GFRA 2023
Scientific Meeting

8-10 November 2023
Kampala, Uganda

Abstract Booklet

Table of Contents

ORAL PRESENTATIONS 6

FMD Research in Africa 6

Immunogenicity of commercial quadrivalent foot-and-mouth disease virus vaccines against Eastern African virus strains 6

Efficacy of a foot-and-mouth disease vaccine against a heterologous SAT1 virus challenge in goats 7

Foot-And-Mouth Disease SAT Specific Virus Peptide Phage Display Libraries For The Identification Of Epitopes 8

Economic Impact of the Foot and Mouth Disease (FMD) on Domestic Small Ruminants Production in the Northern Regions of Cameroon 9

Epidemiology 11

Optimising simple clinical and environmental approaches to sampling foot-and-mouth disease virus for next generation sequencing 11

Molecular characterisation of foot-and-mouth disease virus using 'k-mer' clustering analysis 12

Molecular epidemiology of FMDV in Tanzania: implications for FMD control in East and Southern Africa 13

The role of small ruminants and the environment in the epidemiology and endemicity of FMDV: a longitudinal study in Northern Nigeria in 2021 14

Occurrence and Control Strategies for Foot and Mouth Disease in Uganda: Systematic Review and Meta-analysis 15

Sero-survey of foot-and-mouth disease virus serotypes O and A in selected livestock-wildlife interface areas in Tanzania 16

Genetic and antigenic characterization of variants of serotype O/ME-SA/PANASIA sublineage circulating in Pakistan during 2012-2020 17

Detection And Characterization Of Foot And Mouth Disease Viruses In Small Rumminants In Nigeria 18

Diagnostics 19

Complete genome sequencing of FMDV using nanopore sequencing 19

Preliminary validation of multiplex lateral flow devices LFD1 and LFD2 for on-field identification and serotyping of foot-and-mouth disease viruses 20

Challenge methods for FMD studies in pigs compared 22

Revisiting an old classic: Using the IgG1 avidity ELISA to predict cross-protection in the Brehm et al. sample set 23

Pathogenesis 24

Leaderless FMDV O is fully attenuated in cattle and does not establish persistent infections 24

Leaderless FMDV O does not establish a persistent infection in multilayered cells derived from bovine dorsal soft palate 25
Association between FMDV Persistent Infection and High Aryl Hydrocarbon Receptor Activation of Cattle Sera 26
Incubation phase transmission of FMDV in cattle 27
Virology 28
Interferon stimulated genes correlate with mammalian resistance to foot-and-mouth disease 28
Inter-serotypic recombination of foot-and-mouth disease virus following superinfection of carrier cattle 29
Development of an FMDV minigenome, pKLS3, and its applications 30
Mapping of FMDV antigenic sites recognized by single-domain antibodies reveals different 146S particle specific sites and particle flexibility 31
Immunology 32
Heterologous cross-neutralization with different Foot and Mouth Disease vaccine schemes against serotype O strains 32
Maternally derived antibodies to FMDV modulate the antigenic specificity of the antibody response in vaccinated cattle 33
Impact of Fasciola hepatica infection on the long-term immune response induced by Foot-and-Mouth disease vaccination 34
Optimising prime-boost interval to Enhance FMD Vaccination Efficacy in Cattle 35
Vaccines 36
An overview on the efficacy of novel FMDV vaccines carrying antigenic signatures 36
Potential live-attenuated FMDV serotype O IND R2/1975 vaccine candidate strain generated through genome re-coding approach 37
Rethinking next-generation FMDV vaccines: use of codon deoptimization strategies 38
Tackling FMD in Eastern Africa using stabilized virus-like particles vaccines 39
POSTER PRESENTATIONS 40
Diagnostics 40
Molecular Differentiation and Phylogenetic Analysis of the Egyptian Foot-and-Mouth Disease Virus Collected from Ismailia Governorate, 2021 40
Development of foot and mouth disease serotype-specific blocking ELISAs using monoclonal antibodies 41
Development and Validation of a Field-Applicable CMOS Image Sensor-Based Multi-Well Real-Time Reverse Transcription PCR System for Rapid and Accurate Detection and Serotyping of Foot-and-Mouth Disease Virus 42

Filter paper as an alternative method for transportation of samples in resource-limited areas in Africa for diagnosis of foot-and-mouth disease virus....43
Operation and Utilization of a Foot-and-Mouth Disease Serum Bank as a National Reference Laboratory for Foot-and-Mouth Disease Diagnosis in South Korea....44
Lateral flow devices for the rapid detection of FMDV....45
Foot-and-mouth disease laboratory diagnosis in the framework of the PCP-FMD in Nigeria (2011-2022)....46
Epidemiology....47
Phylogenetic and genotypic characteristics of the foot-and-mouth disease virus from outbreaks in South Korea, 2023....47
Genotypic identification of Foot and mouth disease virus isolated from Cambodia between 2018-2022....48
Genetic Analysis of Foot-and-Mouth Disease Virus O/ME-SA/SA-2018 in Bangladesh, 2022....49
Phylogenetic analysis of Foot-and-mouth disease viruses circulating in Vietnam in 2022....50
Clinical Prevalence of Foot-and-Mouth Disease in Smallholder Dairy Cattle in Northern Cameroon 51
Cross-sectional study of Senecavirus A infection in pig farms in Taiwan....52
Serological Study and Comparison of Various Types of Cattle Groups in the Zambezi Region of Namibia: A Cross Sectional Study....53
Immunology....54
Analyses of Bovine Immunoglobulin Constant Heavy Chain Protein Sequences and the Inferred Properties Regarding Antibody Binding to FMDV Epitopes....54
Mechanisms of Tissue Susceptibility to FMDV Persistent Infection Based on Genes Differentially Expressed between Target and Non-target Tissues....55
Pathogenesis....56
Investigation of Foot-and-Mouth Disease Virus serotypes from Livestock in Kenya in the year 202056
Vaccines....57
Development and evaluation of multivalent FMD vaccine using local field isolates....57
Selection and use of a reference antigen panel to assess the regional relevance of foot-and-mouth disease vaccines in East Africa....58
Improved in vitro and in vivo assays to assess the quality of FMD vaccine: Preliminary evidence from the Indian experience....59
Biogénesis Bagó FMD vaccines: immunogenicity studies in small ruminants and cross immunity against prevalent FMDV lineages in Middle East Asia....60
FMD vaccine strain Asia 1/Sindh-08: immunogenicity studies in cattle and cross immunity against prevalent Asia 1 field viruses....61

Coverage of a Novel Monovalent O1 Campos Vaccine Against Foot and Mouth Disease (FMD) Viruses Circulating in Africa and Asia....62
Post Vaccination Monitoring (PVM) to Assess the Efficacy of the FMD Vaccine Used in Jordan....63
Evaluation of swine protection with commercial foot-and-mouth disease vaccines against challenge with an A/ASIA/Iran-05 lineage virus from South East Asia....65
The early efficacy study on the vaccination of commercial and experimental foot-and-mouth disease vaccines against the challenge with an O/CATHAY isolate from South East Asia in pigs....66
Construction of a FMDV-specific recombinant antibody phage-display bovine library for epitope identification and diagnostic reagents....67
Evaluation of Foot-and-Mouth Disease Virus Inactivation Kinetics with Binary Ethylenimine for Vaccine Production in South Korea....68
Stabilized SAT1 virus-like particles protect cattle against foot-and-mouth disease....69
Stabilized SAT2 virus-like particles protect cattle against foot-and-mouth disease....70
Virology....71
Development of a primary cell model derived from porcine dorsal soft palate for foot-and-mouth disease virus research and diagnosis.....71
Comparison of formaldehyde inactivation of dried FMDV with 3M Attest 1294-S and discs containing Bacillus atrophaeus spores.....72
Comparison of VP1 sequences between samples transfected with FMDV and clinical samples....73

ORAL PRESENTATIONS

FMD Research in Africa

Immunogenicity of commercial quadrivalent foot-and-mouth disease virus vaccines against Eastern African virus strains

Susan D Kerfua[1], Donald King[2], Daniel T Haydon[5] ,Anna Ludi[2], Ginette Wilden[2], Rose Ademun Okurut [3], Stella Atim[3], Ivan Kyakuwa[1], Moses T Dhikusooka[1],Paolo Motta[4], and David J Paton[2]

[1]National Livestock Resources Research Institute, National Agricultural Research Organisation, Uganda
[2]World Reference Laboratory for foot-and-mouth disease, the Pirbright Institute, United Kingdom
[3]Ministry of Agriculture Animal Industry and Fisheries,Entebbe, Uganda
[4]The Food and Agriculture Organization of the United Nations, Regional Office for Asia and the Pacific, *Phra Atit Road Bangkok 10200, Thailand*
[5]School of Biodiversity, One Health and Veterinary Medicine, College of Medical Veterinary and Life Sciences, University of Glasgow, Scotland

Corresponding author: Susan Diana Kerfua, P.O.Box 5704 Kampala; kerfuas@gmail.com or susan.kerfua@naro.go.ug; Tel: +256 706392098

Foot-and-mouth disease (FMD) is a highly contagious disease affecting cloven-hoofed animals. The disease is caused by any of the seven FMD virus serotypes; O, A, C, SAT 1, SAT 2, SAT 3, and Asia 1, and has severe economic consequences. For FMD endemic countries, vaccination is critical alongside other interventions, however FMD vaccine immunogenicity studies are uncommon despite substantial funds spent on vaccine purchase. Our study evaluated antibody responses over 360 days to two African vaccines containing antigens of serotypes O, A, SAT 1 and SAT 2, and compared two primary vaccination regimes followed by a booster dose at six months. The experiment used 37 Viking Jersey calves from which sera were collected monthly. Additionally, 10 calves were vaccinated and sampled for up to 180 days to compare antibody responses to a second production batch of each vaccine. Samples were subjected to Solid Phase Competitive Blocking ELISA and representative sera were tested for their ability to neutralize eight Eastern African virus strains. We used General Linear Models to determine differences between vaccines and vaccination regimes, and parameterize a simple model that predicted duration of protection. Antibody responses varied significantly according to vaccine, regimen, post vaccination day interval and virus strains used for VNT assessment. Vaccine 1 elicited stronger immune responses. VNT performance suggested protection of 50% of calves beyond 6 months for only one virus strain, only when administered as a two-dose primary course. In general, low levels of antibodies were induced, particularly for Vaccine 2, and generally for all calves that did not receive two dose primary vaccination, with protective immune responses lasting less than two months. Overall correlation between the ELISA and VNT data was 0.49 with observed variation between serotypes, whilst antibody half-life was estimated at 99 days. Our findings demonstrate the need to evaluate and improve commercial FMD vaccines and vaccination regimes.

Keywords: Foot-and-mouth disease, Serotype, Post vaccination monitoring, Vaccine control, Uganda, Eastern Africa

Efficacy of a foot-and-mouth disease vaccine against a heterologous SAT1 virus challenge in goats

David Dazhia Lazarus[1], Faith Peta[2], Juanita Van Heerden[2], Paidamwoyo Barry Mutowembwa[2], Livio Heath[2], Belinda Blignaut[2], Pamela Anne Opperman[2], Geoffrey Theodore Fosgate[3]

[1] National Veterinary Research Institute, Infectious and Transboundary Animal Diseases, Vom Nigeria
[2] Agricultural Research Council, Onderstepoort Veterinary Research, Transboundary Animal Diseases, Onderstepoort, South Africa
[3] University of Pretoria, Faculty of Veterinary Science, Department of Production Animal Studies, Onderstepoort, South Africa

Goats are susceptible to infection with foot-and-mouth disease virus (FMDV), but their role in the epidemiology of the disease and response to vaccination is poorly understood. In southern Africa, FMDV serotypes Southern African Territories (SAT) 1, 2 and 3 are known to be endemic and the cause of disease in livestock. In this study, we evaluated the efficacy of a pentavalent FMD vaccine containing serotypes SAT1, SAT2 & SAT3 in goats against heterologous challenge with a pool of field SAT1 FMDV. Forty FMD sero-negative goats (6-12 months of age) of mixed sexes were randomly allocated to one of five treatment groups: full cattle dose (2 ml), 1/3rd (0.67 ml), 1/6th (0.33 ml), 1/12th (0.16 ml) or unvaccinated placebo control. Goats were vaccinated with the inactivated pentavalent vaccine on day 0 and revaccinated at day 20 post vaccination. Thereafter, thirty-four goats were challenged by tongue inoculation at day 41 post-vaccination using 104.57 50% tissue culture infective dose (TCID50) FMDV SAT1 pool. Animals were examined daily, and clinical signs were scored. Rectal temperatures were measured daily, with temperatures ≥40°C defined as fever. Clinical specimens (nasal, oral and rectal swabs) were collected on days 0, 2, 4 and 6 post challenge. Viral shedding was determined using reverse-transcriptase real-time PCR. None of the goats vaccinated with the full cattle dose developed secondary lesions. All vaccinated groups had lower temperatures compared to the unvaccinated controls ($P<0.001$). Based on RT-PCR results, goats in the unvaccinated control group shed more virus compared to all groups except for the 1/12th group ($P<0.05$), while goats in the full dose group shed less virus than goats in the 1/12th and unvaccinated control groups ($P<0.005$). Findings suggest that the 1/3rd (0.67ml) dose of the vaccine is sufficient to reduce viral shedding after heterologous challenge with FMDV SAT1.

Foot-And-Mouth Disease SAT Specific Virus Peptide Phage Display Libraries For The Identification Of Epitopes

NPB Sekgobela[1,2], J Fehrsen[1,2], PA Opperman[1,3], M Chitray[1,2]

[1] Agricultural Research Council, Onderstepoort Veterinary Research Institute, Vaccines and Diagnostic Development, Private Bag X05, Onderstepoort, Pretoria 0110, South Africa
[2] University of Pretoria, Faculty of Veterinary Science, Department of Veterinary Tropical Diseases, Pretoria, South Africa
[3] University of Pretoria, Faculty of Veterinary Science, Department of Animal Production Studies, Pretoria, South Africa

The WOAH ranks foot-and-mouth disease (FMD) as an economically important infectious animal disease affecting cloven hoofed animals. There are seven serologically distinct serotypes i.e. A, O, C, Asia1 and the Southern African Territories (SAT) types: SAT1, SAT2 and SAT3. Three of the seven serotypes exist in South Africa and considering the virus maintenance host i.e., the African buffalo, eradication is near impossible. Thus, emphasis is placed on control e.g., improved vaccines. Vaccination against one serotype does not confer protection against another due to high antigenic variation of the virus. Commonly, variations occur on the capsid coding (P1) region of the genome. Knowledge of the FMD virus (FMDV) antigenic sites can be useful in production of recombinant FMD vaccines.

Due to limited knowledge regarding SAT antigenic sites, phage display technology was utilised. Consequently, three FMDV peptide phage display libraries were constructed using the fragmented P1 regions of a FMDV SAT1, SAT2 and SAT3 and biopanning with immunoglobulin (IgG) purified from FMDV SAT bovine infected sera. The advantage of utilising immune sera and biopanning against virus-specific peptide libraries is that affinity maturation has already occurred in immunized animals and the recognized epitope regions are identifiable.

Antigenic regions to which IgGs bound were identified from screening biopanning output clones followed by Sanger and Miseq sequencing. The 18 amino acid residues from the VP1 C-terminus including three residues of the N-terminus of 2A was identified as a SAT3 epitope. The data also revealed other potential epitopic regions for SAT3.

This study improved FMD knowledge on SAT antigenic sites and significantly contributes towards the future development of improved vaccines. Through recombinant, reverse genetics technology, identified epitopes can be incorporated into the FMDV genome and recombinant viruses can be used for vaccine production, thus producing vaccines that offer a broad immunogenic response and protection.

Economic Impact of the Foot and Mouth Disease (FMD) on Domestic Small Ruminants Production in the Northern Regions of Cameroon

Simon Dickmu Jumbo[1], Hamidou Orriskey[2], Victor Ngu Ngwa[2], Ranyl Nguena Guefack Noumedem[1], Abel Wade[1], Silas Lendzele Sevidzem[3], Jean Marc Feussoum[4], Cyrille Mbu'u Mbanwi[5], Aziwo Tatanja Niba[6], Julius Awah-Ndukum[6]

[1] The National Veterinary Laboratory, Garoua, Cameroon
[2] ESMV University of Ngaoundere, Cameroon
[3] Université Libreville Nord (ULN), Transmissible Diseases Laboratory (TDEL) Okala, Libreville, Gabon
[4] Directorate of Veterinary Services, Ministry of Livestock, Fisheries and Animal Industries, Cameroon
[5] Faculty of Sciences, University of Yaoundé I, Yaoundé, Cameroon
[6] Department of Animal Production Technology, College of Technology, University of Bamenda, Bambili, Cameroon

A study was carried out in the northern regions of Cameroon, from August 2022 to January 2023, with a view to assessing the economic impact of Foot and Mouth Disease (FMD) in small ruminants production. A sample of 175 farmers was interviewed using a survey form and 63 serum samples were collected from small ruminants for the detection of antibodies against non-structural proteins of foot-and-mouth disease virus by a competitive ELISA technique. It appears from the study that the morbidity rate of FMD was 96.13% and the mortality rate was 20.05% in sheep and 24.22% in goats. The total economic losses associated with this disease were high in the North and Far North regions with respective averages of 722.54 ± 668.12 USD an 644.80 ± 962.90 USD. The cost of treating 2,716 animals affected by FMD was estimated at 6,932.66 USD, with an average value of 39.61 ± 51.35 USD and an average of 2.55 USD per head of small ruminants with FMD. Mortality losses were higher in the Far North region with an average of 424.23 ± 652.76 USD and differed significantly from the other two regions. Variations were observed in terms of mortality losses by species with an average of 314.50 ± 629.46 USD (sheep) and 109.73 ± 143.66 USD (goats). Thirty (30) samples were positive out of the 63 analyzed. The animals that tested positive were susceptible to serotype O of the Aphtovirus. Because of these heavy losses due to FMD, it is important to rigorously apply the national FMD control strategy program. It is also important to study the phyologenetics of bovine and small ruminants FMD viruses to assess the evolutionary pathways and transmission among domestic ruminants species.

