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Oral Presentations

Diagnostics

A simple approach for pen-side testing, to rapidly detect foot-and-mouth disease virus and Seneca Valley virus

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Background

Seneca Valley virus (SVV), a picornavirus that infects pigs, has been detected in a growing number of countries since 2014. Although not notifiable, SVV causes clinical signs indistinguishable from other notifiable vesicular diseases, including foot-and-mouth disease (FMDV), potentially complicating disease investigations. A simple field-testing strategy to rapidly detect FMDV and SVV would be a valuable tool to allow rapid differentiation of infection by these viruses.

Methods

Published RT-LAMP assays for FMDV (n=2, targeting the 3D RNA polymerase), SVV (5'UTR), and an additional sample inhibition control (MS2), were optimised into a simple-to-use protocol. A single buffer is used for both sampling and rehydration of lyophilised reactions. At the pen-side, a vesicular lesion swab is placed into the buffer tube. A disposable pastette is utilised to transfer 40 µl of sample buffer to the test strip, which is disinfected while crossing the biosecurity boundary. Reactions are performed on a portable Genie® II (OptiGene Ltd., UK), with an anonymised coded result output.

Test performance was evaluated after incubation of FMDV (n=6) and SVV (n=4) positive and negative (n=4) swabs in the sample buffer at either room temperature or +4oC at various time points (0, 1, 2, 3 and 5 hours) to simulate real-world scenarios. Additionally, the protocol was piloted in a mock-field scenario.

Results

Across the various sample, temperature and time combinations, 328 RT-LAMP reactions were performed. Sensitivity and specificity for the FMDV and SVV assays within the suggested 20-minute cut-off was 100% for all time/temperature combinations. Sensitivity of the MS2 assay was 95.1%.

Conclusions

The protocol is easy-to-use and suitable for use by field teams with limited/no experience in laboratory methodology and is compatible with the reduced dexterity imparted by PPE. It allows differentiation of vesicular viruses in the field, to aid disease control measure decision making.

DEVELOPMENT AND EVALUATION OF A SEROTYPING, REAL TIME RT-PCR ASSAY FOR SOUTHERN AFRICAN TERRITORIES (SAT) SEROTYPES OF FOOT AND MOUTH DISEASE VIRUS (FMDV)

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Introduction

The co-circulation of multiple FMDv serotypes, combined with the lack of cross-protection among them, poses a major challenge for foot-and-mouth disease (FMD) control. Accurate serotyping is critical for effective vaccine selection and disease management. Current serotyping utilises Ag-ELISA (low sensitivity) and/or RT-PCR/VP1 sequencing (lengthy and reagent-consuming). This study aimed to develop and evaluate serotyping RT-qPCR assay for SAT serotypes of FMDv circulating in Southern Africa.

Methods

Primer and probe sets targeting the VP1 region of FMDv SAT serotypes were designed using Primer-BLAST and GenBank sequences. Specificity was evaluated against RNA from vaccine strains representing serotypes O, A, and SAT1-3. Primer sets demonstrating serotype-specific amplification without cross-reactivity were selected for further analysis. Amplification efficiency and linearity (R^2) were determined through standard curve analysis. VP1 sequencing of field isolates confirmed sample serotype identity before RT-qPCR testing. The selected primer/probe sets were validated using 41 representative SAT field samples (SAT1=13, SAT2=18, SAT3=10).

Results

Three primer/probe sets, one per SAT serotype, demonstrated serotype-specific detection with no cross-reactivity. Analytical evaluation demonstrated good amplification efficiency (SAT1: 164.9%, SAT2: 105.9%, SAT3: 100.6%), and linearity ($R^2 \geq 0.94$). Assay validation using field samples showed 100% detection of SAT1 and SAT2 samples by their respective primer/probe sets and 77.8%

detection for SAT3. The assay showed no cross-reactivity, efficient amplification (Ct range, 6.28-36.78), and precise results (CV%=0.38-9.26%), with 100% serotype agreement confirmed by VP1 sequencing.

Conclusion

Serotype-specific RT-qPCR primer/probe sets targeting the VP1 gene of FMDv SAT serotypes were developed and validated. The assay demonstrated high sensitivity and specificity, enabling rapid and accurate serotyping. This advancement supports improved diagnostics and outbreak management in SAT-endemic regions.

Evaluation of a point-of-care molecular test to detect the presence of foot-and-mouth disease virus in milk

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Introduction: Real-time RT-PCR is the currently used diagnostic test for confirmation of the presence of foot-and-mouth disease virus (FMDV) genome in clinical or environmental samples. Although real-time RT-PCR is highly sensitive and specific, it requires a laboratory complete with complex equipment and trained personnel to perform RNA extraction and the assay. There is a need for high performance point-of-care tests to provide rapid testing and reporting in field-deployed settings.

Objective: The objective of this study was to evaluate the ZiP Diagnostics' point-of-care test to detect the presence of FMDV in clinical samples, including milk, serum, oral fluid, as well as oral and nasal swabs collected from experimentally infected cows and pigs. The FMDV serotypes present in the clinical samples were O, A and Asia. A comparison was performed on clinical samples between the ZiP Diagnostics' point-of-care test and the gold standard real-time RT-PCR.

Results and conclusions: The testing results demonstrate good agreement between the ZiP Diagnostics' point-of-care test with the real-time RT-PCR to identify the presence of FMDV in clinical samples. The ZiP Diagnostics' point-of-care test does not require an RNA extraction step, is portable and simple to use. Further evaluation with additional clinical samples including additional FMDV serotypes as well as the determination of the limit of detection are required to validate the diagnostic test in the laboratory as well as evaluation of the point-of-care test in the field.

Fighting FMD on the Frontlines: Lessons from Lateral Flow Device Testing in Indonesia

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Introduction: Foot-and-Mouth Disease (FMD) is a highly contagious viral disease that affects cloven-hoofed animals, posing a significant threat to livestock productivity and trade. Rapid, on-site detection of FMD virus (FMDV) is critical for early outbreak control, especially in resource-limited or remote settings where laboratory infrastructure is lacking. Lateral Flow Assay devices (LFDs) offer a simple, rapid, and cost-effective method for detecting FMDV antigens in field conditions in the form of a "Field lab".

