD diagnostic tests.

### Material and methods

The immune library was prepared from spleen samples from buffalo infected with SAT1/KNP/196/91, SAT2/KNP/19/89 and SAT3/KNP/1/08. Construction of the buffalo library was initiated by extracting RNA from the spleen samples and amplifying the coding sequences for the immunoglobulin variable light and heavy chains by PCR. The constructed buffalo library was bio-panned with representative viruses for each of the SAT serotypes displaying broad neutralising characteristics.

### Results

This is the first time a recombinant antibody phage display library derived from the immune repertoire of FMD–SAT immune buffalo has been constructed. The total library size was 3.84 x107 cfu. Virus-specific binders will be selected and characterised and their use in the development of improved diagnostic assays investigated.

### Discussion

The current LPBE used at ARC-OVR for FMDV diagnosis uses polyclonal sera as both capture and detecting reagents. Polyclonal sera containing a heterogeneous complex mixture of antibodies of different affinities can result in background signals of serological assays. The selected FMDV-specific scFvs will be used to improve the sensitivity and specificity of the current diagnostic ELISA for FMDV.

## INTER-LABORATORY PROFICIENCY TEST FOR SEROLOGICAL DIAGNOSIS OF FMD IN SOUTH KOREA

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### Introduction

FMD is one of the major viral diseases affecting farming industry in Korea. To control the disease, compulsory FMD vaccination and robust sero-surveillance program had been implemented since 2011. To ensure that all regional laboratories perform the reliable FMD serological diagnosis, Animal and Plant Quarantine Agency has been provided training and proficiency test. Here, we described the Inter-laboratory proficiency test performed in 2017 proficiency test in 2017.

### Materials and methods

Forty-six laboratories participated. Test panel contained 6 non-infectious sera originated from 2 bovine and 4 swine. All sera were shipped in frozen and arrived within 24 hours to the diagnostic labs. For analyses, all laboratories used unified commercial NSP ELISAs and SP O ELISA. Test results met the validation criteria were interpreted as described in manufacturer's instruction. Test results were analyzed in two criteria: deviation of controls' value in each ELISA and test results of panel serum.

### Results

Forty-six participants' positive and negative control values of each ELISA tests met the validation criteria and were within the range of 95% confidence interval. For the panel tests, it was noted that results for samples number 1 and 2 showed discrepancy in some of participants in one of the NSP ELISAs. However, all of participants' final interpretation of each sample was coincided with panel description.

### Conclusion

The annual proficiency tests performed in 2017 in Korea demonstrated the FMD serological diagnostic capability of regional veterinary laboratories. Also, it showed the suitability of current NSP antibody diagnosis complemented by using two NSP ELISAs. To eradicate FMD in the country, it is important to maintain the quality of diagnosis and proficiency test can be the means to check the capability of FMD diagnostic laboratories.

## DEVELOPMENT AND EVALUATION OF LINEAGE-SPECIFIC REAL-TIME RT-PCR ASSAYS FOR THE DETECTION AND CHARACTERISATION OF FOOT-AND-MOUTH DISEASE VIRUSES CIRCULATING IN ASIA

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### Introduction

Foot-and-mouth disease (FMD) is endemic in most Asian countries, with field outbreaks occurring regularly due to co-circulating viruses within serotypes A (lineages: ASIA/G-VII, ASIA/Sea-97, ASIA/Iran-05), O (lineages: ME-SA/Ind-2001, ME-SA/PanAsia-2, SEA/Mya-98, ME-SA/PanAsia, CATHAY) and Asia 1 (lineages: ASIA/Sindh-08, ASIA/G-VIII). The ability to rapidly and accurately characterise FMDV lineages is necessary to better understand the epidemiology of FMD and to aid the selection of appropriate vaccines. Currently, FMDV lineage characterisation is determined via sequencing and sequence data analyses, which is not always readily available to laboratories in endemic settings. Therefore, tailored lineage-specific real-time RT-PCR (rRT-PCR) assays for FMDV lineage characterisation in Asia were designed and validated.