Epidemiology

Optimising simple clinical and environmental approaches to sampling foot-and-mouth disease virus for next generation sequencing

Mark Bronsvoort[1], Lina González[1], Wilson Amanyire[2], Richard Orton[3], Adrian Muwonge[1], Lisa Boden[4], Thibaud Porphyre[5], Dennis Muhanguzi[2], Donald P. King[6], Andrew E. Shaw[6]

[1] The Roslin Institute at The Royal (Dick) School of Veterinary Studies, University of Edinburgh, Easter Bush, Midlothian, United Kingdom
[2] Makerere University, Kampala, Uganda
[3] Medical Research Council, University of Glasgow Centre for Virus Research, United Kingdom
[4] Global Academy of Agriculture and Food Systems, University of Edinburgh, Easter Bush, Midlothian, United Kingdom
[5] Laboratoire de Biométrie et Biologie Évolutive, VetAgro Sup, Marcy l'Étoile, France
[6] The Pirbright Institute, Ash Road, Pirbright, United Kingdom

Foot-and-mouth disease (FMD) remains an endemic problem in Equatorial Africa. However, as countries engage with its control, developing novel tools as well as simple and affordable approaches for viral surveillance becomes important. The gold standard for diagnostic samples has been the collection of epithelial samples from lesions, often requiring physical or chemical restraint, followed by tissue culture, antigen ELISA for serotyping and then sequencing. However, this requires technical and laboratory capabilities that are difficult to sustain. Here, we show the utility of a field-deployable clinical and environmental sampling approach and a molecular and diagnostic sequencing pipeline based on the Nanopore sequencing using the pocket-sized MinION device and a universal primer set (described in another presentation by Shaw et al) for the real-time analysis of viral RNA fragments. Samples were collected in 3 districts in South-Eastern Ugandan between October and November 2022. Six outbreak farms were identified and a total of 61 samples were collected from 23 clinically affected animals using a simple synthetic swab. On the same farms, 42 environmental samples were collected using microfibre electrostatic cloths. Surfaces such as ropes, herdsmen's sticks, and feed troughs were swabbed. Markets were also visited and oral swabs collected from animals present at the market and dry swabs collected from surfaces such as ropes, fencing, sticks and transport vehicles. Samples were placed in PBS and inactivated in lysis buffer before processing. We will report the results of the molecular diagnostics and sequencing from different sample types and discuss how these approaches may be practically applied by FMD virus endemic countries in Equatorial Africa as part of an integral strategy for FMD control.

Molecular characterisation of foot-and-mouth disease virus using 'k-mer' clustering analysis

Wilna Vosloo, Nagendrakumar Singanallur Balasubramanian, and Petrus Jansen van Vuren

CSIRO Health and Biosecurity, CSIRO Australian Centre for Disease Preparedness, Geelong Victoria 3220, Australia

Foot-and-Mouth Disease (FMD) is an important disease of livestock that can cause severe loss to the agricultural economy of any nation and can spread quickly from one geographical area to another by biological and mechanical means. Information about an emerging strain is crucial to identify the source and type of control measures, including decisions on vaccines if vaccination is considered one of the options for controlling the incursion.

The phylogenetic analysis based on the Neighbour-Joining (NJ) method is simple. New evolutionary methods like the General Time Reversible (GTR) model use substitution models to establish genetic relationships considering rate heterogeneity (rate of substitution occurs at different rates at different sites). However, all these methods require sequence alignment, a highly computational process that requires a thorough knowledge of the sequence characteristics of the prototypes for proper alignment. An alternative 'k-mer' approach is alignment-free, quick and does not require detailed knowledge of the pathogens' sequence. This study investigated k-mer-based sequence analysis by identifying the optimal k-mer frequency (k-mers are substrings of length k for a sequence with length L) for FMDV sequences. A combination of publicly available and internally generated sequences was used to develop standardised workflows. Scripts were written in R-programming using pre-existing programming libraries. Optimal k-mer frequencies were identified for each virus that depended on the statistical method used.

We compared the tree topologies generated by NJ, GTR and k-mer analysis to evaluate the potential use of the latter for quick investigation in the face of an outbreak. Such an alternative method can confirm other studies' findings or be standalone when sequence alignments prove problematic.

We found that the k-mer analysis is computationally intense and requires high-performance computing systems, therefore not offering any significant advantages over traditional phylogenetic analysis approaches. This was evident when the number of sequences for comparisons increased. However, with cloud computing and online RStudio programs readily available, using the k-mer-based clustering analysis will become increasingly easier. It is advisable to have sets of reference sequences available for each virus's groupings (serotypes/lineages/topotypes/genotypes) to facilitate classification using the k-mer methodology.

Molecular epidemiology of FMDV in Tanzania: implications for FMD control in East and Southern Africa

Christopher Jacob Kasanga[1], Antonello di Nardo[2], Sengiyumva Kandusi[1], Antipachius Msomi[1], Ramadhani Juma Makasali[1], Herbertha Mpete[1], Mathias Mashinagu Mkama[3], Nick Knowles[2], Philemon Nyangi Wambura[1], Mark Rweyemamu[1], David Paton[2], and Donald King[2]

[1] Sokoine University of Agriculture
[2] The Pirbright Institute
[3] Tanzania Veterinary Laboratory Agency

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 viruses (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 2016 and 2020. Tissue epithelia and fluids (n = 1012) 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 VP1 sequencing. The results of this study indicated that 523 out of 1012 (51.7%) samples contained FMDV antigen. Of the 523 positive samples, 159(30.4%) were type A, 227 (43.4%) type O, 97(18.5%) SAT 1, and 40 (7.7%) serotype SAT 2. All four FMDV serotypes were found in the Southern, Northern, Coastal, Central and Eastern zones of Tanzania. Phylogenetic analysis of VP1 nucleotide sequences (using maximum likelihood and neighbour-joining methods) 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. Several virus lineages and sub-lineages were evident per genotype/topotype of the four FMDV serotypes. 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 elsewhere in East Africa. The presence of multiple serotypes, genotypes and lineages complicates FMD control in Tanzania and the region. Further studies are required to investigate the evolutionary characteristics, transmission dynamics and antigenicity of circulating strains for rational FMD control in Tanzania and neighbouring countries.

The role of small ruminants and the environment in the epidemiology and endemicity of FMDV: a longitudinal study in Northern Nigeria in 2021

Claire Colenutt[1], Emma Brown[1], Georgina Limon[1], Yiltawe Wungak[2], Olumuyiwa Oyekan[2], Adeyinka J. Adedeji[2], Sandra I. Ijoma[2], Rebecca B. Atai[2], Moses O. Oguche[2], Banenat B. Dogonyaro[2], Mark Samson[2], Donald P. King[1], Anna B. Ludi[1], Andrew Shaw[1], David O. Ehizibolo[2], Simon Gubbins[1]

[1] The Pirbright Institute, UK
[2] National Veterinary Research Institute, Vom, Plateau State, Nigeria

This study aimed to enhance our understanding of the role of small ruminants and environmental contamination in the epidemiology and endemicity of foot-and-mouth disease and identify reliable and convenient sampling methods for surveillance in endemic settings.

Active surveillance was carried out between March 2021 and October 2021. Monthly samples were collected from five households, one livestock market and one transhumance location in Bassa and Jos South local government areas in Plateau State in northern Nigeria. These regions had been identified as high risk for FMD outbreaks based on previous studies. Blood samples (n=783) and oral swabs (n=424) were collected from selected animals (sheep, goats and cattle) at each site. Environmental samples were also collected at each visit (n=458). Serum, swabs and environmental samples were all tested for presence of FMDV RNA by rRT-PCR. Serum samples (n=780) were also tested for the presence of antibodies against FMDV non-structural proteins. Selected FMDV RNA positive samples (n=8) were sequenced using Illumina based next generation sequencing.

Overall, the proportion of FMDV RNA positive samples was low (<3%) from both regions, although the proportion positive increased (up to 26%) in samples collected in Jos South in September and October 2021. In this period, there was an FMD outbreak reported in the area, with one of the sampled households affected. Fifty-six percent of serum samples were positive for NSP antibodies by ELISA, with the proportion of positive samples highest in cattle, followed by sheep, and lowest in goats, but not differing by month. Seroprevalence increased with age for both cattle and sheep in households sampled in both regions. These data suggest sheep, but not goats, play a role in maintaining FMD. VP1 sequences were generated from five samples (one serum and four environmental swabs), all of which were serotype O, EA-3 topotype. These results demonstrate the utility of oral swabs in sheep and environmental swabs as indicators of ongoing infection, especially when combined with sequencing.

Occurrence and Control Strategies for Foot and Mouth Disease in Uganda: Systematic Review and Meta-analysis

Benedicto Byamuakama[1], Asfor Amin[1], Abel Bulamu Ekiri[1], Frank Nobert Mwiine[2]

[1] University of Surrey
[2] Makerere University

Background: Foot-and-mouth disease (FMD) is a highly contagious viral disease affecting cloven-hooved animals. Despite the availability of effective control measures, occurrence of FMD outbreaks continues to be a big problem in Uganda. This review examined the epidemiological patterns of FMD in Uganda over the past sixty-four years and the existing control strategies and related gaps.

Methods: For this review, the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines were followed. Literature searches were performed in the databases PubMed, ScienceDirect, Scopus, and Web of Science. In addition, FMD reports from the World Reference Laboratories for FMD (WRL-FMD), and the World Organization for Animal Health (WOAH) websites were examined. Data analysis included descriptive and meta-analyses.

Results: Data from 26 eligible articles and 36 reports was reviewed. Serotype O was the most frequently isolated in reported FMD outbreaks in Uganda. Other commonly reported serotypes were A, SAT 1, SAT 2, and SAT 3, and serotype C was rarely reported. Notably ASIA 1 is the only serotype of the seven FMD serotypes that has never been reported in Uganda. High cattle density, low annual rainfall, and pastoralism were identified as major risk factors for FMD, with spatial clustering near international borders. Quarantine and vaccination were the most reported control strategies, and the challenges related to these control strategies were timely response and vaccine distribution. Small and medium herds were reported to incur higher FMD control costs. The gaps highlighted in reviewed studies included insufficient disease control measures, lack of regular sample submissions and reporting of outbreaks, and weaknesses in available laboratory capacity.

Conclusion: The above findings suggest that improved FMD surveillance and reporting combined with increased vaccination coverage are crucial to supporting effective national and regional FMD control strategies and achieving the goals of the Progressive Control Pathway for Foot-and-Mouth Disease (PCP-FMD).

Sero-survey of foot-and-mouth disease virus serotypes O and A in selected livestock-wildlife interface areas in Tanzania

Mathias Mashinagu Mkama[1], Raphael Sallu[1], Robert Fyumagwa[2], Ernest Mjingo[2], Misheck Mulumba[3], Mark Rweyemamu[4], Philemon Nyangi Wambura[5], Francois Maree[3], Donald King[6] and Christopher Jacob Kasanga[5]

[1] Tanzania Veterinary Laboratory Agency
[2] Tanzania Wildlife Research Institute
[3] ARC - Onderstepoort Veterinary Institute
[4] Southern African Centre for Infectious diseases Surveillance
[5] Sokoine University of Agriculture
[6] The Pirbright Institute

Foot-and-mouth disease (FMD) is caused by FMD virus (FMDV). The virus belongs to the genus Aphthorvirus of the family Picornaviridae. There are seven FMDV serotypes, of which the South African Territories (SAT1, SAT2, and SAT3) serotypes cause persistent and inapparent infection in African buffalo (Syncerus caffer), which acts as the reservoir. Work in southern Africa indicated that these serotypes were transmitted from buffalo to cattle. It remains unclear whether the buffalo can also act as carrier of the EurAsian serotypes O and A, which occur in parts of East Africa. This study investigated the seroprevalence of FMDV serotypes O and A in Tanzania where SAT1, SAT2, O and A had been reported to be endemic. A total of 247 buffalo (n =93) and cattle (n =154) positive sera by 3ABC-NSP ELISA test from livestock-wildlife interface areas in Katavi, Mikumi, Mkomazi, and Ruaha ecosystems in Tanzania were used. The typing of sera for serotypes O and A FMDV antibodies was conducted by serotype-specific SPCE using ISZLER kit. Serotyping results revealed 67% (n= 103) FMDV serotype O antibodies in cattle and 55% (n= 51) serotype O antibodies in buffalo whereas the positive sera for FMDV serotype A antibodies were 54% (n= 83) and 25% (n= 23) for cattle and buffalo, respectively. Also, some animals (36%, n= 56 for cattle) and 25% (n= 23 for buffalo) showed evidence for infection with both FMDV serotypes O and A. This study unravels important information for understanding the FMDV epidemiology and infection status in Tanzania and enhances strategic initiatives for the FMD Progressive Control Pathway in livestock-wildlife interface areas in Tanzania and neighbouring countries.

Genetic and antigenic characterization of variants of serotype O/ME-SA/PANASIA sublineage circulating in Pakistan during 2012-2020

Syed M. Jamal[1], Salman Khan[1], Hanif Ur Rahman[2], Syed Asad Ali Shah[3], Muhammad Afzal[4], Asma Riaz[4], Noemi Polo[5], Ginette Wilsden[5], Clare Browning[5], Jemma Wadsworth[5], Nick Knowles[5], Donald P. King[5], Michael Eschbaumer[6], Graham J. Belsham[7]

[1] Department of Biotechnology, University of Malakand, Chakdara, Pakistan
[2] Veterinary Research Institute, Peshawar, Pakistan
[3] Livestock & Dairy Development Department, Khyber Pakhtunkhwa, Peshawar, Pakistan
[4] The Project for Enhancement of Foot and Mouth Disease Control in Pakistan (OSRO/PAK/801/JPN), Food and Agriculture Organization of the United Nations, Islamabad, Pakistan
[5] World Reference Laboratory for FMD, Pirbright Institute, Woking, United Kingdom
[6] Institute of Diagnostic Virology, Friedrich-Loeffler-Institut, Greifswald-Insel Riems, Germany
[7] Department of Veterinary and Animal Sciences, University of Copenhagen, Denmark.

Foot-and-mouth disease (FMD) is endemic in Pakistan and serotypes O, A and Asia-1 of FMD virus (FMDV) are responsible for the outbreaks in the country. The dominant serotype in the country is usually type O and the dominant lineage within serotype O is PanAsia-2, which has diverged into different sub-lineages. Characterization of the viruses in circulation can facilitate appropriate vaccine selection and tracing of outbreaks.

The present study characterized viruses belonging to the O-PanAsia-2 lineage collected during 2012-2020. Phylogenetic analyses using the VP1 coding sequences revealed that viruses belonging to the O/PanAsia-2 lineage grouped into three sub-lineages, of which two sub-lineages are already known, i.e. O/PanAsia-2ANT-10 and O/PanAsia-2BAL-09, while the 3rd one is a new sub-lineage and is designated here as O/PanAsia-2KPK-14. Antigenic profiling of the selected viruses belonging to the O-PanAsia-2ANT-10 and O/PanAsia-2KPK-14 sub-lineages was carried out using antisera raised against reference vaccine strains. Comparison between the antigenic profiling results with the critical amino acid residues present within the complete capsid region of these viruses is reported.

Detection And Characterization Of Foot And Mouth Disease Viruses In Small Rumminants In Nigeria

Olumuyiwa Oyekan[1], Habibu Haliru[1], Abdullahi Jaafaru Gyam[1], Wungak Yiltawe[1], Hussaini Ularamu[1], David Ehizibolo[1], Anna Ludi[2], Valerie Mioulet[2], Georgina Limon[2], Nick Knowles[2], Donald King[2]

[1] National Veterinary Research Institute, Vom, Nigeria
[2] The Pirbright Institute, Ash Road, Pirbright, Woking, UK

Globally, small ruminants represent the largest population of FMD susceptible domestic livestock. However, their role in the epidemiology of FMD is generally neglected and often poorly understood, partly due to the often inapparent nature of clinical disease in these species. Over the years, reported outbreaks of FMD in endemic settings of West and Central Africa have shown that cattle are the dominant FMD-susceptible species. So far, there is paucity of information on the identification of FMDV in small ruminants' population in Nigeria.

Samples from sheep and goats were collected during investigation of reported FMD outbreaks in cattle herd between September – December 2020 and in 2021 from Plateau State, Nigeria. Corresponding blood and oral swab samples (n=178) were taken from small ruminants co-mingling in infected cattle herd, but without any obvious signs of FMD, and epithelial tissue samples (n=9) from sheep and goats with obvious clinical lesions. The serum samples were tested for FMD non-structural proteins (NSP) while the oral swabs and tissue samples were tested for the presence of FMD RNA by real time PCR. Virus isolation was attempted for the rRT-PCR positive tissue samples. Antibodies against FMDV-NSP was detected in 71.9% (128/178) of samples tested. FMDV RNA was detected in 40% (24/60) of oral swab samples that could be tested. Two FMDV serotypes (O and A) were isolated from sheep and goats respectively. Phylogenetic analysis shows that these isolates clustered with isolates from cattle on the same farm.

There is evidence of FMDV infection in small ruminants despite the fact that they most often do not show clinical signs/lesion of the disease. This is the first report of isolation and characterization of FMDV from small ruminants in Nigeria. Whether cattle are the source of infection to small ruminants co-mingling or vis versa is not clear.

Complete genome sequencing of FMDV using nanopore sequencing

Andrew Shaw[1], Lina Gonzalez[2], Ugo Ilhearahu[1], Kebaneilwe Lebani[3,1], Richard Orton[4], Antonello Di Nardo[1], Nick J. Knowles[1], Noemi Polo[1], Graham Freimanis[1]

[1] The Pirbright Institute, UK
[2] Roslin Institute, University of Edinburgh, UK
[3] Botswana International University of Science and Technology, Botswana
[4] MRC-University of Glasgow Centre for Virus Research, UK

The benefits of complete genome sequencing have become increasingly clear, for example the identification of emergent lineages with altered phenotypic characteristics or the fine scale tracking of virus movements. Previously, we showed that it was possible to sequence the capsid-coding region of the FMDV genome using nanopore sequencing technology (Oxford Nanopore Technologies, ONT) following amplification of the P1 region by RT-PCR. Here, we designed a universal RT-PCR based strategy for the amplification of complete FMDV genomes prior to sequencing using the MinION sequencer. The FMDV genome was divided into 20 amplicons (S_scheme). One amplicon encompassed the S-fragment. The remaining 19 amplicons were approximately of equal size and were tiled across the L-fragment. To encompass the global diversity of FMDV, multiple primers were required to target the same footprint. An alternative approach (L_scheme) using three multiplex pools to amplify 7 amplicons was also trialled to minimise the PCR complexity. Initially, primer sets were mixed according to the amplicon. Each mix was screened against a broad panel of serotypes and topotypes isolated from the East African and Southeast Asian pools. As expected, the P2 and P3 regions of all topotypes could be amplified using a limited number of primers, whereas the P1 region required a greater diversity of primers. The sequencing of amplified isolates indicated that the amplification and sequencing approach was broadly universal. A novel computational pipeline was developed in parallel. The pipeline enables the de novo selection of the most closely related sequence to the output reads, in turn allowing real-time assembly of FMDV genomes in the absence of prior information regarding lineage. To evaluate the robustness of the approach, we deployed the amplification and sequencing strategy in Makerere University, Uganda. Near complete genome sequences were amplified using both the S_scheme and L_scheme from environmental and clinical swab samples.