Methods: This study evaluated the diagnostic performance of two commercially available LFDs for the detection of FMDV during confirmed outbreaks in East Java, Indonesia. The sample size requirements were calculated using the Epitools website (https://epitools.ausvet.com.au/prevalencess). Samples (vesicular epithelium and vesicular fluid) from cattle were tested using the LFDs at the point of collection, and results were compared with laboratory-based real-time RT-PCR at the Disease Investigation Centre, Wates, Yogyakarta. We developed logistics and training methods for field teams on the biosecure handling of these devices and the interpretation of results. The LFDs were also used as sample transport devices to extract the nucleic acids in the lab for real-time RT-PCR detection.

Results: We will discuss the learnings from developing field-based applications of these devices, their comparison with laboratory methods, and further applications.

Conclusion: Lateral flow assays provide a valuable tool for rapid FMDV detection at the point-of-care, facilitating timely decision-making and outbreak containment. While slightly less sensitive than laboratory methods, their speed, portability, and simplicity make them well-suited for use in field settings for surveillance and emergency response. Integration of LFA-based diagnostics into national FMD control programs could enhance early warning systems and reduce response times, particularly in endemic or resource-constrained regions.

Keywords: Foot-and-Mouth Disease, lateral flow assay, field diagnostics, outbreak response, point-of-care testing.

Serological and Molecular Detection of Foot-and-Mouth Disease Virus in Livestock Markets of Nigeria: The Role of Nasal Swab Samples in Identifying Sub-clinical Infection

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Background:
Efficient tracking and rapid detection of transboundary animal diseases are critical to controlling their spread. This study aimed to explore the role of international livestock markets in the transboundary movement of foot-and-mouth disease (FMD) virus and evaluate the effectiveness of different sampling methods in endemic regions for timely pathogen detection.
Methods:
Between June 2023 and July 2024, a longitudinal study was carried out at four international livestock markets and one feeder market across three border states in Nigeria (Jigawa, Sokoto, Mubi, Ganye, and Plateau). Weekly collections of blood and nasal swab samples were obtained from cattle, sheep, and goats. In total, 1,150 blood samples from each species were tested for FMDV non-structural protein (NSP) antibodies using the ID-Vet ELISA kit. Samples that tested positive for NSP antibodies were further analyzed for serotype identification using SPCE serotyping kits. Additionally, 225 pooled nasal swab samples were tested using pan-serotypic real-time reverse transcription PCR (RT-PCR) targeting the FMDV 3D gene.
Results:
Out of the 1,150 blood samples tested, 719 (62.5%) cattle, 370 (32.2%) sheep, and 237 (20.6%) goats were positive for FMDV-NSP antibodies. FMDV serotypes O, A, and SAT2 were identified across all three species, with evidence of exposure to multiple serotypes in some animals. Molecular analysis of the 225 pooled nasal swab samples detected FMDV RNA in 20 (8.9%) cattle, 14 (6.2%) sheep, and 6 (2.7%) goats. Overall, cattle showed the highest prevalence of both FMDV antibody positivity and viral RNA detection.
Conclusion:
This study demonstrates that nasal swabs are a valuable tool for early detection of FMD in

livestock markets. It also provides insights into the role of subclinical infections in the epidemiology of FMD in endemic areas. These findings highlight the importance of ongoing surveillance and the implementation of control strategies to mitigate FMD transmission, especially in high-risk market environments.

Stability of foot-and-mouth disease virus stored in cellulose-based FTA cards at different temperatures

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Foot-and-mouth disease (FMD) is a highly contagious viral infection affecting cloven-hoofed animals. In FMD-endemic regions, proper storage and transportation of clinical samples are critical for preserving virological, genomic and epidemiological data. This study evaluated the suitability of Advantec® filter paper, a novel cellulose-based medium, for storing FMD virus (FMDV) RNA for subsequent amplification and sequencing. FMDV RNA stability was tested under four temperatures (-40°C to 25°C) and storage durations (2–12 weeks). Stability was assessed using RT-PCR and sequencing of the VP1 coding region followed by alignment of nucleotides to determine the genomic integrity. The results showed that FMDV genomic RNA remained stable for up to 10 weeks at room temperature (25°C) and 4°C, and over 12 weeks at -20°C and -40°C temperatures. Furthermore, nucleotide sequences generated in samples extracted from filter paper were 100% identical to those from epithelial tissues stored at -80°C. These findings suggest that Advantec® filter paper is a viable alternative to conventional FTA cards for effective preservation of FMDV genomic RNA for up to 10 weeks at moderate temperatures. These findings highlight the potential application of Advantec® filter paper as a cost-effective transport medium for field-based FMDV surveillance and diagnosis, particularly in resource-limited endemic settings in Africa.

Keywords: FTA cards, Advantec® filter paper, FMD virus, viral RNA, storage temperature

Development evaluation of lineage specific assays to detect emerging lineages of concern

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The accurate characterisation of the serotype and lineage of a causative virus of foot and mouth disease (FMD) outbreaks is crucial to accurately inform vaccination and control policies, as well as to facilitate epidemiological studies. Currently, serotype and genotypic characterisation is routinely determined using antigen ELISA and genome sequencing. However, an ELISA can only determine serotype, and sequencing is expensive and time consuming, so therefore rarely practical in countries with limited resources. In contrast, real-time RT-PCR is simple, quick and comparatively cheap. Here, we describe the development and evaluation of an assay to specifically detect the RNA from the O/ME-SA/SA-2018 lineage. The O/ME-SA/SA-2018 lineage emerged in India in 2018, has since spread into pool 3 as far as Iran, and is now considered a threat to Southeast Asia.