### Methods

rRT-PCR assays were designed to specifically detect the A/ASIA/Sea-97, O/ME-SA/Ind-2001, O/ME-SA/PanAsia and PanAsia-2, O/SEA/Mya-98 as well as O/CATHAY lineages by targeting lineage-specific regions within the variable VP1-coding sequence. These assays were validated together with the A/ASIA/Iran-05 (Jamal and Belsham, 2015) and Asia 1/ASIA/Sindh-08 (Reid et al., 2014) lineage-specific assays using a panel of recent field samples. In addition, all samples were evaluated with the pan-specific 3D assay (Callahan et al, 2002).

### Results

The seven lineage-specific assays correctly detected all samples within each of the targeted lineages. Although some cross-reaction was observed with closely related lineages (O/ME-SA/Ind-2001, O/ME-SA/PanAsia and PanAsia-2), the lineage-specific assays can be applied for discrimination between FMDV lineages in Asia.

### Discussion

Together with published A/ASIA/G-VII-specific assays (Saduakassova et al., 2017), the described set of rRT-PCRs constitute a comprehensive panel of assays (or molecular toolbox) for rapid characterisation of the FMDV lineages circulating in Asia at relatively low cost. Thus, this molecular toolbox could enhance the ability of national laboratories in endemic settings to accurately characterise currently circulating FMDV strains and facilitate the prompt implementation of control strategies.

## CHARACTERISATION OF BOVINE DENDRITIC CELLS FOLLOWING FMDV INFECTION

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### Introduction

Dendritic cells (DCs) are considered to be the sentinels of the immune system, responsible for recognising invading pathogens and priming the adaptive immune system to generate appropriate responses. Hence they are considered potential targets for vaccines against pathogens such as foot-and-mouth disease virus (FMDV). However, despite knowing the identity of the receptors used by FMDV and the pathway utilised by this virus to enter moDCs, little is known of the events of FMDV replication in bovine moDCs. Present work therefore, sought

to characterize FMDV and its immune complex (IC) replication in bovine moDCs in vitro.

### Material and methods

A chimeric heparin sulphate FMDV (O1M) was used in this study. Immuno-fluorescence microscopy (IFM) and quantitative RT-PCR was used to analyse viral replication at 0-6, 8, 16 and 24 hpi. Plaque assays were used to investigate the yields of live virus produced in moDCs at 0, 4, 8 and 24 hpi.

### Results

FMDV and IC FMDV could infect moDC. In moDC infected with FMDV alone, or with immune-complexed (IC) FMDV, replication was observed by IFM between 2-4 and 1-16 hpi, respectively. In contrast, for both FMDV and FMDV IC infections RT-PCR analyses showed viral replication peaked at 4 hpi and then decreased between 8 to 24 hpi. Plaque assays using supernatants of the infected moDC showed no evidence of an increase in viral titre at 24 hpi.

### Discussion

The detection of viral nsp (3AB and derivatives) suggests replication of FMDV persists for longer in moDCs when entry is mediated by IC. However, the lack of increase in virus yield suggests replication is abortive in these cells. One possible explanation for this difference could be that bovine moDCs are able to recognise non-immune complexed FMDV more rapidly.

## HUMANS APPLY VACCINES: HOW CAN NEW TRAINING TOOLS BE USED TO BUILD CAPACITY FOR FMD CONTROL?

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### Introduction

Effective foot-and-mouth disease control relies on human resource capacity to apply control measures such as vaccination effectively. A chain of actors, from livestock keepers to veterinary service decision makers, are involved in the effective implementation of disease control measures. Building capacity such that each stakeholder in the chain is able to perform their role effectively is critical to the success of FMD control, either in an endemic country, or a previously free country experiencing an incursion.

### Materials and Methods

The European Commission for the Control of Foot-and-Mouth Disease (EuFMD0 has implemented an innovative needs-based training program which targets both FMD free and non-free countries. The program has been designed following an ongoing needs assessment process and has involved training delivered through a variety of methodologies including online courses, workshops and innovative field based training courses. Training has been delivered online to over 5500 participants from over 50 countries and in nine languages to date.

## Discussion

In this presentation we discuss lessons learned to date from the EuFMD training program, with a focus on areas where we have aimed to build capacity to enable effective use of FMD vaccines. We discuss key target audiences identified and the appropriateness of the training methodologies that have been applied. We project forwards, considering new future audiences for FMD related training and prioritizing critical capacities which will need to built to enable application of new tools for disease control most effectively.

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