Preliminary validation of multiplex lateral flow devices LFD1 and LFD2 for on-field identification and serotyping of foot-and-mouth disease viruses

Efrem Alessandro Foglia[1], Valerie Mioulet[2], Jozhel Baguisi[2], Harry Bull[2], Sena İnel Turgut[3], Abraham Sangula[4], Laura Anfossi[5], Chiara Nogarol[6], Simone Cavalera[5], Lissie Henry[2], Giulia Pezzoni[1], Sergio Rosati[7], Abdulnaci Bulut[3], Donald King[2], Emiliana Brocchi[1], Santina Grazioli[1]

[1] Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna - IZSLER, Brescia, Italy
[2] World Reference Laboratory for Foot-and-Mouth Disease, The Pirbright Institute, Woking, United Kingdom
[3] The Şap Institute, Ankara, Türkiye
[4] Foot-and-mouth Disease National Reference Laboratory, Embakasi, Nairobi, Kenya
[5] Department of Chemistry, University of Turin, Torino, Italy,
[6] In3diagnostic, s.r.l. Grugliasco (Torino), Italy,
[7] Department of Veterinary Science, University of Turin, Grugliasco (Torino), Italy

Introduction

In foot-and-mouth disease (FMD) endemic regions, which often lack adequate transport systems and equipped laboratories, Lateral Flow Devices (LFDs) represent the simplest tool for rapid on-site diagnosis. Other experimental LFD prototypes offer a user-friendly disease confirmation tool, but lack serotyping capacity, whilst serotype identification is crucial to identify regional transmission patterns and to ensure appropriate vaccine selection in countries where multiple serotypes co-circulate. This study aims to depict the preliminary validation of the LFD1 (Pan-FMDV, serotypes O, A and Asia1) and LFD2 (Pan-FMDV, serotypes SAT1 and SAT2) multiplex prototypes, based on characterized monoclonal antibodies (MAbs), for FMD diagnosis (Pan-FMDV) and simultaneous serotyping.

Materials and methods

Diagnostic sensitivity was evaluated on 217 FMDV positive epithelium homogenates; namely 109 for LFD1 (44 serotype O, 43 serotype A and 22 serotype Asia1) and 108 for LFD2 (59 serotype SAT1 and 49 serotype SAT2). FMDV positivity was diagnosed only with evident clinical signs in 70 samples, while it was confirmed by real-time RT-PCR [1], with Ct value between 7 and 30, in the other 147. All LFD results were compared with IZSLER Antigen-ELISA (Ag-ELISA) kit [2]. Additionally, 70 tissue-cultured FMDV strains (40 serotype O, 23 serotype A and 7 serotype Asia1) were tested to evaluate the ability of the LFD1 to recognize the widest spectrum of FMD viruses. For LFD2 only 15 cultured strains were tested (6 serotype SAT1 and 9 serotype SAT2). A score from 0 to 4 was assigned to the results, reflecting the colour-intensity of the line in the test window.

Results

LFD1

- 91% of serotype O-positive and 86% of serotype A-positive were correctly detected with LFD1, whilst Ag-ELISA correctly serotyped 82% and 86% of serotype O and A, respectively for clinical samples positive for serotype O and A;
- a sensitivity of 73% was observed on positive samples serotype Asia1 often showing barely visible lines scored as 1 (confirmed by low Ag-ELISA OD values);

LFD2

- 75% of serotype SAT1-positive and 67% of serotype SAT2-positive were correctly detected with LFD2, whilst Ag-ELISA correctly serotyped 88% and 82% of the two serotypes SAT, respectively;
- 63% of SATs samples gave positive results on Pan-FMDV line, while in Ag-ELISA only 37% showed the

same result.

Both the devices correctly detected and typed all the tested tissue-cultured strains.

Discussion
LFD1 demonstrated a great diagnostic sensitivity and LFD2 an acceptable one. Both the LFDs showed a high concordance with Ag-ELISA (approaching 100%) and confirmed to be able to correctly detect and serotype various FMDV strains, covering all the 7 pools.

Bibliography
[1] Callahan JD, Brown F, Osorio FA, Sur JH, Kramer E, Long GW, Lubroth J, Ellis SJ, Shoulars KS, Gaffney KL, Rock DL, Nelson WM. Use of a portable real-time reverse transcriptase-polymerase chain reaction assay for rapid detection of foot-and-mouth disease virus. J Am Vet Med Assoc. 2002 Jun 1;220(11):1636-42. doi: 10.2460/javma.2002.220.1636. PMID: 12051502.
[2] Grazioli S, Ferris NP, Dho G, Pezzoni G, Morris AS, Mioulet V, Brocchi E. Development and validation of a simplified serotyping ELISA based on monoclonal antibodies for the diagnosis of foot-and-mouth disease virus serotypes O, A, C and Asia 1. Transbound Emerg Dis. 2020 Nov;67(6):3005-3015. doi: 10.1111/tbed.13677. Epub 2020 Jun 29. PMID: 32530134.

Challenge methods for FMD studies in pigs compared

Phaedra Eblé, Aldo Dekker

Wageningen Bioveterinary Research

In animal experiments where animals are challenged with FMDV, different challenge methods are used. For vaccination challenge experiments in cattle, the European Pharmacopoeia prescribes intradermal inoculation in the tongue. For other (e.g. pathogenesis or transmission) studies in cattle, various other challenge methods have been described, which are used because these represent a more natural route of infection.

For pigs, the situation is more complicated. In the European Pharmacopoeia no guidelines are given for vaccination challenge experiments in pigs. In China, intra-muscular inoculation is used. Intradermal inoculation in the bulb of the heel is often used, also in our research institute. The epithelium of the heel bulb is a site where the virus normally replicates after infection, similar to tongue epithelium. After challenge, pigs should be housed individually, to prevent a secondary challenge by other infected pigs. In our hands, this study outline produces robust, reproducible results. Various other challenge methods for pigs have been described, e.g. intranasal inoculation, contact infection by infected pigs, oral or intra-oral-pharyngeal infection. We tested several of these methods, either to compare with our intradermal challenge method or for use in transmission studies. In our hands, intra-dermal inoculation is most consistent in infecting all inoculated pigs. Moreover, the natural route of infection of pigs is less clear as for cattle. It is known that pigs are more resistant for aerosol infection and that oral infection is possible. The oral route can be mimicked by intra-oral-pharyngeal infection. However, for other pig diseases there are indications that pigs infect each other via bite wounds. We believe that this is also the case for FMDV and that the intra-dermal inoculation mimics this infection route.

We will present results of pig studies with different inoculation methods. We strongly recommend using the intradermal inoculation method for vaccination challenge experiments in pigs.

Revisiting an old classic: Using the IgG1 avidity ELISA to predict cross-protection in the Brehm et al. sample set

Alejandra Victoria Capozzo[1], Nancy Patricia Cardoso[1], Michael Eschbaumer[2]

[1] Institute of Virology and Technical Innovations, INTA-CONICET. Buenos Aires, Argentina
[2] Institute of Diagnostic Virology, Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Suedufer 10, 17493 Greifswald-Insel Riems, Germany.

Serology has been proposed as an alternative to challenge trials to predict protection against foot-and-mouth disease virus strains after vaccination provided a good correlation with in-vivo results can be achieved. Our laboratory developed indirect ELISAs that use purified 146S whole viral particles to assess FMDV-specific IgG avidity and subtypes. In this study, we analyzed if avidity and IgG-subtype ELISAs, and the virus neutralization test used alone or combined can produce results that correlate with cross-protection. Sera from 17 non-protected and 23 protected animals from three different PD50 A22/Iraq challenge trials were tested (1). Animals had been vaccinated 21 days before the challenge with monovalent A/Iran96 or A/Iran 99 vaccines. Neutralizing antibody titers against A22/Iraq were below the detection limit in 26 animals (either protected or non-protected), making further analysis impossible. IgG2 ELISA titres were similar between protected and non-protected animals; however, IgG1 titres and avidity indexes of total IgG against A22/Iraq were significantly higher in protected animals ($p<0.05$). ROC curves were built to establish cut-off values and conditional testing was applied to increase sensitivity. In these conditions, the accuracy of the prediction was 86%, and concordance with protection was substantial (Kappa value K=0.7). These results prompted us to test IgG1 avidity using a single dilution isotype-specific avidity ELISA (IgG1-A-ELISA). We found that all protected animals had IgG1-OD values superior to 0.5, and 96% of the samples from protected animals had an avidity index greater than 25%. Using these two parameters, we estimated protection with an accuracy of 93%. Comparing these results with the challenge trial revealed an almost perfect agreement between them (K=0.83) and confirmed the utility of the IgG1-A-ELISA as a single test to predict cross-protection in FMDV-vaccinated cattle.

(1) K.E. Brehm et al. Vaccine (2008) 26, 1681—1687

Pathogenesis

Leaderless FMDV O is fully attenuated in cattle and does not establish persistent infections

Michael Eschbaumer[1], Benedikt Litz[1], Anja Landmesser[1], Florian Pfaff[1], Sandra Blaise-Boisseau[2]

[1] Friedrich-Loeffler-Institut
[2] Laboratoire de Santé Animale de Maisons-Alfort

The leader protease Lpro of FMDV is an important virulence factor. Leaderless FMD viruses of serotype A, wherein a large part of the Lpro open reading frame has been deleted, are strongly attenuated in cattle and pigs during the acute phase of infection. It has not been investigated, however, if leaderless viruses can cause persistent infection of epithelia in the upper respiratory tract of cattle. Neither has there been any in vivo evaluation of leaderless viruses of other serotypes.

In this experiment, two groups of eight cattle each were inoculated with either wildtype FMDV O/FRA/1/2001 or its leaderless derivative O FRA delta Lpro by intranasopharyngeal deposition. Throughout the experiment, serum, saliva, nasal and oropharyngeal fluid were collected for virological analysis. Two cattle from each group were euthanized 24 hours after inoculation and tissue samples were collected to evaluate early-stage virus distribution. The remaining six cattle in the wildtype group developed pronounced generalized disease and shed large amounts of virus during the first 10 to 14 days of the experiment. The O FRA delta Lpro group did not show any clinical signs of FMD nor was there any detectable virus in serum or secretions. Two of six animals in the wildtype group remained persistently infected until the end of the experiment on day 35, while there was no evidence of persistent infection in the delta Lpro group.

Our experiment confirms the strong attenuation of leaderless FMDV in cattle. No clinical disease, virus shedding or persistent infection was observed after inoculation with a leaderless derivative of virulent O/FRA/1/2001 virus.

In a companion study presented at the conference by Michaud et al., O FRA delta Lpro was also found to be strongly attenuated in multilayered air-liquid interface cultures of bovine soft palate cells and was similarly unable to establish persistent infection.

Leaderless FMDV O does not establish a persistent infection in multilayered cells derived from bovine dorsal soft palate

Sandra Blaise-Boisseau

UMR Virologie, Laboratoire de Santé Animale de Maisons-Alfort, INRAE, École Nationale Vétérinaire d'Alfort, ANSES

A subclinical infection may occur if a virus can counteract the host immune response. For foot-and mouth disease virus (FMDV), the major viral protein known to be involved in host innate response evasion during acute infection is the leader protease Lpro. Indeed it has been previously shown that the deletion of the Lpro-coding region results in highly attenuated viruses in vivo. If both wild type and leaderless viruses replicate at the primary site of infection in the nasopharynx, the leader-deleted viruses failed to generalize and to cause viremia with subsequent disease. It was however not explored if the leaderless FMDV could establish a persistent infection.

Here we have investigated if the a recombinant leaderless FMDV O could persist in-vitro in multilayer cells from bovine dorsal soft palate (DSP) grown at the air-liquid interface (ALI). In this assay, DSP cells were cultured on inserts for five weeks, then inoculated with either recombinant wildtype FMDV O/FRA/1/2001 or its leaderless derivative O FRA delta Lpro, or a placebo. The upper multilayer was washed with culture medium 6, 12, 24 and 48h post-infection and thereafter each two/three days until 35dpi. Wash medium was analysed to detect infectious FMDV and viral RNA. Live wt virus was isolated from supernatants of 5/6 cultures while no leaderless live virus had been detected after 48h post-inoculation of DSP-ALI. These results were confirmed by viral titration. Likewise, leaderless viral RNA decreased from 7dpi until 28dpi to the point of becoming undetectable at 35dpi. Proteogenomics analysis will complete these data.

Overall, our results show that the leaderless FMDV O virus cannot establish a prolonged nor persistent infection in DSP-ALI cells.

In a companion study presented at the conference by Litz et al., O FRA leaderless was also found to be strongly attenuated in cattle and unable to establish persistent infection.

Association between FMDV Persistent Infection and High Aryl Hydrocarbon Receptor Activation of Cattle Sera

Shannon Collinson[1,2], Carolina Stenfeldt[1,3], Luis L. Rodriguez[1], Jonathan Arzt[1] and James J. Zhu[1]

[1] Foreign Animal Disease Research Unit, Plum Island Animal Disease Center, Agricultural Research Service, United States Department of Agriculture, Greenport, NY, United States.
[2] PIADC Research Participation Program, Oak Ridge Institute for Science and Education, Oak Ridge, TN, United States.
[3] Department of Diagnostic Medicine/Pathobiology, Kansas State University, Manhattan, KS, United States.

In cattle, foot-and-mouth disease virus (FMDV) causes primary and persistent infection of the nasopharynx. Our previous studies compared gene expression of the nasopharynx between carriers of persistent infection and non-carrier cattle as well as between bovine nasopharynx and lung tissues. The results showed that FMDV persistent infection was associated with several differentially expressed genes (DEG) related to regulatory T cells (Treg) cells that are known to regulate the immune response. The bioinformatic analysis of DEG suggested that FMDV persistent infection was associated with dysregulation of gene expression including enhanced aryl hydrocarbon receptor (AHR) signaling that is known to stimulate Treg cell differentiation. Ruminants can produce a large amount of AHR ligands in the rumen. Excessive production of AHR ligands such as 3-methylindole (a tryptophan derivative) in the rumen causes fog fever or bovine atypical interstitial pneumonia. Therefore, it is hypothesized that high AHR ligand production in the rumen causes FMDV to persist. This follow-up study aimed to test the hypothesis using a commercial AHR assay to measure AHR activation by cattle and pig serum samples and rumen fluid. The results showed that the sera of bovine carriers had significantly higher AHR activity than those of non-carriers. Additionally, rumen fluid samples collected from naïve cattle showed > 10-fold higher AHR activity than naive sera. More interestingly, pigs are known to not develop persistent infection, and pig sera did not show detectable AHR activity. These findings support the hypothesis of a role for AHR signaling in the pathoetiology of persistent infection and provide a molecular basis for further study to develop a countermeasure.

Incubation phase transmission of FMDV in cattle

Jonathan Arzt[1], Monica Rodriguez-Calzada[1,2], Carolina Stenfeldt[3]

[1] Foreign Animal Disease Research Unit, Plum Island Animal Disease Center, Agricultural Research Service, United States Department of Agriculture, Greenport, NY, United States
[2] PIADC Research Participation Program, Oak Ridge Institute for Science and Education, Oak Ridge, TN, United States
[3] Department of Diagnostic Medicine/Pathobiology, Kansas State University, Manhattan, KS, United States, Foreign Animal Disease Research Unit, Plum Island Animal Disease Center, Agricultural Research Service, United States Department of Agriculture, Greenport, NY, United States

Successful control of outbreaks of infectious diseases, such as foot-and-mouth disease (FMD), requires thorough understanding of the pathogen's capability to transmit during distinct phases of infection. Infectious diseases typically have an incubation (pre-clinical) as well as a latent (pre-infectious) period, which often overlap, but are distinct in referring to concepts of signs of disease versus transmissibility. Outbreak control becomes increasingly challenging when the latent period is shorter than the incubation period as this facilitates disease transmission prior to identification of clinical manifestation of infection.

The current investigation was designed to evaluate the occurrence of transmission of FMDV from infected cattle to cohabitating cattle before and after the appearance of objective clinical signs of FMD . For this purpose, two donor cattle were infected with FMDV A24 through intra-nasopharyngeal deposition of inoculum, and were subsequently used for direct contact exposure of 4 groups of 4 contact cattle each, through sequential 24h exposure periods, starting at 24 hours post inoculation (hpi) of the donors.

The 4 cattle exposed to the donors from 24-48 hpi (group 1) were not infected. However, all animals in the three subsequently exposed groups, corresponding to 48-72 hpi (group 2), 72-96 hpi (group 3), and 96-120 hpi (group 4) developed clinical FMD. The first signs of clinical FMD in the two donor cattle consisted of small vesicular lesions on the dental pads of both animals, were observed at 96 hpi, corresponding to the end of the third exposure period. Thus, FMDV was successfully transmitted to the 4 cattle in contact group 2 (48-72 hpi), prior to the occurrence of clinical signs in the donor animals. These findings demonstrate incubation-phase transmission between infected and naïve cattle, which would substantially affect the dissemination of an outbreak compared to scenarios in which incubation-phase transmission did not occur.

Interferon stimulated genes correlate with mammalian resistance to foot-and-mouth disease

Soumendu Chakravarti[1], James Kelly[1], Chris Neil[1], Andrew Shaw[1], Caroline Wright[1], Sam J Wilson[2], and Toby Tuthill[1]

[1] The Pirbright Institute
[2] MRC-University of Glasgow Centre for Virus Research

The cellular antiviral response is part of the mammalian innate immune system and has species-specific components which may contribute to the restriction of viral tropism to particular hosts. The picornavirus foot-and-mouth disease virus (FMDV) infects multiple species of domestic livestock and wild animals, however many other mammalian species such as primates and horses are non-susceptible to FMD. The virus can use integrins of all these species as receptors to enter cells, suggesting that the species-specific tropism of FMDV is determined by a post-entry restriction in the virus life cycle. Interferon-stimulated gene overexpression library screens from human and non-human primate (macaque) identified primate proteins which act as potent restriction factors to FMDV replication. The genes encoding these restriction factors appear to exist only in FMDV non-susceptible species, thus providing a novel potential mechanism for species-level tropism of FMDV. Overexpression of individual primate restriction factors in an otherwise highly susceptible porcine cell line rendered the culture refractory to FMDV infection. Furthermore, targeting endogenous restriction factors by siRNA knock down of protein expression or pharmacological inhibition of protein function made a human cell line more permissive for growth of FMDV. Overexpression of such primate specific FMDV restriction factors did not restrict the human picornavirus EV-71 in primate cells, consistent with host range involving virus co-evolution with specific host species.