A real-time RT-PCR targeting SA-2018 was validated by testing a genetically diverse panel of 78 FMDV isolates originating from 18 different countries in Asia. All (18/18) the samples previously shown by sequencing to be O/ME-SA/SA-2018 had a Ct value from the SA-2018 assay which was approximately equal to the Ct obtained using the 3D assay. For most samples of non-O/ME-SA/SA-2018 lineages, no fluorescence was evident. In a small number of cases (n=14), weak signals were observed with resulting Ct values at least 20 cycles greater than the values from the 3D assay. Therefore, comparing results from this lineage specific assay with those from the 3D assay and applying a straightforward cutoff enables 100% specificity for the O/ME-SA/SA-2018 lineage. Other lineage specific assays have also been developed and validated with similar results, including one to detect the SAT2/XIV lineage which recently emerged in Ethiopia and the Middle East.

This work demonstrates how PCR can be used to more efficiently characterise circulating strains of FMDV to enable timely responses to emerging FMDV threats.

Diagnosis of FMDV infection using mouth and nose swabs is fast and reliable

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To be able to detect an outbreak of FMD as soon as possible, it is very important that the samples that are submitted to the investigating laboratory are fit for purpose, i.e., will contain virus or at least viral RNA. Lesion material (from vesicles) is preferred because it contains the highest amounts of virus. During outbreaks where vesicles are clearly present, this would be the material of first choice.

However, vesicles might not be present or hard to find during early infection or in small

ruminants. When animals are sampled for tracing purposes, e.g. due to an epidemiological link with a confirmed outbreak, or when additional samples are taken on a farm where an outbreak has already been confirmed, not all animals of interest will have lesions.
In the absence of vesicles, blood samples are often collected and sent to the lab, but the duration of viraemia in FMDV infection is usually very short. In contrast, virus can be detected for a longer period in nose and mouth swabs. In practice, these swabs are also very easy to obtain.
We will show data from animal experiments in cattle, sheep and pigs, as well as data from a recent FMD outbreak that confirm that swab samples are a viable alternative (instead of using vesicular lesions or blood samples) to detect an FMDV infection.
We strongly recommend that the use of swab samples for FMD diagnostics is included in contingency plans.

Lyophilised PCR reagents to simplify nanopore sequencing of foot-and-mouth disease virus

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We have previously developed a universal nanopore strategy to sequence foot-and-mouth disease virus (FMDV) genomes based upon multiplex PCR followed by library preparation using rapid barcoding. Whilst straightforward, this method involves the careful allocation of 28 different primers into two amplification pools. Here, we describe the generation of lyophilised versions of the PCR reactions necessary to amplify FMDV genomes.

The SuperFiII mastermix used in the original protocol was found to contain levels of glycerol detrimental to the lyophilisation process, therefore different polymerase mixes were trialled to determine their ability to amplify FMDV in a multiplex format, as well as their suitability for lyophilisation. VeriFi® Hot Start was found to be the optimal choice for lyophilisation and was selected for further development. Trial batches of VeriFi® Hot Start PCR mixes were prepared in 8-well PCR strips and sealed in foil sachets. Three different excipient combinations were trialled, with 15% trehalose found to be optimal for generating a good cake structure. PCR evaluation revealed that multiplex amplification was possible using the lyophilised reactions.

The stability of the lyophilised products was assessed by incubating the trial pellets at either room

temperature or 4 °C for eight days prior to performing the PCRs. No reduction in performance was evident following the incubation, providing evidence that these strips can be shipped and/or stored at room temperature. To demonstrate the utility of pre-made PCR mixes, the strips were trialled in Jordan and Ghana, where complete FMDV genome sequences were obtained. Amplification was successful but reduced compared to SuperFiII; however, this can be compensated for by adjusting the input amount for the library preparation.

In summary, the lyophilised reactions described here simplify the PCR process and are stable at room temperature. Together, it is anticipated that these characteristics will further facilitate sequencing FMDV samples in endemic countries.

Epidemiology

Foot-and-mouth disease (FMD) outbreaks investigation in cattle: insights for FMD virus evolution in the southern highlands of Tanzania

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Abstract

Foot-and-mouth disease (FMD) is one of the most economically devastating viral diseases affecting cloven-hoofed animals globally. FMD is caused by the FMD virus (FMDV) which has seven antigenically distinct serotypes namely A, O, C, Southern African Territory (SAT) 1, SAT 2, SAT 3 and Asia 1. In Tanzania, FMD has been caused by FMDV serotypes SAT 1, SAT 2, O and A. In the Southern highlands of Tanzania, serotype SAT 1 was last reported in cattle in 1999 and in African buffalo in 2013. This study was conducted to investigate the presence of FMDV field strains following the 2023 FMD outbreaks that occurred in Mbeya, Sumbawanga, and Njombe regions, Tanzania. The investigation involved the collection of epithelial tissues from clinically sick cattle. The analysis of samples was conducted by reverse transcription polymerase chain reaction (RT-PCR) targeting the 5'UTR and VP1 coding regions of FMDV genome, nucleotide sequencing of VP1 region and pylogeny. The results revealed that the detection rate of FMDV was 65.8% (n = 48) and serotypes A, O, and SAT 1 strains were involved in causing FMD outbreaks in Makambako-Njombe, Uyole-Mbeya, and Sumbawanga-Rukwa areas of Tanzania. Phylogenic analysis revealed the presence of variant SAT1 lineage(s) from cattle in Sumbawanga suggesting the recent re-emergence of SAT strains in the southern highlands of Tanzania. Further studies are

required to unravel the factors responsible for evolution and re-emergence of SAT1 variants in Tanzania and neighboring countries for appropriate control of FMD in the region.

Key words: FMDV, RT-PCR, Genotypes/Topotypes, Lineages, Phylogeny, Tanzania

Inferring Transmission Dynamics of Foot-and-Mouth Disease Virus: Who Infected Whom?

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Understanding how infectious diseases spread and why some infections propagate while others do not is central to understand the disease ecology and its control. This study investigated the transmission dynamics of foot-and-mouth disease virus (FMDV) in African buffalo (Syncerus caffer), a key wildlife reservoir in endemic regions of southern Africa. For this study a longitudinal cohort of 108 individually identified African buffalo in Kruger National Park, South Africa were sampled (serum, tonsil and probang) approximately every two months for three years. We combined detailed serological, virological profiling, contact network data from proximity collars, and whole-genome viral sequencing, to reconstruct viral transmission chains.
Serological data revealed widespread exposure to SAT1, SAT2, and SAT3 serotypes; however, SAT1 reported for the majority of successfully isolated viruses (>95%). Whole genome sequencing (WGS) was performed on 101 SAT1 viral isolates obtained from approximately 50% of the qPCR-positive individuals. Phylogenetic analysis revealed high diversity within the SAT1 serotype and viruses sampled from later captures tend to be more divergent, indicating evolution over >2 years. The SAT1 phylogeny also explains most late viruses' group with viruses circulating earlier, consistent with the transmission links. However, some of the late viruses are unrelated to the earlier viruses and might have come from different sources or infections. Furthermore, tonsillar samples collected at later time points showed the highest phylogenetic diversity, implicating the tonsils as a potential reservoir for diverse or emerging viral strains.
This study highlights the importance of combining genomic, epidemiological, and ecological data to understand FMDV infection dynamics and also underscores the complexity of FMDV persistence and transmission in endemic settings in African buffalo.

‘Out of Africa’ - incursions of exotic foot-and-mouth disease viruses into the Western Asia

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Incursions of exotic foot-and-mouth disease viruses (FMDV) from East Africa have been historically associated with trade in livestock or products of animal origins. Recent findings of FMD cases in Western Asia led to the isolation of unexpected SAT2/XIV and SAT1/I FMDV lineages originating from East Africa. We assessed these recent FMDV incursions by analysing both sequences encoding the VP1 region and full genomes to reconstruct virus movements from likely sources in Eastern Africa, and to map likely transmission links across Western Asia. Bayesian phylogenetic reconstruction demonstrated that the recent introductions of SAT2/XIV into western Asia were defined by multiple independent incursions. Outbreaks detected in Oman and Bahrain were not directly linked, supportive of independent introductions of the virus during 2022–2023. Furthermore, cases in Iraq appeared to be caused by viruses derived from a single ancestor introduced during late 2022, and those subsequently detected in Turkey likely originated from Iraq; however, outbreaks reported in Jordan were likely caused by a virus of a different origin. Phylogenetic reconstruction of the SAT1/I incursion shows viruses isolated from outbreaks reported in Bahrain and Iraq form sister clades, suggestive of independent introductions again from East Africa (basal to the clade is a virus isolated from Tanzania during 2020). Cases in Türkiye are likely derived from viruses that originated from Iraq. It is interesting to note that these recent SAT1/I outbreaks were phylogenetically distant to earlier cases detected in Qatar during 2023, which were most closely related to a different virus from Kenya isolated in 2020. The emergence of these two exotic SAT1 and SAT2 serotypes, and their rapid onward spread among naive populations, poses threats not only to the region, but also to countries in Europe protected by the vaccination buffer zone in Turkish Thrace.

Does dose availability affect the optimal allocation of vaccines in settings of endemic Foot-and-Mouth Disease?

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Background & Aims of Study
In settings where Foot-and-Mouth Disease (FMD) is endemic, vaccine supply is often limited, making it challenging to determine optimal allocation strategies. These may vary with dose availability—for instance, vaccinating all animals on farms above a certain herd size may be more effective than vaccinating farms at random only when the proportion of farms vaccinated is sufficiently large.

This study investigates how optimal allocation strategies shift as vaccine availability increases. We ask: 1) Is there a consistent ranking of strategies across evaluation criteria? 2) Does this ranking change with the number of available doses?

Methods & Results
We use a previously developed and validated model of FMD transmission in Turkey —an endemic region with high-resolution movement data — and simulate multiple allocation strategies across a range of dose availabilities. Outcomes include average prevalence and probability of eradication. We also conduct a sensitivity analysis to identify key policy drivers.

We find that the resultant ranking of strategies depends on the evaluation criteria used. Random allocation is usually most efficacious when prioritising reduction in prevalence, but minimising annual incidence exhibits a more complex ranking – the most efficient depending strongly on doses available.

Implications
Our findings underscore the importance of tailoring vaccination strategies to available resources. In particular, it is necessary not just to increase the number of doses available but also to ensure that doses are optimally targeted based upon the availability of vaccines, so that the impact of any vaccination campaign is maximised.

Evolutionary Emergence of the A/ASIA/G-VII FMDV lineage in Western Asia

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Trans-pool movements of foot-and-mouth disease viruses (FMDV) can substantially impact a region's risk profile. The A/ASIA/G-VII lineage has been historically circulating within the FMD endemic Pool 2, primarily in India. However, outbreaks due to this lineage were reported in Western Asia (Pool 3) from 2015 and onwards. This study aimed to investigate the molecular evolution, geographic spread, and selective pressures driving the emergence and spread of the A/ASIA/G-VII lineage. We analysed 327 FMDV sequences encoding for VP1 and a further 48 complete genomes, retrieved from FMDbase (www.fmdbase.org). Bayesian inference of the A/ASIA/G-VII lineage using VP1 data estimated the ancestral origin during 1980 and an evolutionary rate of 5.97 × 10^-3 substitutions/site/year. Phylogeographic analysis reconstructed the westward spread from Pool 2 (primarily India) into the FMD endemic Pool 3, beginning in 2012 and coinciding with the emergence of viruses with a deletion at residue 59 of the VP3 coding region. Spread of this lineage involved repeated geographic virus transitions from India into Saudi Arabia, and from India to neighbouring Pool 2 countries. Branch-specific analysis revealed evidence of positive selection at residues flanking the VP3-59 deletion for viruses introduced into both Saudi Arabia and Pool 2. Additional positively selected sites were identified within the VP1-coding region. Signals of within-lineage recombination were detected in 5 out of the 48 complete genomes analysed. In summary, the A/G-VII lineage expanded geographically and evolved under selective pressure, with recombination likely contributing to its diversification. These findings underscore the need for a continued FMD genomic surveillance to better inform risk-based regional control strategies.