Inter-serotypic recombination of foot-and-mouth disease virus following superinfection of carrier cattle

Carolina Stenfeldt, Jonathan Arzt, Ian Fish

United States Department of Agriculture, Agricultural Research Service

We have previously reported on the emergence of recombinant foot-and-mouth disease viruses (FMDV) following a scenario of superinfection of persistently infected cattle, in which initial infection with FMDV serotype A was followed 3 or 5 weeks later by infection with FMDV serotype O. In those studies, we found that all recombinant viruses detected featured the major antigenic regions (capsids) of the second, superinfecting virus. Here, we present a follow-up cattle experiment in which the sequential order of infection and superinfection were reversed, i.e. type O initial infection was followed by type A. Consistent with previous experiments, post-acute phase samples from half of all animals included recombinant viruses. While some recombinant genomes contained the same patterns denoting antigenic evasion, samples from later timepoints in some animals were dominated with further (re-)recombined viruses that encoded capsids belonging to the initial O virus whilst encoding 5'- and 3'-regions belonging to the second (A) virus. Beyond confirming our earlier findings, these studies further demonstrate superinfection of carriers as a source of recombinant viruses in natural settings. These findings are important to understanding the molecular epidemiology of FMDV and may influence policies for management of persistently infected FMDV carriers following FMD outbreaks.

Development of an FMDV minigenome, pKLS3, and its applications

Ploypailin Semkum[1], Porntippa Lekcharoensuk[1], Nattarat Thangthamniyom[1], Challika Keawborisuth[2], Sirin Theerawatanasirikul[3], Penpitcha Chankeeree[1], Wantanee Tommeurd[1]

[1] Department of Microbiology and Immunology, Faculty of Veterinary Medicine, Kasetsart University
[2] Virology and Cell Technology Research Team, National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency
[3] Department of Anatomy, Faculty of Veterinary Medicine, Kasetsart University

The reverse genetics is an essential technology for molecular studies of picornaviruses. Various reverse genetics platforms have been developed for decades, mostly depending on in vitro transcription and RNA transfection, which the effectiveness is limited by the rapid degradation of the RNA template. More recently, DNA-based reverse genetics systems driven by mammalian RNA polymerase I and/or II promoters facilitate transfection of the engineered Foot-and-Mouth Disease Virus (FMDV) into cells; however, both enzymes present only in the nuclear compartment. Herein, we have developed a novel DNA-based vector called pKLS3 which is a T7 polymerase dependent, FMDV minigenome. pKLS3 contains all essential cis-acting elements required for intracytoplasmic transcription and translation of a foreign gene. In addition, we have also generated a helper plasmid to enhance the production of an uncapped foreign mRNA. When the green fluorescent protein (GFP) gene was inserted into pKLS3, the green fluorescent signals were visualized within the transfected cells. In addition, we have shown that the pKLS3 system could facilitate the construction of both homologous and heterologous FMDV infectious clones, which are an essential tool for molecular virological study and vaccine development. We have demonstrated the practical application of pKLS3 for the full-length FMDV infectious clone construction by combining with Gibson Assembly (GA) technique. The full-length FMDV infectious clones were simply generated by the vector-insert assembly in a single GA reaction, which was directly transfected into baby hamster kidney-21 (BHK-21) cells. The infectious FMDVs were rescued which demonstrated growth kinetics and antigenicity similar to their parental viruses. Moreover, we also demonstrated the application of the pKLS3 system in a cell-based antiviral drug screening. Conclusively, the pKLS3 is a useful tool for the generation of FMDV infectious clones and shown to be a potential tool for other applications for the molecular studies and novel vaccine developments.

Mapping of FMDV antigenic sites recognized by single-domain antibodies reveals different 146S particle specific sites and particle flexibility

Michiel Harmsen[1], Haozhou Li[2], Shiqi Sun[2], Wim van der Poel[1], Nishi Gupta[1], Aldo Dekker[1]

[1] Wageningen Bioveterinary Research
[2] Lanzhou Veterinary Research Institute

Vaccination with intact (146S) FMDV particles is used to control FMD. However, 146S particles easily dissociate into pentameric 12S particles which are less immunogenic. We isolated a panel of single-domain antibody fragments (VHHs) that specifically bind either 146S or 12S particles of serotypes A, O, Asia 1 and SAT2. These particle-specific VHHs are excellent tools for vaccine quality control. We mapped the antigenic sites recognized by serotype A specific VHHs by various biochemical techniques, including competition ELISAs. Two separate antigenic sites were found for both 12S-specific and 146S-specific VHHs. The major 146S specific site recognized by two broadly strain reactive VHHs was further mapped by cross-linking mass spectrometry (XL-MS). The epitopes were located close to the 2-fold and 3-fold symmetry axes of the icosahedral virus 3D structure, mainly on VP2 and VP3. Since the epitopes were located on a single 12S pentamer, the 146S specificity cannot be explained by the epitope being split due to 12S pentamer dissociation. In an earlier study the cryo-EM structure of the 146S-specific VHH M170 complexed to type O FMDV was resolved. The 146S specificity was reported to be caused by an altered conformation of this epitope in 12S and 146S particles. This mechanism probably also explains the 146S-specific binding by the two type A VHHs mapped by XL-MS since their epitopes overlapped with the epitope recognized by M170. Surprisingly, residues internal in the 146S quaternary structure were also cross-linked to VHH. This probably reflects particle flexibility in solution. Having a complete panel of 146S-specific VHHs suitable for all relevant FMDV vaccine strains enables their broad application for replacing animal experiments in vaccine batch release testing. However, some important vaccine strains are not recognized by the currently available 146S-specific VHHs. Therefore, we currently optimize the broadness of strain recognition of 146S-specific VHHs by molecular evolution.

Immunology

Heterologous cross-neutralization with different Foot and Mouth Disease vaccine schemes against serotype O strains

Melanie Benito-Barrios[1], María Cruz Miraglia[2], Sabrina Galdo-Novo[1], Ana Taffarel[1], Danilo Bucafusco[2], Alejandra Capozzo[2], Manuel Borca[3], Mariano Perez-Filgueira[2]

[1] Direccion de Laboratorio, Servicio Nacional de Sanidad y Calidad Agroalimentaria
[2] Instituto de Virología e Innovaciones Tecnológicas, INTA-CONICET
[3] Plum Island Animal Isease Center, ARS-USDA

Foot-and-mouth disease (FMD) is a highly infectious disease with potential for rapid spread and severe economic impact. The development of good quality vaccine banks with broad antigenic spectrum coverage is paramount and requires in deep studies to understand how heterologous protection is gained. We have previously shown that increased intra-serotypic cross-protection may be reached for serotype A FMD virus (FMDV) with vaccines including heterotypic strains. Here, we determine the heterotypic cross-reaction using different vaccine schemes against FMDV serotype O strains from different lineages, evaluating the effect of revaccination, different antigen loads, and incorporation of additional FMDV serotypes. Naïve cattle were immunized with seven FMDV vaccines carrying different strain compositions and antigenic payloads. To test the effect of revaccination, each experimental group (n=3) received three immunizations of the same formulation at 0, 28 and 56 days post-primary vaccination (dpv) and serum samples were taken up to 70 dpv. Immune sera were tested by a virus-neutralizing test (VNT) against four serotype O FMDV strains from the same topotype as the vaccinal strain (O1/Campos) and other topotypes from East Asia (EA). Starting at the second revaccination, the heterologous neutralizing capacity was improved particularly in vaccines containing the O1/Campos strain, as monovalent (high payload), bivalent o trivalent formulations against most of FMDV strains tested. High payload O1/Campos monovalent vaccines reached high VNT titers (> 2.0 log10) against heterologous strains faster than lower payload formulations. Neutralizing responses against EA region strains (O Taiwan and O SKR 84 ydm) showed high VNT titers after the first revaccination at 28 dpv. Both South American strains obtained during the same outbreaks (O Ecu 46/10 and O Ecu 56/10) showed divergent responses, indicating that even subtle differences in capsid proteins may impact the ability of the immune sera to neutralize the infective FMDV.

Maternally derived antibodies to FMDV modulate the antigenic specificity of the antibody response in vaccinated cattle

Jamaliah binti Senawi[1], Ginette Wilsden[2], Clare Browning[2], Anna Ludi[2], Kasia Bankowska[2], Simon Gubbins[2], Don King[2], David Paton[2]

[1] Department of Veterinary Services, Malaysia
[2] The Pirbright Institute

Foot-and-mouth disease (FMD) is an important livestock disease and vaccination is a critical tool for its control in the many countries where the disease is endemic. Where vaccination is performed routinely, adult cattle develop antibodies to the FMD virus (FMDV) which may be passed to calves in colostrum and interfere with the response to calfhood vaccination. This study was designed to optimize the vaccination regime of calves in Malaysia by measuring the neutralising antibody responses of 52 calves before and after vaccination with a one or two dose vaccination regime starting at 2-7 months old. The presence of maternal antibody was associated with poor post-vaccination antibody responses after a single dose of vaccine in calves less than 6 months old. However, a second dose of vaccine given three weeks later, improved the antibody responses in all ages of calves. Sera from cows and pre-vaccinated calves neutralised homologous serotype A vaccine virus more strongly than a heterologous serotype A field virus, but this pattern was reversed in some calves after vaccination. The strength of heterologous response in calves at 49 days post vaccination correlated to the amount of transferred maternal antibody, so epitope masking might have modulated the specificity of the active antibody response. If confirmed, such an effect by pre-existing antibodies could have wider implications for broadening the coverage of FMD vaccine responses.

Impact of Fasciola hepatica infection on the long-term immune response induced by Foot-and-Mouth disease vaccination

Alejandra Victoria Capozzo[1], Monique Costa[2], Florencia Celeste Mansilla[1], Juan Manuel Sala[3], Teresa Freire[2]

[1] Instituto de Virología e Innovaciones Tecnológicas "IVIT", CONICET-INTA, Buenos Aires, Argentina
[2] Laboratorio de Inmunomodulación y Vacunas, Departamento de Inmunobiología, Facultad de Medicina, Universidad de la República, Montevideo, Uruguay
[3] Estación Experimental Mercedes INTA, Corrientes, Argentina

Fasciola hepatica is a worldwide distributed helminth that causes a robust immunoregulatory effect in the host, increasing the susceptibility to secondary infections. The impact of fasciolosis on the immune response induced by Foot-and-Mouth disease (FMD) vaccination in cattle has never been assessed. Our objective was to evaluate whether the infection by F. hepatica in cattle influenced the long-term immunity elicited by a currently used commercial FMD-inactivated vaccine.

Aberdeen Angus steers (n=36) negative for F. hepatica were vaccinated twice against FMD virus (FMDV) during the first 6 months of age using a commercial oil vaccine formulated with A24/Cruzeiro and O1/Campos strains. Animals were divided into two groups and infected with 500 metacercariae/animal (n=24) or mock-infected (n=12) when maternal antibodies against F. hepatica were weaned. Individual serum samples were collected at 0, 15, 28, 43-, 59-, 7- and 87 days post-infection (dpi). Indirect ELISAs were used to assess A24/Cruzeiro-specific bovine IgG and IgG subtypes.

FMD IgG antibody levels and IgG-avidity did not show significant differences between all the groups. IgG1 levels significantly decreased in infected animals at 28 dpi compared to controls and their avidity was also statistically lower ($p<0.01$). These results show that F. hepatica infection modified anamnestic responses against FMDV. This is the first report of immune regulation of F.hepatica modifying the immune response elicited by a commercial FMD vaccine. Vaccine efficacy studies in endemic areas should consider the impact of helminth infection.

Optimising prime-boost interval to Enhance FMD Vaccination Efficacy in Cattle

Ranjitha Bommanna[1], Eva Perez-Martin[1], Marie Bonnet-Di Placido[1], Helen M. E. Duyvesteyn[2], Elizabeth E. Fry[2], John Hammond[1], Ian Jones[3], Sophie Jegouic[3], Carina Kahl[4], Silvia Loureiro[3], Claudine Porta[2], Angela W. Steyn[1], David I. Stuart[2, 5], Michiel Verwoolde[4], Erwin van den Born[4], Bryan Charleston[1]

[1] The Pirbright Institute
[2] Division of Structural Biology, Wellcome Trust Centre for Human Genetics, Oxford, UK
[3] University of Reading, Health and Life Sciences Building, Reading, UK
[4] MSD Animal Health, Boxmeer, The Netherlands
[5] Diamond Light Source, Oxford, UK

Enhancing the duration of immunity of foot-and-mouth disease (FMD) vaccines holds substantial benefits for effective disease control. Our study investigated the optimal prime-boost immunisation strategy using thermostable tetravalent virus-like particles (VLPs) vaccine containing O/TUR/5/2009, A/Sau/1/2015, Asia1/Shamir/ISR/89 and SAT2/ETH/65/2009 antigens, that would provide longer-term protection in cattle. Three distinct groups of cattle were prime-vaccinated and subsequently boosted at different intervals: Group 1 at one month, Group 2 at three months, and Group 3 at six months post-prime vaccination. Neutralising antibody responses were evaluated each month by virus neutralisation test (VNT) over a period of 1 year. All three groups showed an increase in neutralising antibody titres (>1.6log10) after prime immunisation. Group 1 showed an increase in neutralising antibody titres after boost that declined to below the threshold of protection (<1.6log10) by 4 months after booster vaccination, thus antibody titres were >1.6log10 for 5 months in total. Group 2 showed robust sustained neutralising antibody titres of >1.6log10 after prime and boost and so providing 12 months of predicted protection. In Group 3, the antibody titres were >1.6log10 for three months post prime and were then boosted to >1.6log10 for 6 months after boost. Therefore, there was a period of three months over the 12-month period where animals will probably not be protected. Group 2 showed the highest frequency number of antigen-specific antibody-secreting cells identified as CD40+/IgL+ SSChiCD138+ B cells at 3 days post-boost, correlating with the higher VNT titres. Furthermore, all groups received a second booster at twelve months after the first immunisation and no differences in the magnitude of neutralising antibodies were found between groups. Collectively, our findings suggest that a three-month prime-boost interval using thermostable tetravalent VLPs yields an optimal antibody response against FMDV in cattle. This study highlights the importance of fine-tuning prime-boost intervals to enhance FMD vaccination protocols, thereby supporting more effective disease control strategies.

An overview on the efficacy of novel FMDV vaccines carrying antigenic signatures

Elizabeth Rieder[1], Katherine Pflaum[1], Ignacio Fernandez Sainz[1,2], Jessica Canter[1,2], Arlind Mara[1], Michael Oldakowski[1], Fayna Diaz-San Segundo[1], Will Fischer[3]

[1] U.S. Department of Agriculture, Agricultural Research Service, Plum Island Animal Disease Center, NY 11944, USA.
[2] Kansas State University College of Veterinary Medicine, Manhattan, KS 66506, USA.
[3] Group T-6 (Theorical Biology), MS K-710, Los Alamos National Laboratory, Los Alamos, NM 87545, USA

Foot-and-mouth disease (FMD) is a highly contagious an economically important disease of cloven-hoofed animals. It is an OIE notifiable disease that remains a global threat to trade in livestock and livestock products. In endemic countries like those in Africa, FMD causes billions of dollars (USD) in losses each year. Although inactivated whole antigen vaccines and effective policy measures have been applied to control FMD; only a limited number of strains are available as commercial vaccines, as a result, animals vaccinated against one virus strains do not warrant protection against infection from another viral isolate. We have applied bioinformatic design methods that incorporate natural antigenic variation to the capsid coding P1 region of the FMDV genome and tested these novel vaccines in proof of concept. Here we discuss the progress that has been achieved in the development of improved vaccine seed viruses using reverse genetics and their performance in animal efficacy studies.

Potential live-attenuated FMDV serotype O IND R2/1975 vaccine candidate strain generated through genome re-coding approach

Jitendra Kumar Biswal, Rajeev Ranjan, Jajati K. Mohaptra, Saravanan Subramaniam, Rabindra Prasad Singh

ICAR-National Institute on Foot-and-mouth Disease, India

FMD is endemic in many parts of the Asian and African continents and is primarily controlled by cell-culture-derived inactivated whole virion vaccine formulated with a suitable adjuvant. However, one of the major limitations of the current FMD vaccine is the short duration of protective immunity offered by the vaccine. It is well-known that rapid, and long-lasting protective immunity against virus infection is usually best achieved by the use of the live-attenuated vaccine. In the past, using the live-attenuated vaccine, viral diseases such as Rinderpest and smallpox had been eradicated from the globe. Therefore, the availability of live-attenuated vaccine against FMD could be a game-changer for the control and subsequent eradication of the disease.

In our endeavor to generate a live-attenuated FMD vaccine strain, we modified the genome of the FMDV O IND R2/1975 virus to harbor serine and leucine codons with non-sense mutation targets (i.e the codons that could generate stop-mutations/non-sense mutations after a single nucleotide substitution). Through the synthetic biology approach, serine and leucine amino acid codons (n=101) located on the capsid-coding region of FMDV O IND R2/1975 genome were re-coded to codons that could generate stop-/non-sense mutations after a single nucleotide substitution during virus replication. We have also deleted the non-essential region of the 3A-NSP region (amino acid 93-142 region) in order to provide negative-marker vaccine characteristics to the potential live-attenuated vaccine strain. Subsequently, using reverse genetics technology recombinant genetically defined FMDV O IND R2/1975 virus was rescued in cell culture. After rescuing the modified virus, the growth kinetics and plaque morphologies were studied on various cell monolayers. The results suggested that the rescued recombinant virus is 100 times slower to replicate compared to the parental virus. In the suckling mouse model study, it was determined that all the suckling mice (n=6) inoculated with parental FMDV (106.3 TCID50/ml) died by 24 hours post-inoculation, while the suckling mice (n=6) inoculated with the same virus-titer of re-coded FMDV O IND R2/1975 died on 5-6 days post-inoculation.

Therefore, the results highlight the potential use of genome re-coded FMDV as a strategy to generate live-attenuated FMDV vaccine strain and its subsequent application in FMD-susceptible species.