Foot-and-mouth disease in small ruminants in North-western Pakistan

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Foot-and-mouth disease (FMD) is endemic in Pakistan and three serotypes of FMDV i.e. serotypes O, A and Asia-1, are responsible for outbreaks in the country. Clinical signs are more severe in cattle than buffalo. Exotic and crossbred cattle are particularly susceptible to the disease. However, sheep and goats usually show milder or inapparent clinical signs. Small ruminants are not vaccinated against FMD in Pakistan and samples are not usually collected for diagnosis of FMD due to inapparent clinical signs. Thus, the role of small ruminants in the epidemiology of FMD is unknown in Pakistan. This study investigated the role of small ruminants in maintenance and spread of FMDV in North-Western Pakistan.

Both serological and virological surveillance of FMD was carried out in small ruminants. Serological surveillance was conducted by collecting a total of 1743 blood samples from sheep (n = 366) and goats (n= 1377). These samples were tested for the presence of antibodies against non-structural proteins (NSP) of FMDV. Samples that scored positive for antibodies against NSP of FMDV were further tested to ascertain serotype of FMDV. Virological surveillance was carried out by collecting a total of 100 oral swab samples from small ruminants. RNA was extracted and tested for the presence of FMD viral genome using pan-FMDV real time RT-PCR assay. Samples showing CT values <32 were subjected to conventional RT-PCR for determination of serotype of

FMDV and sequencing the PCR products for determination of FMDV subtype.

A total of 593 samples scored positive for antibodies against NSP, showing an overall 34.0% prevalence of FMD. Species-wise, 128 sheep and 465 goats scored positive for antibodies against NSP, showing 35 and 33.8% prevalence of FMD, respectively. No significant difference ($P>0.05$) in the prevalence of FMD between sheep and goats was noted. However, a significantly higher prevalence ($P<0.001$) was noted in small ruminants reared with large ruminants compared to those which were not reared together with large ruminants. As NSP antibodies is a herd-level test, each village/flock was taken as epidemiological unit and village-wise/herd-wise prevalence was determined. Of a total of 187 villages/flocks, 124 were found positive for FMD, showing village/flock level prevalence as 66.3%. A total of 404 samples (73.2%) scored positive for antibodies against structural proteins of either serotype O, A or Asia-1 FMDVs or combination thereof. Of the positive samples, 284 (70.3%) tested positive for antibodies against structural proteins of single serotype, while 120 (29.7%) scored positive for antibodies against more than one serotype of FMDV. Antibodies against serotype O FMDV were predominant (59.9%), while those against serotype Asia-1 FMDV were the least prevalent (0.7%).
In virological surveillance, 16 swab samples scored positive for FMDV RNA. CT values of the positive samples ranged from 19.6 to 37.4. Serotype of the 11 positive samples were established as O (n=2) and A (n=9). The VP1 coding nucleotide sequences were successfully generated from a total of two serotype O and seven serotype A FMDVs. Phylogenetic analysis of serotype O FMDVs shows that both the viruses belong to the O/ME-SA/PanAsia-2PUN-16 sublineage. Phylogenetic analysis of serotype A FMDVs reveals that all the seven viruses sequenced here belong to a new/unnamed sublineage within the A-Iran05 lineage.

Germany's First FMD Outbreak in 37 Years: Detection, Recovery, and Lessons Learned

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On 10 January 2025, Germany detected its first FMD outbreak since 1988, affecting a water buffalo herd in the state of Brandenburg. Three of 14 buffaloes had died within two days, and one was submitted for post-mortem examination, which revealed extensive oral and ruminal mucosal erosions, necrotizing myocarditis, and pulmonary edema. FMDV was detected by real-time RT-PCR in multiple tissues and successfully isolated from a lung sample. The remaining 11 buffaloes on the outbreak farm as well as 325 susceptible animals from nearby holdings and a high-risk

contact were culled immediately. Movement restrictions for susceptible animals were imposed for Brandenburg and Berlin.
Serological analysis showed no FMDV antibodies in the deceased animal, while the culled buffaloes tested positive for both antibodies and viral RNA. The 325 animals that had been preemptively culled were negative. Phylogenetic analysis identified the virus lineage as O/ME-SA/SA-2018, sharing 99.8% nucleotide identity in VP1 with a 2024 isolate from Eastern Anatolia. Epidemiological investigations suggest virus introduction in late December 2024. The source remains unknown, with hypotheses including deliberate introduction or accidental exposure to illegally imported contaminated animal products. The outbreak farm is located near Berlin and is a popular spot for outdoor activity.
Comprehensive surveillance of 150 holdings with nearly 7,000 animals within a 10-km radius, six other contact holdings, and thousands of animals shipped from Brandenburg in December and early January detected no additional cases. No FMDV or specific antibodies were found in over 500 wild animals tested. Germany regained its FMD-free status in April 2025, though some third-country trade restrictions remain. The total economic impact has been estimated at 1 billion Euros.

A cross-sectional study of FMDV in African Buffalo in the Kruger National Park, South Africa: Diagnostic and Genetic characterization

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Introduction

South Africa lost its foot-and-mouth disease (FMD) free status in 2019 as a result of outbreaks The relationship between FMD and its virus maintenance hosts, the African buffalo, in the Kruger National Park (KNP) is a critical area of study due to virus transmission risks. Thus, circulating FMD viruses (FMDV) were investigated by sampling buffalo herds along a 300km North to South transect of KNP.

Materials and Methods

FMD serological and molecular diagnostic methods were used to analyse buffalo samples. Further characterisation of FMDVs were performed by analysing Sanger and next generation sequences.

Results

Seropositivity of buffalo for FMDV non-structural and structural proteins was >90% for all serotypes. Lower qPCR positivity for probang samples was observed than for tonsil swabs. Virus isolations were successful from 75 tonsil swabs. Spatial distribution for SAT1 and SAT2 was similar showing mixture of viruses, whilst SAT3 showed three clusters. Variation between serotypes in terms of persistence revealed that clusters were derived from KNP historical viruses with SAT2 having one dominant cluster. Interestingly, the FMDV SAT1/SAR/35/2024 outbreak strain (topotype II, a new introduction into the KwaZulu-Natal province of South Africa) was genetically similar to viruses isolated from this study.

Discussion and Conclusion

Diagnostics of probang samples have a low success rate compared to tonsil swabs. African buffalo harbor multiple FMDV serotypes and theevidence of evolution shows that surveillance of FMD is important. Genetic resemblance of SAT1 and SAT3 to historical strains suggest continued circulation among wildlife populations. The similarity of recent outbreak strains to the buffalo strains proves the threat of buffalo to cattle transmission still exists thus, robust and well-maintained fencing in areas where buffalo reside, can limit transmission. This study's data is preliminary but when complete, the results can inform mitigation strategies in identified high-risk areas and inform decisions regarding the FMD buffer zone based on the spatial distribution of SAT serotypes.

Machine Learning and Artificial Intelligence Frameworks for Foot-and-mouth Disease Molecular Epidemiology and Vaccine Matching Predictions

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Molecular epidemiology and vaccine matching studies of Foot-and-mouth disease (FMD) are crucial to implement its control strategies including vaccination and containment. The existing approaches for the same are biological in nature, which are time-consuming and risky due to live virus handling. Thus, novel Artificial Intelligence (AI) tools are highly required for large-scale molecular epidemiology and vaccine matching of FMD virus. This study reported machine learning algorithms and AI tools for FMD molecular epidemiology and vaccine matching. Initially, ten machine learning algorithms were evaluated on cross-validated and ten independent

secondary datasets for serotype/topotype/lineage and vaccine matching score predictions through accuracy, sensitivity and 14 other metrics. Next, four best performing algorithms (Support vector machine, random forest, Xtreme Boosting, and Gradient Boosting) were identified with higher predictive accuracies for molecular epidemiology and vaccine matching. These four algorithms are implemented in the computational tools. Then, performance of these tools was assessed on five independent secondary datasets, never seen before and wet-lab generated experimental data. The cross-validated and independent evaluation of learning algorithms revealed that support vector machine, random forest, XGBoost, and Gradient boosting algorithms outperformed others. For instance, these four algorithms achieved accuracy ≥96% and precision ≥95% on cross-validated data, when evaluated for vaccine matching. These algorithms are implemented in the AI tools, i.e., MolEpidPred (https://nifmd-bbf.icar.gov.in/MolEpidPred) and FMDVacMac (https://github.com/ICARNIFMD/FMDVacMac) for molecular epidemiology and vaccine matching, respectively. The independent validations of MolEpidPred and FMDVacMac tools revealed their satisfactory performance in terms of higher accuracy (>90%), sensitivity and specificity (>95%). The utility of these tools was also demonstrated on wet-lab data, which suggested their quick prediction of results with ≥95% accuracy when benchmarked with their wet-lab counter parts. MolEpidPred and FMDVacMac tools provide innovative platform for quick and large-scale analyses in FMD research, which are crucial for tracking FMD virus infection and assessing the vaccination program.

Immunology

Amino Acid Substitution at VP2-72 Drives Antigenic Diversity in Contemporary FMDV O/ME-SA/Ind-2001 Field Isolates in Asia

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Foot-and-mouth disease virus (FMDV) serotype O is globally circulating and exhibiting genetic and antigenic diversity. Although five independent neutralizing antigenic sites have been identified for serotype O, the specific amino acid substitutions driving antigenic variation require further investigation. We compared virus neutralizing (VN) titers of bovine serum immunized with O Manisa vaccine against fourteen serotype O representative and field strains, classified into ME-SA/Ind-2001, ME-SA/PanAsia2, SEA/Mya-98, and CATHAY topotype/lineages and isolated from Thailand, Myanmar, Pakistan, Chinese Taipei, Hong Kong and Japan between 1997 and 2019, aiming to identify the amino acid substitution contributing to antigenic variation.
The VN titer of the serum against the homologous O Manisa was 362 (28.5), whereas titers against SEA/Mya-98 and CATHAY strains were >8-fold lower, suggesting reduced vaccine serum

reactivity to these strains. The serum showed similar VN titers to the homologous stain against three ME-SA/Ind-2001 strains isolated in 2016 and 2019. However, it showed an approximately 8-fold lower against two other ME-SA/Ind-2001 strains isolated in Myanmar 2019 . Further antigenic characterization using panels of monoclonal antibodies (mAbs) established against ME-SA topotype strains revealed that a VP2- targeting mAb reacted with O Manisa and the three ME-SA/Ind-2001 strains, but did not react with the two Myanmar 2019 strains, indicating the structure of this region is not conserved among ME-SA/Ind-2001 strains. Comparison of the amino acid sequences revealed that O Manisa and other strains showing similar VN titers harbored Ser at VP2-72 in site 2. However, substitution to Asp were confirmed on this position in the two Myanmar 2019 strains. This Ser-to-Asp substitution at VP2-72 likely alters epitope structure, reducing antibody reactivity. Reduced reactivity with both polyclonal and monoclonal antibodies suggests its antigenic relevance. Further analysis using reverse genetics and structural studies will help elucidate its role in immune escape among recent FMDV serotype O strains.

Cattle antibodies identify a cross-serotype broadly neutralising foot-and-mouth disease virus epitope

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Foot-and-mouth disease virus (FMDV) remains a significant threat to global livestock, undermining food security due to its highly contagious nature and antigenic diversity. Neutralizing antibodies play a crucial role in protection, but vaccine effectiveness is compromised by the virus's serotypic variation. In this study, cattle were sequentially vaccinated with FMDV antigens from multiple serotypes to uncover cross-reactive neutralizing epitopes. Using a combination of single-cell isolation and sequencing of FMDV-specific B cell receptors, we selected and expressed 50 recombinant antibodies from 143 identified heavy and light chain pairs. Three antibodies demonstrated broad neutralizing activity, effectively neutralizing 23 viruses from serotypes O, A, Asia1, and C. Cryo-electron microscopy revealed a common epitope with flexible binding confirmed by ELISA, SPR, and bio-layer interferometry (BLI). Co-crystal structures further identified a minimal epitope binding across a shared groove in the antibody paratope. Moreover, immunoinformatics tools enabled the identification of antigen-specific antibody clusters from

whole antibody repertoire analysis. These findings provide new insights into the identification of FMDV cross-specific antibodies and inform the design of improved vaccines capable of eliciting cross-serotype protection.