Rethinking next-generation FMDV vaccines: use of codon deoptimization strategies

Gisselle N. Medina, Sarah Attreed, Monica Rodriguez-Calzada, Christina Silva, Edward Spinard, Paul Azzinaro, Amy Burklund, Ryan Heimroth, Carolina Stenfeldt, Elizabeth A. Rieder, Bruce Taillon, Steffen Mueller, Fayna Diaz-San Segundo and Teresa de los Santos

United States Department of Agriculture

Foot-and-mouth disease virus (FMDV) is a rapidly replicating virus whose natural infection in susceptible species does not elicit a complete and durable protective immunity. Vaccination with the inactivated adjuvanted FMDV vaccine continues to be a vital strategy for prevention and response against the disease. However, various challenges remain in using this vaccine such as the necessity for a highly controlled biosafety III laboratory for production, the lack of intra- and inter-serotype elicited protection and the relative short-term induced immunity. To effectively control outbreaks in endemic areas and ultimately eradicate the disease, it would be highly desirable to develop new vaccine platforms. In this study, we have, for the first time, evaluated a codon deoptimized live-attenuated vaccine candidate in cattle. The vaccine was developed by recoding the P2/P3 coding region in an A24-Cruzeiro-DIVA backbone by using codon pair bias deoptimization. One group of animals were inoculated intradermolingually with 106 PFU of vaccine candidate to evaluate safety, and two groups were intramuscularly with 106 or 105 PFU of vaccine to evaluate efficacy. Efficacy groups were boosted at 14 days post-inoculation (dpi). None of the animals developed clinical signs of disease post inoculation, and all of them were protected from clinical disease after challenge with wild type A24 FMDV. Strong neutralizing antibody responses were detected prior to challenge, peaking by 28 dpi (14 days post challenge). At 3 days post-challenge (dpc), vaccinated animals in efficacy groups exhibited a notable increase in the percentage of NK cells and both CD4+ and CD8+ effector memory T cells (Tem) positive for IFNγ. We also assessed the DIVA compliance of our vaccine through a 3ABC ELISA and found that all animals remained clear of these antibodies up to the time of infection with WT virus. Our work demonstrates that codon deoptimization is a viable technology to derive novel LAV candidates that are both immunogenic and efficacious against FMD in cattle.

Tackling FMD in Eastern Africa using stabilized virus-like particles vaccines

Eva Perez-Martin[1], Ranjitha Bommanna[1], Helen Duyvesteyn[2], Liz Fry[2], Ian Jones[3], Sophie Jegouic[3], Silvia Loureiro[3], Claudine Porta[2], David Stuart[2], Michel Verwoolde[4], Erwin van den Born[4], Bryan Charleston[1]

[1] The Pirbright Institute, Pirbright, Surrey, GU24 0NF, United Kingdom
[2] Division of Structural Biology, University of Oxford, Wellcome Trust Centre for Human Genetics, Roosevelt Drive, Oxford, United Kingdom
[3] University of Reading, Health and Life Sciences Building, Reading, United Kingdom
[4] MSD Animal Health, Boxmeer, Netherlands

Given recent outbreaks and the endemic nature of foot-and-mouth disease (FMD) throughout Eastern Africa, there is an urgent need to make high-quality FMD vaccines more widely available to combat the disease in the region. To address this, we evaluated the potential of a FMD vaccine platform based on virus-like particle (VLPs). We expressed the VLPs of several regionally relevant Eastern Africa foot-and-mouth disease virus (FMDV) antigens (O/Eth/8/2013, A/Egy/18/2012, A/Ken/5/2012; SAT2/Eth/65/2009; SAT2/Sud/3/13, and SAT1/Ken/80/10) with amino acid substitutions that confer enhanced thermostability of the VLP, as demonstrated by 75s-specific ELISAs. We assessed their immunogenicity in cattle in a prime-boost immunization separated 28 days. All vaccines elicited high neutralizing antibody titres (>1.6log10) against the homologous virus from day 7 to day 42 post-vaccination. As expected, these titres further increased after the booster vaccination. Sera from vaccinated cattle at days 21 and 35 post-vaccination (one-week post-boost) were screened by virus neutralizing test, against the AgResults panel. The AgResults panel comprises four FMDV isolates representing each of the four serotypes assembled by the WRLFMD (UK), tailored to cover the genetic diversity within the FMDV lineages that circulate in Eastern Africa countries. All vaccines tested except for A/Ken VLP met the AgResults pass criteria at day 21. After the booster, all tested vaccines met the pass criteria, demonstrating their potential to be used in the region. VLP vaccines show great promise as a tool in the fight against FMD in Eastern Africa.

POSTER PRESENTATIONS

Diagnostics

Molecular Differentiation and Phylogenetic Analysis of the Egyptian Foot-and-Mouth Disease Virus Collected from Ismailia Governorate, 2021

Nourhan Ahmed Ashour[1], Mohamed Fawzy[2], and Mohamed Mahmoud[3]

[1] Department of Botany and Microbiology, Faculty of Science, Suez Canal University, Ismailia 41522, Egypt.
[2] Department of Virology, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, 41522, Egypt.
[3] Department of Animal Medicine (Infectious Diseases), Faculty of Veterinary Medicine, Suez Canal University, Ismailia, 41522, Egypt.

Foot-and-mouth Disease (FMD) is a serious contagious viral disease affecting cloven-hoofed animals. Egypt is endemic by three FMDV serotypes out of seven, serotype O, A and SAT2. This study aimed to detect and characterize FMDV serotype O and SAT2 in clinically affected cattle and buffaloes in Ismailia governorate, Egypt during 2021. Furthermore, compare between virus isolation (VI) and real time RT-PCR (rRT-PCR) in diagnosis of FMDV. Twenty oral epithelial tissues were collected then tested by virus Isolation (VI) and real time polymerase chain reaction (rRT-PCR) that was applied only for FMDV serotypes. All the tested twenty tissue samples were positive by rRT-PCR. However, only 17 (85%) samples successfully isolated by VI that confirmed by rRT-PCR. All the positive samples by rRT-PCR were subjected to RT-PCR for detection of FMDV serotype SAT2 and O with subsequent amplification of VP1 region for further characterization. Out of 20 samples, 11 samples (55%) were positive and further phylogenetic analysis revealed circulation of serotype O and SAT2. Phylogenetic analysis demonstrate presence of East Africa-3 topotype (EA-3) of serotype O which is closely related to O/SUD/8/2008 with identity 95%, but differs from vaccine strain (O/PanAsia-2) of ME-SA topotype by 15.4%. Serotype SAT-2 was closely related to the previously detected strains in Egypt 2012 outbreak (SAT2/EGY/A/2012 of topotype VII, lineage SAT2/VII/ALX-12) with percentage of identity ranged from 99.1% to 99.8%. In conclusion, rRT-PCR is highly sensitive and specific assay for diagnosis of FMDV. In addition, FMDV Serotype O topotype EA-3 strains and serotype SAT2 topotype VII is still circulating and causing clinical disease in cattle and buffaloes population in Ismailia governorate.

Development of foot and mouth disease serotype-specific blocking ELISAs using monoclonal antibodies

Shawn Babiuk, Patrycja Sroga, Hamza Amjad, Kate Hole, Sean Yeo, Ming Yang Charles Nfon

National Centre for Foreign Animal Disease

Introduction: Serology is used routinely to identify the presence of serotype specific antibodies to foot and mouth disease virus. Currently, a solid-phase competitive enzyme-linked immunosorbent assay (cELISA) is used for diagnostics. These cELISAs use serotype specific rabbit anti-FMD antisera to capture inactivated foot and mouth disease virus antigen. Test sera is added to compete with guinea pig anti-FMDV antiserum and incubated. A conjugate consisting of anti-guinea pig IgG-horse radish peroxidase (HRP) is used to detect the amount of guinea pig antibodies bound and detection is performed using a chromogenic substrate.

Objective: The objective of this study was to develop blocking ELISAs using conjugated monoclonal antibodies specific for A, Asia1, O, SAT 1, SAT 2 and SAT 3 FMDVs to replace the guinea pig anti-FMDV antiserum currently used for these assays. A comparison between the currently used FMD cELISAs and the blocking ELISAs were completed.

Results and conclusions: Sera that were negative for antibodies specific for FMDV from cattle, swine, sheep and goats were used to determine the specificity of each serotype specific blocking ELISA. FMDV serotype specific antibody positive sera from cattle, swine and sheep was used to determine the sensitivity of the blocking ELISAs. Controls were tracked for each ELISA and demonstrated the repeatability of the ELISAs. The sensitivities and specificities of the serotype specific blocking ELISAs are comparable to the competitive ELISAs. The use of conjugated monoclonal antibodies to replace guinea pig polyclonal sera reduces the number of reagents required and shorten the turn-around time, allowing for improved assays which can be more easily transferred between laboratories.

Development and Validation of a Field-Applicable CMOS Image Sensor-Based Multi-Well Real-Time Reverse Transcription PCR System for Rapid and Accurate Detection and Serotyping of Foot-and-Mouth Disease Virus

Seong-Min Seo[1], Sang-Eun Han[1], MinSik Song[1], Jae-Hyoung Seo[1], Doyoung Lee[1], Soyoon Ryoo[2], Da-Rae Lim[2], Hyeonjeong Kang[2], Sang-Ho Cha[2], Jae-Myung Kim[2], HyeonWoo Hwang[2], Seoyeon Park[2]

[1] OPTOLANE Inc.
[2] Animal and Plant Quarantine Agency

This study was initiated based on the necessity of rapid and accurate detection and serotyping of the Foot-and-Mouth Disease Virus (FMDV) for effective management of FMD outbreaks. Existing methods, which require complex gene extraction processes, are difficult to apply directly in the field. Also, the increasing rate of serotyping failure with current methods has called for the need for a more accurate, sensitive, and field-applicable molecular diagnostic technology.

To solve this problem, our research has developed a Complementary Metal-Oxide-Semiconductor (CMOS) image sensor-based multi-well real-time reverse transcription PCR (rRT-PCR) system. This novel system integrates PCR functionalities into a semiconductor chip, overcoming the limitations of traditional real-time PCR equipment that required optical system calibration. This offers significant advantages in portability and direct molecular diagnostics in the field.

Moreover, this system enables accurate detection of viral RNA from various sample types through a simple sample pretreatment process, without the need for complex gene extraction. Using this system, we fixed specific primers and probes in each of the nine multi-wells to distinguish between FMDV and non-FMDV similar viruses and classify O, A, and Asia1 serotypes of FMDV. This allows for the detection and classification of the virus simply by injecting the sample.

Our system has shown equivalent analytical sensitivity and specificity compared to the lab-based rRT-PCR analysis method recommended by the World Organization for Animal Health (OIE), demonstrating its ability to accurately and quickly detect and classify viruses.

Through these findings, we have proposed a new direction that offers a rapid and accurate diagnostic tool that can be used directly in the field for effective management of FMD. Following on, we plan to verify the clinical sensitivity and specificity of this system, striving to achieve our research goal of providing a more practical diagnostic tool.

Filter paper as an alternative method for transportation of samples in resource-limited areas in Africa for diagnosis of foot-and-mouth disease virus

Sengiyumva Kandusi[1], Herbertha Mpete[1], Ramadhani Juma Makasali[1], Mathias Mashinagu Mkama[2], Abel Lupala[1], Philemon Nyangi Wambura[1], and Christopher Jacob Kasanga[1]

[1] Sokoine University of Agriculture
[2] Tanzania Veterinary Laboratory Agency

Foot-and-mouth disease (FMD) is a highly contagious viral vesicular disease of cloven-hoofed animals and poses major constraints to international trade in livestock production. The disease is caused by FMD virus (FMDV), an RNA virus classified within the genus Aphthovirus of the family Picornaviridae. FMD is endemic in most resource-limited countries where maintenance of cold chain during sample storage and transportation is a major constraint. In this study, we evaluated the ability of novel filter paper(s) in sample transportation, storage, RNA recovery and RNA detection of FMDV using one-step RT-PCR. The FMDV positive epithelial tissues were grounded in 10% PBS, placed on the filter paper(s) and allowed to air dry for 30 minutes. The filter papers were placed at different temperatures for four weeks. To recover the sample from filter paper, the area with the sample was aseptically punched and placed in 43ppendorf tube containing 500µL of nuclease-free water and allowed to dissolve overnight. Filter paper was removed and Viral RNA extracted from supernatant using the Qiagen Viral Mini kit according to manufacturer's instruction. The one-step RT-PCR was performed onto supernatant for detection of FMDV genome. The findings indicated that the RT-PCR results obtained on RNA recovered from filter papers kept at different temperature conditions were similar to the results obtained from the positive control FMDV samples. These findings indicate that the novel filter papers have the ability to maintain the integrity of RNA genome and could be used for transportation, storage and subsequent analysis of FMDV viral RNA in resource-limited areas where cold chain condition is a major barrier. This could enhance molecular epidemiological studies of FMDV and related viruses and hence improve the diagnosis of FMDV and selection of the rational control options of FMD in endemic settings in Africa.

Operation and Utilization of a Foot-and-Mouth Disease Serum Bank as a National Reference Laboratory for Foot-and-Mouth Disease Diagnosis in South Korea

Tae-Woon Kim, Mi-Young Park, Hyun-Ji Seo, GyeongMin Lee, JaeMyoung Kim, Eun-Jin Choi

Animal and Plant Quarantine Agency

The Animal and Plant Quarantine Agency (APQA) in South Korea operates a Foot-and-Mouth Disease (FMD) diagnostic laboratory, which is the national standard laboratory for FMD and supports studies related to FMD diagnosis. APQA has been collecting and storing sera that support studies on FMD infection occurring outdoors or assist FMD vaccination policies in South Korea. Since 2017, APQA has established a standardized serum bank for FMD, supporting the use of the sera collected. This study aims to secure and utilize FMD sera according to the rules of the FMD standardized serum bank. APQA collects sera, including sera from outdoor infection, FMD vaccine sera, field sera, and sera from countries introducing FMD-free status. These are evaluated using antibody testing methods and are then added to the serum bank, according to its management procedures. The stored sera are classified into categories based on characteristics, such as animal species, collection date, FMD virus infection status (infected/non-infected), vaccination status (vaccinated/non-vaccinated), structural protein (SP) serotype (O-type, A-type, and Asia1-type), and non-structural protein (NSP) type (positive/negative). Currently, there are approximately 1,100 FMD-related samples in the serum bank. Among these samples, a panel of 524 standardized sera are used for specific purposes, such as standardization of antibody diagnostic methods, evaluation of effectiveness for antibody diagnostic kit development, proficiency testing for regional animal hygiene laboratories, validation of SP and NSP serum screening ELISA diagnostic kits, and determination of antibody titers for vaccine-derived SP-O type. APQA utilizes or distributes these standardized sera panels for its own use. Our organization envisages further collection of national standardized serum panels to support FMD diagnosis and research and to secure panels with performance equivalent to international standards by collaborating with overseas institutions, such as the world Reference Laboratory, thereby supporting our role as an international standard laboratory for FMD.

Lateral flow devices for the rapid detection of FMDV

Valérie Mioulet[1], Jozhel Baguisi[1], Elizabeth Henry[1], Harry Bull[1], Britta Wood[1], Amy McCarron[1], Donald King[1], Efrem A. Foglia[2], Santina Grazioli[2], Andrew Bentham[3], Kerry Mitchell[3], Alison Wakeham[3]

[1]The Pirbright Institute
[2]Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna
[3]Global Access Diagnostics

FMDV is an economically devastating disease of livestock and is one of the most contagious viral diseases. Its rapid detection is critical to efficient control in outbreak situations.
Lateral flow devices (LFDs) are designed to rapidly detect FMDV antigen. Here we describe the recreation of LFDs that were previously available from Svanova/Boehringer-Ingelheim through a partnership between the Pirbright Institute, IZSLER and Global Access Diagnostics (UK). These rely on the 1F10 antibody, which has been extensively characterised through its use in both LFD and ELISA tests. We also present the evaluation of new LFD devices that rely on the use of bovine integrin αvβ6 and the 1F10 antibody.

Both devices underwent optimisation in terms of membranes and buffers. A representative panel of FMDV isolates (n=85) belonging to serotypes O, A, Asia1, SAT1, 2 and 3 and a variety of currently circulating lineages was tested on both devices and signal intensities were recorded using a score card (0 to 10). Scores were recorded after 20 minutes and 89.4% of the isolates were detected on the 1F10:1F10 devices (line intensity 1 or higher), while 94.1% were detected with the 1F10:integrin devices.

Epithelial suspension prepared from field samples were evaluated (n=72). FMDV antigen was detected in 76.4% of these with the 1F10:1F10 devices, 73.6% with the 1F10:integrin devices and 56.9% with the 1F10 component of the IZSLER/Pirbright ELISA. The lower detection levels are likely due to an expected lower viral load in field material, compared to laboratory-propagated isolates.

Specificity was assessed using viral isolates that cause diseases indistinguishable from FMDV, namely vesicular stomatitis, swine vesicular disease, vesicular exanthema of swive and Seneca valley. No signal was detected with any of these isolates (n=8) on either device. Epithelial suspensions prepared from field samples where no virus was detected also generated no signal in either device.

SAT2 remains the more challenging serotype for detection in all test formats. However, it should be noted that epithelial suspensions belonging to the SAT2/XIV lineage currently circulating in Middle East countries were successfully detected using both devices.

The ongoing evaluation and validation of these devices presents a solution for the rapid field and laboratory testing of samples associated with a suspicion of FMD. We are now investigating simple field solution for sample preparation with field tests being planned.

Foot-and-mouth disease laboratory diagnosis in the framework of the PCP-FMD in Nigeria (2011-2022)

Wungak Y. S., Ularamu H. G., Ehizibolo D. O., Lazarus D. D., Agom D., Onoja M., Oyekan O. A., Abdullahi J.G., Habibu H., Isa Z. A., William E., and Muhammad M.

National Veterinary Research Institute, Vom, Nigeria

The National Veterinary Research Institute, Vom, Nigeria was established in 1924 as a Veterinary Department by the colonial powers to control the scourge of rinderpest which was a major cattle plague in the West African coast. However, with the successful eradication of rinderpest in 2010 and the global efforts to control FMD using the Progressive Control Pathway (PCP), the FMD research and diagnostic laboratory of the institution was repositioned to provide the needed capacity for the progressive FMD control programme.