Field Evidence of Fasciola hepatica-Mediated Modulation of Antibody Responses to Foot-and-Mouth Disease Vaccination in Water Buffaloes

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We previously showed that experimental Fasciola hepatica infection reduces antibody avidity in cattle vaccinated against foot-and-mouth disease virus (FMDV), despite stable total FMDV-specific antibody levels. Here, we assessed the humoral response of water buffaloes (Bubalus bubalis), a species relevant to FMDV epidemiology, naturally infected with F. hepatica under field conditions. Two herds (n = 50 each) were selected based on fecal egg detection and anti-F. hepatica seropositivity. All animals had received two doses of FMDV vaccine, with the last dose administered 264 days before sampling. Serum samples were analyzed for virus-neutralizing titers (VNT), total anti-FMDV IgG levels, and IgG avidity against the A24/Cruzeiro vaccine strain. Infected animals showed significantly lower VNT, IgG levels, and avidity compared to their non-infected counterparts. These findings suggest that F. hepatica infection can impair the humoral response to FMD vaccination, highlighting the need to monitor immune status in vaccinated buffaloes in endemic areas.

Socioeconomics of Disease Control

Quantifying the impacts of landscape ecology and environmental events on livestock contacts and their relevance for foot-and-mouth disease control in East Africa

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In East Africa over 80% of livestock management systems depend on the movement of herds to communal resource areas. These movements are largely uncontrolled and difficult to restrict because they are essential for livestock survival and for the sustenance of rural economies. Herd movements to critical resources (e.g. grazing and watering points) can be represented in a network comprising nodes (key locations of livestock aggregation) linked by movement trajectories (edges) that can facilitate the local spread of transboundary diseases such as foot-and-mouth disease (FMD). Network analyses can therefore reveal locations of high herd traffic, hence of great transmission potential, towards which intervention programmes could be targeted. However, in East Africa, this approach has been constrained by a lack of livestock movement data. To address this paucity of data, we used participatory mapping, involving consultation with local community stakeholders, to generate high-resolution data on local herd movements and contacts at key resource areas in Longido district, Tanzania. We constructed networks of livestock herd connectivity and used generalised linear mixed models to investigate the impacts of landscape and environmental features on connectivity and to predict locations of high disease transmission risks. Normalised Difference Vegetation Index and rainfall were key predictors of livestock contact risks: herds travelled longer distances and had higher contacts in the dry and drought periods compared to wet spells. Village landscape features such as higher gradients of hills, appropriate soil health and reduced greenness had the highest impacts on connectivity and drove herd contacts throughout the network. Here, we provide a framework that enables the use of open-source data on landscape ecological and environmental indicators to determine locations and times of increased risks of transmission, and ultimately to inform vaccination strategies in low-resource settings where FMD is endemic.

Decision tree analysis for evaluation of management practices to reduce FMD outbreaks in beef cattle replacements under the Egyptian conditions

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Foot and Mouth Disease (FMD) is one of the highly contagious viral diseases with a major impact on animal health and productivity. More than 60% of the Egyptian beef industry are small and medium-sized herds (<50 animals and 50–500 animals, respectively) and they follow different

management protocols during receiving newly purchased animals. Receiving newly purchased replacements into feed lot farms represents a major risk for the occurrence of FMD outbreaks in endemic countries. Physical inspection of replacements in living animals' markets and/or at receiving in farms and the timing of FMD vaccination may affect the risk of occurrence of FMD outbreaks under endemic conditions. The aim of the current study was to evaluate the management practices (examination of replacements and the timing of FMD vaccination) using a decision tree model to on the occurrence of FMD outbreaks accompanied with entry of new replacement cattle on Egypt's medium sized feedlot beef farm. A personal interview survey on FMD control and prevention practices was done on a convenient sample of 34 beef herds in Egypt revealed that more than 50% of the surveyed herds relied on live animal markets as a source for replacements and reported more FMD outbreaks (P- value=0.09), FMD herd morbidity > 50% (p-value=0.05), and weight loss > 15 kg/animal for FMD clinical cases in comparison to herds that received replacements from other sources. There were more than 70% of surveyed farms received new animals ≤1year old and had considerably higher FMD outbreaks (P-value=0.02) in comparison to farms that received older animals. Physical inspection of newly purchased animals before entering their premises was done on more than 80% of the surveyed farms. The decision tree model identified the lowest probability of occurrence of FMD outbreak (8.9%) associated with applying physical examination of newly purchased animals prior to arrival and mixing with premises followed by vaccination against FMD upon arrival. In contrast, herds that did not perform physical examination and delay the FMD vaccination for two or more weeks had the highest probability of FMD outbreaks (33.5%).

Investigating the impact of heterogeneity in farmer behaviour on the control of foot-and-mouth disease

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Background & aims of study

Disease management behaviours of farmers are crucial to disease control in their livestock, whilst also contributing to the success of wide-scale disease control. Heterogeneity in farmer behaviour towards disease management therefore warrants consideration when establishing veterinary health policies. However, analytical approaches that can contribute insights to livestock disease control plans, such as mathematical modelling, traditionally omit variation in farmer disease management behaviours.

To address this, we explored how heterogeneity in farmers vaccination behaviour could be incorporated to inform mathematical models. Our ambition was to develop a methodological pipeline to generate novel quantitative data on farmer beliefs with respect to disease management, process the data for use in livestock disease models and then the models according to the findings of the data.

Methods & results

We formulated a novel graphical user interface to dynamically show the progress of a foot-and-mouth disease outbreak in Great Britain; this was used to elicit when farmers would use an available vaccine. We studied how psychosocial and behaviour change factors were predictive of farmer vaccination behaviour. We used the attained behavioural groups within an infectious livestock disease model, exploring how incorporation of heterogeneity in behaviour towards disease management impacted epidemiological and health economic outcomes.