During the period under review (2011-2022), laboratory capacity for FMDV antibody detection, antigen detection, virus isolation, and reverse transcriptase polymerase chain reaction platform was established. Currently, a total of 12,702 sera, 572 epithelial tissue, 396 bovine meat juice, and 69 swine meat juice specimens were received from the field as laboratory submissions for analysis. Of the total sera received 48.5% (95%CI: 47.3-49.7) were from cattle, 11.7% (95%CI: 11.0-12.5) from sheep and goats, 33.8% (95%CI: 32.7-35) from swine, 5.5 % (95%CI: 4.90-6.11) from camels and 0.43% (95%CI: 0.29-0.61) from wildlife species.

Human capacity development involved several staff members obtaining Ph.D., MSc, and other laboratory training over the same period. The laboratory has also participated in several FMD Proficiency tests within this period and has published and presented research outputs at several scientific conferences and workshops. The laboratory also enjoys a good working relationship with the WRLFMD, the Pirbright Institute, UK, Canadian National Centre for Foreign Animal Disease and has had an OIE Laboratory Twinning Programme with the Sciensano, Belgium.

Keywords: Foot and Mouth Disease, capacity, Progressive Control Pathway

Epidemiology

Phylogenetic and genotypic characteristics of the foot-and-mouth disease virus from outbreaks in South Korea, 2023

Hyeonjeong Kang, Soyoon Ryoo, Da-Rae Lim, Jae-Myung Kim, Sang-Ho Cha

Animal and Plant Quarantine Agency, Ministry of Agriculture, Food and Rural Affairs, Republic of Korea.

Foot-and-mouth disease (FMD) is endemic and the most important disease of livestock, having a huge economy of many countries worldwide. Most FMD outbreaks in South Korea since 2000 are investigated to be introduced from foreign countries. In this study details a FMD outbreak in livestock in South Korea, first reported in May 2023. FMD was introduced into South Korea in 2023, which subsequently caused 11 outbreaks of FMD and emerged in 1 provinces in South Korea. In the past 4 years, FMD in South Korea has been well controlled using current vaccines. However, there were several occurrences of FMD outbreaks in cattle farms in North Chungcheong where vaccination against the disease was implemented. Therefore, this study aimed to determine the genotypic characteristics of FMDV strains in these outbreaks.

A total 208 samples (serum(155), tissue(29) and saliva(24)) were collected from cattle(10) and goats(1). Virus isolation was attempted to LFBKavb6 cells. Then, The VP1 region was amplified for sequencing. Following sequencing, the VP1 coding sequences were aligned using CLC Main Workbench 13(QIAGEN). Phylogenetic trees were constructed using MEGAX software with the maximum likelihood method based on the generalized time reversible model and bootstrap values were calculated on 1000 replicates.

Sequencing and phylogenetic analysis of VP1 sequences revealed that this determined strain belongs to O/ME-SA/Ind-2001e and is closely related to strains that have caused recent outbreaks of FMD in Vietnam, Cambodia, Laos and Mongolia. The FMDV field strains in the study shared very high sequence similarity(99.5-100% and 99.53-100% at nucleotide and amino acid levels, respectively). Compared to reference isolated belonging to the same topotype O/ME-SA/Ind-2001e, they also had very high genetic and amino acid homology with South Korean strains in 2019 (94.7% and 98.1%, respectively), in 2020 Vietnam (98.4-98.8% and 98.42-99.5%, respectively), in 2019 Cambodia(98.4-98.8% and 98.58~99.5%, respectively), in 2020 Laos(98.4-98.8% and 98.74-99.5, respectively) and in 2021 Mongolia(97.7-98.7% and 97.95%-99.0%, respectively).

Genetically, the field strains had a very close genetic relationship and probably shared a common origin with some Cambodia and Vietnam strains. These findings of the present study demonstrate that the recently circulating FMDV strains in South Korea in 2023 belong to serotype O, topotype ME-SA, and sublineage Ind-2001e.

Genotypic identification of Foot and mouth disease virus isolated from Cambodia between 2018-2022

Da-Rae Lim[1], Hyeonjeong Kang[1], Soyoon Ryoo[1], Seoyeon Park[1], TaeYoon Eom [1], Eunjin Jo[1], Hyeonwoo Hwang[1], JunSeong Lim[1], Jae-Myung Kim[1], Seng Bunnary[2], Sothyra Tum[2], and Sang-Ho Cha[1]

[1] Animal and Plant Quarantine Agency
[2] National Animal Health and Production Research Institute

Genotypic identification of foot-and-mouth disease (FMD) virus (FMDV) is essential for implementing control policies against emergent FMD outbreaks. FMDV is divided into seven immunologically distinct serotypes (O, A, C, Asia and SAT 1-3] and serotypes O, A, and Asia 1 have been threatening the livestock industry in the Southeast Asian countries. Most of the FMD outbreaks in South Korea are expected to have been brought in from endemic countries in the Southeast Asia including Cambodia. Therefore, the purpose of this study is to identify the genotype of FMDV circulating in Cambodia between 2018-2022 to predict and prevent FMD outbreaks in South Korea.

A total of 152 clinical samples were collected from cattle or buffalo in the FMD outbreak regions in Cambodia between 2018-2020. Viruses were isolated using LFBKavb6 cells, and the isolated viruses were used for RNA extraction. The 3D real time PCR was conducted to detect FMDV and amplification of the VP1 coding region was performed for sequencing. Following sequencing, the VP1 coding sequences were aligned using BioEdit 7.2.5. Phylogenetic trees were constructed by MEGAX software with the maximum likelihood method based on the generalized time-reversible model and bootstrap values were calculated on 1000 replicates.

Among 152 clinical samples subjected to virus isolation, 92 clinical samples showed cytopathic effect and the viral isolation was successfully completed (92/152, 65%). Sequencing and phylogenetic analysis was performed using the VP1 sequences of the isolated viruses(n=92). It showed that they were classified into three lineages; O/ME-SA/PanAsia(n=36), O/ME-SA/Ind-2001e(n=43) and A/ASIA/Sea-97(n=13).
In Cambodia, O/ME-SA/PanAsia, O/ME-SA/Ind-2001e and A/ASIA/Sea-97 were detected before 2020, but only O/ME-SA/Ind-2001e was detected from 2020 to 2022. It is thought that O/ME-SA/Ind-2001e is dominant in the Southeast Asia these days. Therefore, rapid and accurate detection assays of these lineages and lineage-specific preventive controls are necessary in South Korea.

Genetic Analysis of Foot-and-Mouth Disease Virus O/ME-SA/SA-2018 in Bangladesh, 2022

Hyeonjeong Kang[1], Soyoon Ryoo[1], Da-Rae Lim[1], Tae-Yoon Uom[1], Hyunwoo Hwang[1], G. A. Chowdhury[2], Jae-Myung Kim[1], Sang-Ho Cha[1]

[1] Animal and Plant Quarantine Agency, Ministry of Agriculture, Food and Rural affairs, Republic of Korea
[2] Central Disease Investigation Laboratory, Ministry of Fisheries and Livestock, People's of Republic of Bangladesh

FMD is highly endemic in Bangladesh, where serotype O is most prevalent among four reported FMDV serotypes (O, A, Asia1, and C). A review of different research articles showed that serotype O accounts for about 31%–85% of the outbreaks followed by types A (7%–47%) and Asia-1 (5%) at different times and in different regions of Bangladesh. The purpose of this study is to identify the genotype of the foot-and-mouth disease virus circulating in Bangladesh in 2022.

A total of 14 clinical samples were collected from regions(6 provinces) in Bangladesh where FMDV outbreaks occurred in cattle. Viruses were isolated using LFBKavb6 cells, RNA was extracted using Nextractor(Genolution) from the isolated virus, and the VP1 region was amplified using the RT-PCR for sequencing. Following sequencing, the VP1 coding sequences were aligned using BioEdit 7.2.5. Phylogenetic trees were constructed using MEGAX software with the maximum likelihood method based on the generalized time-reversible model and bootstrap values were calculated on 1000 replicates.

We confirmed in a previous study that O/ME-SA/Ind-2001e and A/AISA/G-VII are circulating in Bangladesh in 2020-201. In 2022, Sequencing and phylogenetic analysis of the VP1 sequences of the isolated viruses showed that they were classified into the O/ME-SA/SA-2018. Isolated viruses in the study shared very high sequence similarity. These viruses were classified into subgroups different from Ind-2001e viruses previously isolated from Bangladesh in 2021.

Our study confirms evidence that O/ME-SA/SA-2018 is circulating and evolving across Bangladesh in 2022, suggesting that countries in the pool 2 region, including India, should prepare for the emergence of the O/ME-SA/SA-2018.

Phylogenetic analysis of Foot-and-mouth disease viruses circulating in Vietnam in 2022

Hyeonjeong Kang[1], Soyoon Ryoo[1], Da-Rae Lim[1], W Theppangna[2], Jae-Myung Kim[1], Sang-Ho Cha[1]

[1] Animal and Plant Quarantine Agency, Ministry of Agriculture, Food and Rural affairs, Republic of Korea
[2] National Animal Health Laboratory, Department of Livestock and Fisheries, Ministry of Agriculture and Forestry

Foot-and-mouth disease virus (FMDV) is a highly contagious and economically devastating disease of domestic livestock and wild animals. FMDV belongs to the genus Aphthovirus genus of the Picornaviridae family, with seven immunologically distinct serotypes: O, A, C, SAT1-3, and Asia 1. Most of the FMD outbreaks in South Korea are expected to have been brought in from countries in the Pool 1 region, which includes Korea. Therefore, through the Pool 1 region FMDVs analysis, we have attempted to predict and prevent FMD outbreaks in South Korea.

A total of 4 clinical samples were collected from cattle, buffalo and pig in the FMD outbreak regions (3 provinces) in Vietnam. Viruses were isolated using LFBKavb6 cells, RNA was extracted from the isolated virus, and the VP1 region was amplified for sequencing. Following sequencing, the VP1 coding sequences were aligned using BioEdit 7.2.5. Phylogenetic trees were constructed using MEGAX software with the maximum likelihood method based on the generalized time-reversible model and bootstrap values were calculated on 1000 replicates.

Sequencing and phylogenetic analysis of the VP1 sequences of the isolated viruses showed that they were classified into the O/ME-SA/Ind-2001e. These viruses were classified into subgroups different from Ind-2001e viruses previously isolated from countries in the pool 1 region(Cambodia, Laos, Mongolia, and South Korea), and the sequence homology ranged from 91.71 to 97.97%.

As a result of analyzing genotypes that have been mainly isolated since 2014, O/SEA/Mya98 and A/ASIA/Sea97 have been continuously occurring from 2014 to the latest. In 2017, O/ME-SA/PanAsia and O/CATHAY mainly occurred, and O/ME-SA/Ind-2001e mainly occurred in 2019-2021. Except for the O/Cathay genotype, all genotypes occurred in South Korea and most occurred at similar times.; O/ME-SA/PanAsia (2000·2002), O/SEA/Mya98 (2010·2014·2015·2016), A/ASIA/Sea97 (2010·2017·2018), O/ME-SA/Ind-2001e (2017·2019)

Monitoring FMDV circulating in Vietnam provides information to prepare for the possible introduction to the South Korea. Our study confirms evidence that O/ME-SA/Ind-2001e is circulating and evolving across Vietnam in 2022, suggesting that countries in the pool 1 region, including South Korea, should prepare for the re-emergence of the O/ME-SA/Ind-2001e.

Clinical Prevalence of Foot-and-Mouth Disease in Smallholder Dairy Cattle in Northern Cameroon

Kong Anita Burinyuy[1], Sevidzem Silas Lendzele[2]

[1]Université de Ngaoundere
[2]Université Libreville Nord

Background: Foot-and-Mouth Disease (FMD) endemic hyper-endemic in Cameroon hampers production and the health of animals with no systematic vaccination in dairy farms leading to significant economic losses to farmers. This study aimed at determining the prevalence of clinical FMD as well as to evaluate farmer's knowledge and practices in selected dairy herds in Adamawa.

Methods: Clinical diagnosis using classical FMD signs and semi-structured interviews were conducted during the 2019 outbreaks in this administrative region. Clinical diagnosis was carried out on 401 animals in 11 dairy farms, the overall clinical FMD prevalence was 22.19% and most cases were identified in Herd 2 of Mbidjoro village that was adjacent to the regional capital city (Ngaoundere) market. Of the 21 dairy farmers interviewed, 100% of them could identify the clinical cases. Low biosecurity level during hand milking practiced by 100% of farmers could spread the disease. About 100% of farmers witnessed a drop in milk production in clinically sick animals. Furthermore, 100% used veterinary pharmaceuticals especially antibiotics to treat clinical cases.

Conclusion: FMD clinical cases are frequent in dairy herds and are easily recognized and managed by farmers using antibiotics. The local milking techniques could spread the disease during outbreaks. Further investigations on the epidemiology and economic impact of FMD in dairy herds are required.

Keywords : Foot-and-mouth disease, cattle, Dairy farms, Adamawa

Cross-sectional study of Senecavirus A infection in pig farms in Taiwan

Cheng-Ju Pan[1], Yu-Liang Huang[1], Kuo- Jung Tsai[1], Ming-Chung Deng[1], Wen-Yuan Yang[2]

[1] Veterinary Research Institute, Ministry of Agriculture
[2] Department of Veterinary Medicine, National Taiwan University

Senecavirus A (SVA), a single-stranded RNA virus belonging to the family Piconaviridae, is an emerging pathogen associated with vesicular diseases in swine. This study aimed to investigate the seroprevalence of SVA infection and the evolution of SVA in pig farms to gain insights into the transmission of SVA within the pig population in Taiwan. A total of 304 pig farms in the nursery stage and 301 pig farms in the finishing stage were randomly selected for collecting blood and oropharyngeal samples to detect SVA infection. The seroprevalence of SVA in nursery pigs was 44.4%, while it was 11.6% in finishing pigs. SVA was isolated in pigs from three different farms at the finishing phase. The alignment of the whole genome sequence between Taiwan isolates and other SVAs revealed that the SVA isolates in this study shared nucleotide identities of 96.9%-97.4% with the SVA isolate from the United States (KY618836). The findings highlight the subclinical circulation of SVA in Taiwan. The infections were mainly observed in intensified pig farming regions of Taiwan. This study has provided valuable insights into the distribution of SVA among pig populations, laying an essential groundwork for further research on SVA prevention and control.

Serological Study and Comparison of Various Types of Cattle Groups in the Zambezi Region of Namibia: A Cross Sectional Study

Freddy Samuntu

University of Pretoria

Trade in beef products was limited to geographic areas free from foot-and-mouth disease until the inception of commodity-based trade. This study will experimentally examine the equivalence of two vaccination protocols as part of a commodity-based trade initiative in the Zambezi Region of Namibia. This study aims to test whether the proportion of animals protected by vaccination in the quarantine camp is equal to the proportion protected by field vaccination by attempting to match non-specific and specific antibody titres at the beginning and end of the study through serum collection. Cattle (N = 299) will be used for the purposes of this research. Cattle will be allocated to four groups. Group A will contain cattle which have been vaccinated twice in the field, group B will contain cattle which have been vaccinated once in the field, group C will contain naive cattle from south of the veterinary cordon fence which have never been vaccinated and group D will contain cattle which are positive for antibodies to natural infection with foot-and-mouth disease virus. These animals will be quarantined at Kopano quarantine facility and slaughtered at Katima Mulilo's abattoir at the end of the project. This contribution to the limited information available for commodity-based trade will significantly help improve the lives of Namibian farmers north of the veterinary cordon fence.

Keywords: equivalence, vaccination protocols, commodity-based trade, Zambezi region, Namibia, foot-and-mouth disease

Analyses of Bovine Immunoglobulin Constant Heavy Chain Protein Sequences and the Inferred Properties Regarding Antibody Binding to FMDV Epitopes

Shannon Collinson[1,2], Paul Azzinaro[1], Teresa De Los Santos[1], Sarah E. Attreed[1], and James J. Zhu[1]

[1] US Department of Agriculture, Agricultural Research Service, Foreign Animal Disease Research Unit, Plum Island Animal Disease Center, Orient, NY 11957, USA
[2] Plum Island Animal Disease Center Research Participation Program, Oak Ridge Institute for Science and Education (ORISE), USA

Immunoglobulins (Ig) or antibodies are the critical humoral factors of adaptive immunity, whose functions depend on antigen and Fc receptor binding and/or complement fixation. In this study, bovine Ig protein sequences from the NCBI databases were compared to human counterparts to exploit what is known of the immune properties of human Ig to infer those of bovine antibodies. The analyses show that all bovine Ig classes and subclasses are transcribed as secreted antibodies and membrane-bound B cell receptors. The majority of bovine Ig EST consist of IgA (18%), IgG1 (37%), IgG2 (15%) and IgM2 (19%), while the remaining Ig range from 1 - 3.5% of total EST. The Ig domain sequences are conserved between humans and cattle (identities > 60% for IgA, IgG and IgM and > 40% for IgD and IgE). The hinge regions are highly variable in sequence, length, and the number and position of cysteine residues forming disulfide bonds among bovine Ig classes and even within subclasses, which could alter Fab reach to epitopes and Fc functionalities. Bovine IgG1 and IgG3 have very similar FcγRIII binding sequences, with 85.7% identity and have 76.2% identity respective to human IgG1, compared to only 47.6% identity between bovine IgG2 and human IgG1. Conversely, bovine IgG2 and human IgG2 show less than 62% identity in the binding sequences. Analysis of bovine Ig hinge sequences and reported 3D structures of antibody binding to epitopes on FMDV capsids indicate that bovine IgA, IgG1 and IgG2 cannot bivalently bind most identified epitopes on the same capsid whereas IgG3 can do so. The inferred immune properties agree with reports regarding FMD immune protection. These described sequence variations provide the molecular basis for further investigation to understand immune protection and guide vaccine designs.

Mechanisms of Tissue Susceptibility to FMDV Persistent Infection Based on Genes Differentially Expressed between Target and Non-target Tissues

James J. Zhu, Shannon Collinson, Jessica A. Canter, Theresa Aponte, Luis L. Rodriguez, Jonathan Arzt

US Department of Agriculture, Agricultural Research Service, Foreign Animal Disease Research Unit, Plum Island Animal Disease Center

Foot-and-mouth disease virus (FMDV) infects the nasopharynx and lung tissues of cattle after aerosol infection; however, it can only persistently infect the nasopharynx. Our previous studies comparing gene expression between carriers and non-carrier showed that persistent infection was associated with differentially expressed genes (DEG) associated with regulatory T cells (Treg) cells and chemokines recruiting cytotoxic cells and phagocytes. In this study, gene expression profiles were compared between the bovine nasopharynx (target tissues of persistent infection) and the lung (non-target tissue) to understand why the nasopharynx is susceptible to persistent infection. Bioinformatic analysis of DEGs indicated that the lung and nasopharynx recruited different immune cells and adapted different mechanisms for defense. Tissue susceptibility to persistent infection was also found to be associated with downregulated expression of chemokines recruiting cytotoxic cells and phagocytes and upregulated genes associated with Treg cells, adenosine signaling, inhibition of IL-17 signaling, increasing production of AHR ligands, and TGF-β and retinoic acid activity. The differential expression of these DEGs could significantly hinder virus clearance in the nasopharynx. Many DEGs identified in this study including CD39 that is known to play a key regulatory role in clearing viral infections were also found to be differentially expressed between carriers and non-carriers, indicating that similar immune mechanisms underlying the differences in FMDV persistence between these tissues and between carriers and non-carriers are at play.

Pathogenesis

Investigation of Foot-and-Mouth Disease Virus serotypes from Livestock in Kenya in the year 2020

Hellen Nduku Mutua[1], Nyabuga Nyariki[2]

[1] Foot And Mouth Disease Reference Laboratories
[2] Technical University Of Kenya

(FMD) is an infectious virus of ungulate animals, that affects over seventy livestock and wildlife species in the world. The causal agent is a single-strand non-enveloped RNA virus of the family Picornaviridae, genus Aphthovirus, FMD results in huge economic loss in Kenya, where serotypes O, A, SAT1, and SAT2 are frequently encountered. Despite of the previously reported field circulating strains in Kenya, there is limited data on the current circulating FMDV serotypes, prevalence, and vaccination coverage. Therefore, this study intended to generate spatiotemporal data and the respective prevalence of the current circulating FMDV serotypes at the county level and nationwide. The Antigen detection approach was deployed to determine the presence of FMDV in collected study samples, and performed as indicated in the kit instructions manual.

Samples with O.DS greater than 1 were considered positive while those with O. Ds less than 1 were negative.

Out of 133 samples tested, 88 samples were FMDv positive, 39.1% (n=52) tested positive for FMDV type O, whereas 27.07 % (n=36) were positive for type-SAT1, 33.83 % (n=45) of the analysed (n=133) samples were FMDV negative. Comparable levels of serotype O and serotype SAT1 in all sampled counties were observed using a pie chart.

The FMDV type O was the most predominant compared to type SAT1, as it was prevalent in 17 and SAT1 in 9 out of the 25 study counties respectively.

The findings of this study are pertinent for enhancing knowledge of 2020 Kenya circulating strains, and it enables the setting of suitable strategies for effective control of FMD especially when vaccination is considered the main option. However; the policy for tailored FMD vaccines for Kenya needs to prioritize FMDV type O and SAT1 in the available vaccines to strengthen the FMD control outcomes.

Keywords: FMD, FMDV, Serotype O, Serotype SAT1, Kenya

Vaccines

Development and evaluation of multivalent FMD vaccine using local field isolates

Qaiser Akram

University of Veterinary and Animal Sciences, Lahore, Pakistan

Foot-and-mouth disease (FMD) is a serious disease affecting the global graziery industry. Once an epidemic occurs, it can lead to economic and trade stagnation. In recent decades, FMD has been effectively controlled and even successfully eradicated in some countries or regions through mandatory vaccination with inactivated foot-and-mouth disease vaccines. Nevertheless, FMD still occurs in some parts of Africa and Asia. The transmission efficiency of foot-and-mouth disease is high. Both disease countries and disease-free countries should always be prepared to deal with outbreaks of FMD. The development of vaccines has played a key role in this regard. This paper summarizes the development of several promising vaccines including progress and design ideas. It also provides ways to develop a new generation of vaccines for FMDV and other major diseases.

Keywords: foot-and-mouth disease; inactivated vaccine; adenovirus vector vaccine; synthetic

Selection and use of a reference antigen panel to assess the regional relevance of foot-and-mouth disease vaccines in East Africa

David Paton[1], Anna Ludi[1], Don King[1], Ginette Wilsden[1], Clare Browning[1], Sarah Belgrave[1], Nick Knowles[1], Antonello Di Nardo[1], Nick Nwankpa[2], Ethel Chitsungo[2], Cisse Rahamatou[2], Moustapha Boukary[2], Gelegay Ayelet Melesse[2], Sanne Charles Bodjo[2], Santina Grazioli[3], Efrem Alessandro Foglia[3], Emiliana Brocchi[3]

[1] The Pirbright Institute
[2] The Pan African Veterinary Center of the African Union
[3] Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna

Africa has a great diversity of foot-and-mouth disease viruses (FMDV) against which killed vaccines are provided by several companies, incorporating diverse strains of different serotypes. To help determine the regional suitability of these vaccines, 16 FMDV were selected to represent the antigenic variation found in East Africa. The panel comprised four viruses for each of the serotypes O, A, SAT 1, and SAT 2 commonly affecting livestock in this region. They were selected from 1,124 isolates made between 2011 and 2019. Sixty-eight isolates were chosen initially, to represent regional genetic lineages, with a final antigenic selection made from differences in patterns of virus recognition by monoclonal antibodies and neutralisation by monospecific antisera. The ability of vaccine-specific antisera to neutralise the panel viruses can indicate which formulated vaccine products are likely to protect in the field. This approach complements vaccine matching, can assess multivalent vaccines, does not need vaccine viruses, and takes account of batch-specific vaccine potency. Antisera were prepared from groups of 5-10 cattle immunised with 13 multivalent FMD vaccine batches produced for use in East Africa by five companies. The ability of the antisera to neutralise the panel viruses was compared to threshold titres associated with cross-protection to provide a measure of each vaccine's batch-specific potency and regional antigenic relevance as well as indicating the reactivity signatures of the virus strains and serotypes. Marked differences were observed in the immunogenicity of different vaccine batches and between cattle given one and two doses of vaccine. Further virus panels could be developed and deployed for vaccine selection in other regions.

Improved in vitro and in vivo assays to assess the quality of FMD vaccine: Preliminary evidence from the Indian experience

Tamil Selvan R. P, Donald King, Anna Ludi, David Paton, Simon Gubbins, Madhusudan Hosamani, Toby Tuthill, Stephen Berryman, Saravanan P, Eva Perez, Sreenivasa, B. P, Danny Goovaerts, Patel B.H.M., Narayanan K, Ginette Wilsden, Amin Yasmin, Yvonne Sewell, Ranjitha Bommanna, Sumana, K, Ramakrishnan M. A, Suresh, H.B, Dechamma H. J, Gnanavel, V, Bhanuprakash V, Muthuchelvan, S.D Bhure, Mohanty N. Nihar, Gupta V, Singh S. K. Biswal, J. K, Mohapatra, J. K, Pallab Chaudhuri, Singh, S.K, Nick Juleff, Sanyal A, Singh R. P, Praveen Malik, Ashok Kumar, Jena, J.K, Abhijit Mitra and Triveni Dutt

The Indian Pharmacopoeia mandates the use of vaccination/challenge studies in cattle for FMD vaccine potency testing but also allows alternate assays to be used provided a statistical correlation is established with levels of protection in cattle. As cattle experiments are constrained by difficulties to source sero-negative cattle, welfare, and throughput, approaches that use (i) assays for quantification of intact FMD vaccine antigen and (ii) a guinea pig (GP)-serology model were evaluated.

Two different ELISAs were applied to differentiate and quantify intact (146S) and dissociated (12S) vaccine antigens as in both process and batch-release assays. The first used serotype-independent monoclonal antibodies targeting VP4 and VP2 proteins whereas the other used serotype-specific Llama nanobodies for detection of the FMDV antigens in the Indian vaccines. Batches of FMD antigen and formulated oil-adjuvanted FMD vaccine were tested in both ELISA formats. Comparison of predicted antigen content in vaccines with serological results obtained from cattle and GP showed the potential application of these assays.

Parallel testing of vaccine batches revealed a positive correlation between VN titers in cattle and GP. However, the GP titers were generally lower relative to cattle sera at least for serotype O and the GP test may require further harmonization across different laboratories including harmonization of the GP source.

These data show that the combination of ELISA-based intact antigen quantification and GP-based serology can simplify the assessment of FMD vaccine potency.

Biogénesis Bagó FMD vaccines: immunogenicity studies in small ruminants and cross immunity against prevalent FMDV lineages in Middle East Asia

C. Caldevilla, C. Malnero, R. Scian, T. Rico, J. Filippi, M. Martinez, E. Smitsaart, S. Cardillo

Biogénesis Bagó S.A. Garín, Buenos Aires, Argentina

Introduction: sheep and goats play an important epidemiological role in the spread of Foot -and-Mouth Disease (FMD) in Middle East. Typically, outbreaks are due to FMD viruses of either the O or A serotypes. In the present study, the immunogenicity elicited by a polyvalent oil vaccine and the cross immunity against lineages considered of high risk for the region were assessed.

Materials and Methods: water-in-oil single emulsion vaccine, manufactured by Biogénesis Bagó, which contains O1 Campos, A24 Cruzeiro and A2001 Argentina was evaluated for its immunogenicity in two independent clinical trials that included sixteen sheep and goats with no previous history of FMD vaccination. Two groups of eight animals were included in which group 1 received single dose of 1 mL, and group 2 received two doses of 1 mL (0-30 days post vaccination -dpv-). Homologous titers were determined by Liquid Phase Blocking ELISA (LPBE) at different times between 0 and 90 dpv. Virus Neutralization Test (VNT) on ovine post vaccinal serum samples was performed against A/ASIA/Iran-05 and O/ME-SA/Ind-2001e field viruses carried out by the Pirbright Institute.

Results: A single administration of trivalent FMD vaccine in sheep and goats induced a high antibody response against the three vaccine strains at 30 dpv which continued to increase up to at least 90 dpv. A second dose administered at 30 dpv, induced statistically higher LPBE titers in both species against the three vaccine strains.
Mean group VNT titers > 2 log10 were obtained against A/IRN/18/2021 and O/MOG/3/2021 viruses at 30 dpv in ovine sera.

Discussion: Biogénesis Bagó FMD polyvalent vaccine conferred satisfactory immune response in sheep and goats and broad coverage against high-risk lineages for the Middle East. These results demonstrate its usefulness as a tool for prevention and control of FMD caused by serotype O and A in these target species.

FMD vaccine strain Asia 1/Sindh-08: immunogenicity studies in cattle and cross immunity against prevalent Asia 1 field viruses

C. Caldevilla[1], C. Malnero[1], L. Niño[1], R. Scian[1], J. Filippi[1], M. Barrios[1], AI Taffarel[2], S Galdo Novo[2], E. Smitsaart[1], S. Cardillo[1]

[1] Biogénesis Bagó S.A., Garin. Buenos Aires, Argentina
[2] WOAH FMD Reference Laboratory, Servicio Nacional de Sanidad y Calidad Agroalimentaria (SENASA), Buenos Aires, Argentina

Introduction: As part of the development project for new vaccine strains, an FMD virus Asia 1/Sindh-08 was developed as a new vaccine strain. Clinical trials in cattle were performed to assess the potency (duration of immunity), safety and purity after one and two doses of monovalent vaccine.

Materials and Methods: water in oil single emulsion vaccine, manufactured by Biogénesis Bagó, which contains Asia 1/Sindh-08 vaccine strain was evaluated for its immunogenicity in sixteen FMDV antibody free cattle with no previous history of FMD vaccination. Two groups of eight animals were included in which group 1 received single dose of 2 mL, and group 2 received two doses of 2 mL at 0- and 30 days post primary vaccination (dpv). Neutralizing titers against de homologous strain were determined by WOAH FMD reference laboratory at SENASA at different times post vaccination (between 0 and 210 dpv). In addition, VNT titers against Asia 1 heterologous field virus were performed by the Pirbright Institute at 30 dpv and 30 days post revaccination (dprv).

Results: After a single administration of FMD monovalent vaccine containing Asia 1/Sindh-08 in cattle, antibody titers increased rapidly in the first 30 dpv (mean/group 1.90 log10). In the following months the antibody titers remained at high levels or still increased. At 210 dpv antibody titers were higher than at 30 dpv (mean/group 2.52 log10). Antibody titers increased after second dose. High heterologous antibody titers against Sindh-08 and G-IX lineages were observed at 30 dpv and 30 dprv (> 2 log10).

Discussion: high quality FMD vaccines containing a newly developed Asia 1/Sindh-08 vaccine strain induced a high-potency and long-lasting immune response in cattle. In addition, cross immunity studies demonstrated broad antigenic coverage predicting protection against relevant Asia 1 field isolates.

Coverage of a Novel Monovalent O1 Campos Vaccine Against Foot and Mouth Disease (FMD) Viruses Circulating in Africa and Asia

Sofia Gomez Bustillo, Anahí Fernandez Acevedo, Paulo Di Tella

Centro Diagnóstico Veterinario

Introduction: Vaccination is the main strategic tool to control FMD infections. Therefore, the development of new high-quality vaccines that show great efficacy, long lasting immunity and broad antigenic cross-protection are essential to ensure disease control. In this report, we explore the protective capacity of a EURO-SA O1 Campos monovalent vaccine against FMD in cattle and analyze cross-protection against circulating lineages in Africa and Asia.

Methodology: The monovalent w/o simple oil emulsion vaccine, CDVac AFTOSA MONOVALENTE was manufactured in CDV (Centro Diagnóstico Veterinario, Argentina). Naïve cattle were vaccinated with a 2 ml dose intramuscularly (IM). Sera was analyzed by virus neutralization test and Liquid Phase Blocking ELISA at different time points up to 180 days post-vaccination to determine the onset and immunity duration. In vitro vaccine matching to determine heterologous titers against serotypes O field isolates in Africa and Asia (Panasia, Ind2001e, Mya-98, O-MANISA, EA-3, Panasia2) were performed by the WOAH reference laboratory in Argentina, SENASA.

Results: r1 values were determined after 30 Days post vaccination, all the field isolates tested, showed high r1 values from 0.42 up to 1.00. Moreover, early immune response was confirmed by virus neutralization test, high neutralizing antibody titers were produced after only 14 days post vaccination, surpassing the cut off limit established at 1.65 log10. Lastly, antibody titers remained high up to 180 days post vaccination indicating the protective capacity of the vaccine.

Conclusions: r1 values show the close relatedness between the representative field isolates of O serotypes circulating in endemic regions and the vaccine strain O1 Campos, suggesting it is very likely to confer protection. What is more, these results prove that this high-quality vaccine, CDVac AFTOSA MONOVALENTE confers early and long-lasting immune response.

Post Vaccination Monitoring (PVM) to Assess the Efficacy of the FMD Vaccine Used in Jordan

Giampietro Maccabiani[1], Cornelis van Maanen[2], Vito Tranquillo[1], Tiziana Trogu[1], Emad M. Bennour,[3] M. Hashem[4], Nussieba A. Osman[5], M. Khalifeh[6], Maisa S. Al Ameer[7], Moh'd-Saddam F. Bintarif[7], Saeda A. Salah[8], Shahin Baiomy[2], Francesca Ambrosini[2], Fabrizio Rosso[2], Santina Grazioli[1], Efrem Alessandro Foglia[1]

[1] Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna - IZSLER, Brescia Italy
[2] European Commission for the control of Foot-and-Mouth Disease (EuFMD), FAO, Rome, Italy,
[3] Department of Internal Medicine, Faculty of Veterinary Medicine, University of Tripoli, Tripoli, Libya
[4] The Central Laboratory for Evaluation of Veterinary Biologics, Abbassia, Egypt
[5] Department of Pathology, Parasitology and Microbiology, College of Veterinary Medicine, Sudan University of Science and Technology, Khartoum-North, Sudan
[6] Department of Basic Veterinary Medical Science, Faculty of Veterinary Medicine, Jordan University of Science and Technology, Irbid, Jordan
[7] Virology Unit, Animal Wealth Laboratories Directorate, Amman, Jordan
[8] Epidemiology Unit, Veterinary and Animal Health Directorate, Amman, Jordan

Introduction

In countries endemic for foot-and-mouth disease (FMD), routine or emergency vaccinations are strategic tools to control disease. According to WOAH/FAO guidelines, the estimation of vaccine effectiveness is recommendable to optimize control programs. This study reports the results of a Post Vaccination Monitoring (PVM) performed in Jordan across 2022 and 2023.

Materials and Methods

A polyvalent inactivated vaccine, including one serotype O strain (O1-Campos), was administered to 60 goats and 56 sheep. All animals were naïve and a sub-group received a second vaccination 28 Days-Post-Vaccination (DPV). Antibody titres were determined in sera, collected at 0, 14, 28 and 56 DPV, by Virus Neutralization Test (VNT) versus a strain closely related to O1-Campos (O-BFS) and O-JOR 6/21 (O/ME-SA/PanAsia-2/ANT-10 sublineage, recently circulated in the country).

Results

All animals were VNT negative against both FMDV O strains at day 0.
Against O-BFS:
(i) A good seroconversion was detected at 14 DPV, goats showed even higher values (median log10 titres 2.7, 98% positivity) than sheep (median log10 titres 2.1, 78% positivity).
(ii) The immune response reached its peak at 28 DPV, confirming a stronger reaction in goats (median log10 titres 3.2, 100% positivity) than in sheep (median log10 titres 2.7, 82% positivity).
(iii) Antibody levels were maintained until 56 DPV in single-vaccinated animals, with only a minor decrease of the median log10 titres and the positivity percentage (goats, 3, 100%; sheep, 2.4, 79%).
(iv) In the re-vaccinated sub-population, a clear booster response was detected, characterized by high median log10 titres and positivity percentage (goats, 3.3, 100%; sheep, 3, 100%).
Against O-JOR 6/21:
(i) At 14 DPV animals showed a significant immunization (goats, median log10 titres 2.7, 100% positivity; sheep, median log10 titres median 2.4, 78% positivity).

(ii) At 28 DPV goats reached the highest antibody level (median log10 titres 3) while sheep showed a small decline in antibody titres with median log10 titres of 2.1 and 76% positivity.
(iii) Sheep displayed unchanged immune levels from 28 to 56 DPV, while goats showed a very small reduction of log10 titres (median 2.7).
(iv) The second administration of the vaccine induced an effective booster response and re-vaccinated sub-populations showed an increase of median log10 titres (goats 3.3, sheep 3).

Discussion
Overall: (i) the evaluated vaccine induced a good response, revealing a better and faster immunization in goats; (ii) a second administration stimulated an appropriate booster effect; (iii) the induced antibodies demonstrated to be able to neutralize both O-BFS and O-JOR 6/21.
The results of this PVM, according to previous studies [1], suggest that after the vaccination almost all animals should have at least 75% probability to be protected from O-BFS and O-JOR 6/21, but 88% of goats and 68% of sheep should reach 95% probability of protection.

[1] Gubbins S, Paton DJ, Dekker A, Ludi AB, Wilsden G, Browning CFJ, Eschbaumer M, Barnabei J, Duque H, Pauszek LL, King DP. Predicting cross-protection against foot-and-mouth disease virus strains by serology after vaccination. Front Vet Sci. 2022 Dec 1;9:1027006. doi: 10.3389/fvets.2022.1027006. PMID: 36532344; PMCID: PMC9751447.

Evaluation of swine protection with commercial foot-and-mouth disease vaccines against challenge with an A/ASIA/Iran-05 lineage virus from South East Asia

Jaejo Kim, Seon Woo Kim, Ha-Hyun Kim, Yong Bin Kim, Seung Heon Lee, Jong-Hyeon Park

Animal and Plant Quarantine Agency

The vaccination with Foot-and-mouth disease (FMD) vaccines, formulated with chemically inactivated purified whole viruses, can be highly effective in limiting the spread of infection. However, due to high antigenic diversity of the FMD viruses, the current vaccine, a purified inactivated virus vaccine derived from a specific strain, can confer protective immunity against only a limited range of field strains. In addition, type A FMD virus is considered to be antigenically and genetically the most diverse FMD virus serotypes. In this study, the efficacy trial was conducted to determine if the commercial vaccines were appropriate against the A/ASIA/Iran-05 lineage virus recently isolated. Fifteen FMD sero-negative pigs were randomly divided into three vaccinated groups (each four pigs; O1/Manisa + O/3039 + A22/Iraq, O1/Campos + A24/cruzeiro + A/Argentina/2001, O/Primorsky/2014 + A/Zabaikalsky/2013) and one unvaccinated control group (three pigs). All pigs were challenged with A/PAK/1/2020 strain (2x10^6 TCID50/200µl), by tongue inoculation at 28 days post-vaccination. And clinical signs, viremia, nasal shedding and neutralizing antibody responses of all pigs were investigated during 7 days. In each vaccinated group, protection percent in pigs varied from 25% to 100% in heterologous challenge test with A/PAK/1/2020 strain. Comparing with those of non-protected pigs, none or relatively low levels of viremia and nasal shedding were detected in protected individuals. In the group in which all pigs were protected, the neutralizing antibody titers against field strain were maintained at a stable level and increased. In addition, the relatively less clinical score and viral loads were detected in non-protected vaccinated animals, comparing with pigs in the control group. From these results, it is demonstrated that these commercial FMD vaccines could have the potential to protect against the A/PAK/1/2020 strain of A/ASIA/Iran-05 lineage field virus, although there are differences among vaccines.

The early efficacy study on the vaccination of commercial and experimental foot-and-mouth disease vaccines against the challenge with an O/CATHAY isolate from South East Asia in pigs

Jaejo Kim, Seon Woo Kim, Yong Bin Kim, Ha-Hyun Kim, Seung Heon Lee, and Jong-Hyeon Park

Animal and Plant Quarantine Agency

Among eight topotypes in type O Foot-and-mouth disease virus (FMDV), O/CATHAY lineage virus is known as a porcinophilic virus that has caused devastating damage to the pig industry. According to vaccine matching and efficacy results reported in other studies, there have been concerns that many commercially available type O vaccines could not confer effective protection against O/CATHAY lineage viruses. In this study, in vivo efficacy tests were conducted to find the applicable regimen for three commercial vaccines (O1/Manisa + O/3039 + A22/Iraq, O1/Campos + A24/cruzeiro + A/Argentina/2001, O/Primorsky/2014 + A/Zabaikalsky/2013) and one experimental vaccine (O/SKR/Boeun/2017) to confer the protective immunity against heterologous challenge with a CATHAY field virus in pigs. Four efficacy trials were conducted with different booster status and administration intervals. According to the efficacy results in all efficacy trials, the protective immunity induced by vaccination increased over time and more rounds of vaccination, while all pigs in the control developed typical FMD clinical signs. Interestingly, all pigs in trial with twice vaccination at 2-week intervals were protected against the challenge with the O/CATHAY lineage virus, probably due to the highly induced heterologous virus neutralization (VN) titer. As the results of these clinical trials, it might be concluded that the proper modification of the vaccination regimen could be the alternative measure to control the possible future FMD outbreak more efficiently.

Construction of a FMDV-specific recombinant antibody phage-display bovine library for epitope identification and diagnostic reagents

Maruping Mangena[1], Jeanni Fehrsen[1,3], Maria Cruz Miraglia[4], Pamela Opperman[1,2], Florencia Mansilla[4], Mariano Perez-Filgueira[4], Alejandra Capozzo[4], Melanie Chitray[1,3]

[1] Agricultural Research Council, Onderstepoort Veterinary Research Institute, Vaccines and Diagnostic Development, Private Bag X05, Onderstepoort, Pretoria 0110, South Africa.
[2] Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria
[3] University of Pretoria, Faculty of Veterinary Science, Department of Veterinary Tropical Diseases, Pretoria, South Africa. ChitrayM@arc.agric.za 4 Institute of Virology and Technical Innovations CONICET-INTA, Centro de Investigaciones en Ciencias Veterinarias y Agronómicas (CICVyA), INTA CNIA, Hurlingham, Buenos Aires, Argentina

Foot-and-mouth disease (FMD) is caused by the foot-and-mouth disease virus (FMDV). It is the most wide-spread transboundary disease due to its highly infectious nature and is endemic in many African countries including, South Africa. There are seven serotypes of FMDV i.e., SAT1, SAT2, SAT3, A, O, C, and Asia1. The disease affects cloven-hoofed animals such as cattle, sheep, pigs and goats. Furthermore, it causes significant economic losses due to high morbidity in infected animals and stringent trade restrictions imposed on animal products from affected countries. An important control measure is vaccination, however, the FMDV high mutation rate results in antigenic variation rendering vaccines less effective. Knowledge of FMDV epitopes is advantageous and can be included in FMDV recombinant vaccine development resulting in vaccines that induce a broad immunological response and thus offer improved protection. In this regard, a bovine immune recombinant phage antibody library was constructed, using tissue samples taken from the (right) prescapular lymph nodes of six Jersey steers vaccinated with a commercial tetravalent FMD vaccine used in Argentina. Following RNA extraction, variable heavy (VH) and light chain (VL) regions of the immunoglobulin G antibody (IgG) genes were amplified by RT-PCR, joined by a flexible linker and cloned into a phagemid vector. The construct was transformed into electro-competent E.coli TG1 cells and an immune library constructed consisting of 1.8 X 107 different clones. Biopanning will be used to identify FMDV-specific single-chain variable fragments (scFvs). These scFvs will be utilised to identify FMDV epitopes and investigate its potential as reagents in a diagnostic ELISA. This is the first report of a FMDV-specific bovine phage library construction.

Evaluation of Foot-and-Mouth Disease Virus Inactivation Kinetics with Binary Ethylenimine for Vaccine Production in South Korea

Sang Hyun Park, Jae Young Kim, Sun Young Park, Jong Sook Jin, Dohyun Kim, Jong-Hyeon Park, Young-Joon Ko

Animal and Plant Quarantine Agency

Foot-and-mouth disease (FMD) vaccines must be produced in a biosafety level 3 facility, so the FMD virus (FMDV) must be completely inactivated after amplification. The inactivation kinetics of FMDV during vaccine antigen production were assessed by evaluating whether the viral titer dropped below 10-7 TCID50/mL within 24 h of binary ethyleneimine (BEI) treatment. This study deals with four FMD vaccine candidate strains for efficacy of BEI treatment at different concentrations and temperatures to determine the optimal inactivation condition of each virus. Two domestic isolates, O/SKR/Boeun/2017 (O BE) and A/SKR/Yeoncheon/2017 (A YC), and two recombinant viruses, PAK/44/2008 (O PA-2) and A22/Iraq/24/64 (A22 IRQ), were investigated. The O BE and A22 IRQ required 2 mM BEI at 26°C and 0.5 mM BEI at 37°C for complete inactivation. The O PA-2 and A YC required 2 mM BEI at 26°C and 1 mM BEI at 37°C. Crucially, the yield of FMD virus particle (146S) in the viral infection supernatant was relatively higher (>4.0 µg /mL) than those previously reported; additionally, there was little antigen loss, even after 24 h of treatment with 3 mM BEI. Overall, it is considered economical to produce FMD vaccines using these four kinds of viruses; therefore, these candidate strains will be prioritized for the manufacture of FMD vaccines in South Korea.

Stabilized SAT1 virus-like particles protect cattle against foot-and-mouth disease

Michel Verwoolde[1], Eva Perez-Martin[2], Ranjitha Bommanna[2], Helen M E Duyvesteyn[3], Elizabeth Fry[3], Ian Jones[4], Silvia Loureiro[4], Sophie Jegouic[4,] Claudine Porta[3], David I. Stuart[3], Carina Kahl[1], Bryan Charleston[2], Erwin van den Born[1]

[1] MSD Animal Health, Boxmeer, Netherlands
[2] The Pirbright Institute
[3] Division of Structural Biology, Wellcome Trust Centre for Human Genetics, Oxford, United Kingdom
[4] University of Reading, United Kingdom

Foot-and-mouth disease (FMD) is a significant concern in Eastern Africa due to its endemic nature, and a high-quality, low cost FMD SAT1 vaccine is crucial to control the spread of the disease. In this regard, virus-like particle (VLPs) based on SAT1/KEN/80/2010 were evaluated. The VLPs were equipped with amino acid substitutions that confer enhanced thermostability as demonstrated by an intact-capsid-specific ELISA. The protective capacity of the stabilized SAT1 VLPs was assessed in cattle. The VLP vaccine elicited a robust immune response, with cattle exhibiting high neutralizing antibody tiers (>1.6 log10) against the homologous virus. Notably, VLPs also induced substantial heterologous virus neutralizing antibody titres, showcasing their potential for broader protection. Moreover, VLP vaccinated animals were protected against homologous virus when challenged 21 days post-vaccination, further demonstrating efficacy of the VLP-based approach. These findings indicate that VLP vaccines show great promise for controlling FMD in Eastern Africa.

Stabilized SAT2 virus-like particles protect cattle against foot-and-mouth disease

Eva Perez-Martin[1], Ranjitha Bommanna[1], Elizabeth E. Fry[2], Helen M. E. Duyvesteyn[2], Ian Jones[3], Silvia Loureiro[3], Sophie Jegouic[3], Claudine Porta[2], David I. Stuart[2], Carina Kahl[4], Michiel Verwoolde[4], Bryan Charleston[1], Erwin van den Born[4]

[1] The Pirbright Institute
[2] Division of Structural Biology, Wellcome Trust Centre for Human Genetics, Oxford, United Kingdom
[3] University of Reading, United Kingdom
[4] MSD Animal Health, Boxmeer, Netherlands

Given the recent SAT2 outbreak and the endemic nature of foot-and-mouth disease (FMD) throughout Africa, there is an urgent need for high-quality FMD SAT2 vaccines in the region. To address this, virus-like particle (VLPs) based on SAT2/SAU/6/2000 were evaluated. The VLPs were equipped with amino acid substitutions that confer enhanced thermostability as demonstrated by an intact-capsid-specific ELISA. The protective capacity of the stabilized SAT2 VLPs was assessed in cattle. The VLP vaccine elicited high neutralizing antibody titres (>1.6log10) against the homologous virus. Additionally, good heterologous virus neutralizing antibody titres against other SAT2 isolates potentially present in the region, were also obtained. When vaccinated animals were challenged with the homologous strain, at 21 days post-vaccination, they were effectively protected against the disease. These results indicate that VLP vaccines hold great potential as a valuable tool in the fight against FMD.

Virology

Development of a primary cell model derived from porcine dorsal soft palate for foot-and-mouth disease virus research and diagnosis.

Cindy Bernelin-Cottet[1], Caroline Michaud[1], Anthony Relmy[1], Aurore Romey[1], Anne-Laure Salomez[1], Hélène Huet[1], Grégory Jouvion[2], Sara Hägglund[3], Jean-François Valarcher[3], Labib Bakkali Kassimi[1] and Sandra Blaise-Boisseau[1], Morgan Sarry[1]

[1] UMR VIROLOGIE, INRAE, École Nationale Vétérinaire d'Alfort, Université Paris-Est, ANSES Laboratoire de Santé Animale, 94700 Maisons-Alfort, France
[2] Unité d'Histologie et d'Anatomie pathologique, Ecole Nationale Vétérinaire d'Alfort, 7 avenue du Général de Gaulle, 94700, Maisons-Alfort, France
[3] Host Pathogen Interaction Group, Section of Ruminant Medicine, Department of Clinical Science, Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden

Foot-and-mouth disease virus (FMDV) persistence has been evidenced in dorsal soft palate (DSP) epithelial cells in ruminants. While the DSP is one of the primary sites of FMDV replication in both ruminants and swine, no persistent infectious virus was found in swine DSP.

The lack of suitable in vitro models is an obstacle to knowledge progress regarding FMDV persistence. Although we have developed a bovine DSP cell model adapted to FMDV study, no model derived from porcine tissues of interest are currently available. Indeed, the porcine cells used are kidney cells such as IBRS-2.

Primary cells were isolated from DSP collected from 6-month-old swine. These cells were then processed to isolate and maintain the epithelial cells. The presence of epithelial cell markers and FMDV-specific integrin receptors were assessed by immunofluorescence. Once characterised, DSP cells were infected in monolayers and multilayers grown at the air-liquid interface (ALI) with a type-O FMDV. Cell culture supernatant were regularly collected until 115 dpi and were tested for the presence of infectious virus, viral RNA and viral antigen. Analysis of supernatants from the multi-layered model infection led to the detection of infectious virus up to 7 dpi, as well as viral RNA up to 23 dpi.

Consistent with in vivo observations, no evidence of FMDV persistence was observed in our model as no infectious viruses were detected after 28 dpi. The relevance of this model having been confirmed by our results, it would be considered to 71mmortalize these cells in view of developing a FMDV-sensitive porcine epithelial cells lineage. Regarding their sensitivity to a wide diversity of reference and field FMDV strains, as well as other viruses such as Vesicular Stomatitis Virus, Seneca Valley Virus and Swine Vesicular Disease Virus, we consider these cells as a very promising tool for FMDV research and differential diagnosis.

Comparison of formaldehyde inactivation of dried FMDV with 3M Attest 1294-S and discs containing Bacillus atrophaeus spores.

A. Dekker, D. Kuperus, C.A. Baars-Lorist, R. van der Heide and X. Luinenburg

Wageningen Bioveterinary Institute

Knowledge on inactivation kinetics of foot-and-mouth disease virus (FMDV) is essential for a risk-based decision on procedures to reduce escape of FMDV from laboratories. In the current study we compare the inactivation of dried FMDV by formaldehyde gas to the result obtained using the 3MTM Attest 1294-S and Bacillus atrophaeus spores on discs (106 Bacillus atrophaeus spores Apex Discs, MesaLabs).

A predefined volume of 37% formaldehyde solution is boiled dry to achieve a concentration of 0.5 - 7.7 gram formaldehyde gas per m3. We used an airlock, 11 m3, with several sampling points to be able to take samples in time. In total 100 µl FMDV, strain A10 Holland, virus was dried on coverslips for 4 hours in a class II safety cabinet. At the time of sampling the dried FMDV was suspended in EMEM 5% FBS and 2% antibiotics, the disc with Bacillus spores was suspended in Inositol serum. The 3MTM Attest 1294-S tube was processed according to instructions. FMDV titration was performed by plaque count, using 200 µl of 10-fold serial dilutions in duplicate on primary lamb kidney cells and Bacillus spore titration was performed by counting colonies using, using 100 µl of 10-fold serial dilutions on heart infusion sheep blood agar. In total 11 separate experiments were conducted.

In all tests where the 3MTM Attest 1294-S did not show growth, measured by fluorescence, after 4 hours the reduction of dried FMDV was >3.8 log10. The reduction in Bacillus spores, however, ranged from 1 - 5.3 log10. The correlation between reduction of Bacillus atrophaeus spores and dried FMDV was low (R2 = 0.2).

The 3MTM Attest 1294-S indicator is a useful bioindicator for FMDV inactivation by formaldehyde. The poor correlation between 3MTM Attest 1294-S and Bacillus atrophaeus spores on stainless steel discs on one hand and FMDV titre reduction and Bacillus atrophaeus reduction on the other hand was not expected.

Comparison of VP1 sequences between samples transfected with FMDV and clinical samples

Da-Rae Lim, Hyeonjeong Kang, Soyoon Ryoo, Seoyeon Park, TaeYoon Eom, Hyeonwoo Hwang, JunSeong Lim, Jae-Myung Kim, and Sang-Ho Cha

Animal and Plant Quarantine Agency

Foot-and-mouth disease (FMD), caused by the FMD virus (FMDV), is a highly contagious disease of cloven-hoofed livestock that massively affects the domestic livestock industry. Virus isolation is essential for the analysis of FMDV or subsequent experiments to control FMD. But virus isolation may not be successful due to insufficient amount of the samples. Therefore more sensitive methods are required to efficiently isolate viral RNA. Lipofectamine shows remarkable performance when compared to the common infection methods. For that reason, this study is to sequence FMDV viral RNA after transfection using Lipofectamine 3000 and compare with clinical samples.

Clinical samples (serum) from the FMD-affected pigs were used for virus isolation and it was attempted on LFBK αvβ6 cell using Lipofectamine 3000® according to the manufacturer's instructions. After 48 hours of incubation, cytophathic effects(CPE) were observed and the cells and culture media were harvested. Viral RNAs were extracted from the FMDV transfected in cells. The 3D real time PCR was conducted to detect FMDV and sequencing was performed after VP1 coding region amplification. Following sequencing, VP1 sequences of transfected FMDV were aligned using BioEdit 7.2.5. and compared with sequence of clinical samples.

LFBK αvβ6 cells transfected with clinical samples using Lipofectamine 3000® showed clear CPE. Virus isolation was successfully completed showing the Ct value 16.26 on average. Following sequencing, sequences of VP1 coding region(636bp) were obtained. Aligned sequences indicate that the nucleotide homology between the isolated virus and clinical samples was 100%.

This suggests that the VP1 sequences of samples transfected with FMDV are completely same as those of clinical samples. Although it is confirmed that the sequences of VP1 coding region are identical to each other, studies of full-sequence through transfection method and further detailed investigations are needed to confirm their indentity.