When assuming homogeneity in farmer behaviour versus configurations informed by the psychosocial profile cluster estimates, the modelled scenarios revealed a disconnect in projected distributions and threshold statistics across outbreak size, outbreak duration and health economic measures.

Implications

Our work demonstrates the utility of model frameworks integrating epidemiological and socio-behavioural elements, revealing how omitting heterogeneity in farmers' disease management of livestock infections can result in ill-judged assessments of the likely epidemiological outcomes. The concepts developed also offer a pipeline deployable in other public and veterinary health settings, particularly for incorporating human behaviour into mathematical models of vaccination behaviour.

Predictors of productivity within small-scale beef cattle enterprises in the foot-and-mouth disease (FMD) protection zone of South Africa

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Communal livestock farming is an important component of agricultural production in South Africa and has the potential to contribute to food security and poverty reduction. The objective on this study was to quantify productivity and identify its predictors in small-scale communal cattle enterprises within South Africa's foot-and-mouth disease (FMD) control zone. Both cross-sectional and prospective cohort approaches were employed within 271 participants from 44 dip-tanks (animal assembling points). Profitability of cattle enterprises was estimated using a farm enterprise budget. Farmers with a net income greater than zero were categorized as profitable for the evaluation of predictors. Mixed-effects logistic regression was used to evaluate demographic predictors associated with cattle enterprise profitability and associations were reported as odds ratios (OR) with their corresponding 95% confidence intervals (CI). Correlations between cost inputs and the net income were assessed using Spearman's rank correlation coefficient (rho). Nineteen percent (51/271) of participants were female and 81.0% (218/271) male. The mean (standard deviation) cattle herd size was 15.0 (±17.3) animals. Sales were the major reason of herd exits accounting for 53% of total exits and annual offtake was 12%. The median annual cash revenue was $427 (range, $0 to 10 657). The median annual variable and fixed cost were $44 (range, $0 to 3 983) and $208 (range, $0 to 3 927), respectively. The average (sd) annual net income was $42 (±1 399) with a median of -$26 (range, -$5 285 to 8 495). One hundred and seventeen (43.1%) of the cattle enterprises were profitable (net income greater than $0). The most important parameter influencing the net income was the number of cattle sold (Spearman's rho of 0.58 (95 % CI 0.45, 0.68)). Male farmers were significantly more likely to have profitable cattle enterprises (OR 2.02, 95% CI 1.02, 3.98, P=0.042). Farmers who had more than 21 years of farming experience were more likely to have profitable cattle enterprises (OR 1.82, 95% CI 1.07, 3.08, P=0.047) compared to farmers with less farming experience. Farmers aged between 51-60 years (OR 0.39, 95% CI 0.19, 0.81, P=0.012) were less likely to have profitable enterprises compared to all other age groups. Farmers who supervised their own cattle during grazing were significantly more likely to have profitable cattle enterprises (OR 1.8, 95% CI 1.07, 3.08, P=0.026). Agricultural policies aimed at increasing the productivity of small-scale communal cattle farmers should prioritize improving women's access to essential resources such as animal health services, credit, and training. Additionally, targeted investments and support for communal cattle farmers should focus on reducing mortalities and the costs of medicines

Profound financial and welfare impacts of Foot-and-mouth disease: a case series in two dairy farms in Indonesia in June 2022

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Foot-and-mouth disease (FMD) reemerged in Indonesia in April 2022, causing widespread production and economic losses in the cattle sector. In August 2024, as part of a feasibility study

for registration of a wound pain therapeutic formulation (Tri-Solfen, Medical Ethics, Australia), two dairy farmers in East Java were interviewed to obtain on-farm clinical FMD-outbreak information. Animal-specific data was obtained from 5 animals from each farm (n=10 total) to quantify the acute and chronic impacts of FMD. The mean age of the 10 dairy cattle was 3.4 years, with breeds including Holstein-Friesian (7), Red Holstein-Friesian (1), Friesian Holstein-Friesian x Simmental (1) and Jersey (1). All cattle became infected with FMD between the 21 June and 15 August 2022, although did not receive FMD vaccination until 12 September 2022. The average liveweight prior to and following FMD was 462 kg and 230 kg respectively; an average loss of 50%. The average value of cattle pre-FMD was USD $1,390, declining to $165 during active FMD, and recovering to $797; a loss of 43%. Farmers reported all cattle displayed clinical signs including foot lesions, lameness, mouth vesicles, milk drop and weight loss, with udder oedema and necrosis also reported. Treatments with a range of products included Oxytetracycline, Lidocaine, Metamizole, Vitamin B and Tumeric, with an average treatment cost of $31.70 per animal. On-farm labour hours increased from an average of 9.5 to 22 hours per day, with enhanced animal care required for 75 days. The average number of days to recover was 272 days. Compromised reproduction was also reported as a major issue post-FMD. This case-series demonstrates the cataclysmic nature of an FMD outbreak on both animals and producers. Urgent research to improve therapeutic strategies including non-antibiotic therapies, is required to assist limiting the impacts of FMD and aid faster recoveries.

Vaccines

Characterization and Antigenicity of Novel Foot-and-Mouth Disease Virus Serotype O Mosaic Vaccine Constructs

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Foot-and-mouth disease virus (FMDV) is a high consequence pathogen in dire need of an efficacious and immunogenic vaccine. The mosaic vaccine strategy was applied to FMDV serotype O to rationally design an array of full-length (FL) vaccine constructs. These FMDV-FL-O Mosaic viruses were cloned and characterized in vitro, illuminating their efficient replication as well as enhanced stability when compared to parental FMDV O viruses. The antigenic profile of these FMDV-FL-O Mosaics offer a broader range of epitopes than parental FMDV O viruses when tested using a monoclonal antibody panel. When utilizing two candidate Mosaic viruses in their leader-

less form, FMDV-LL3B3D-O Mosaic 2.1 + 2.2.7, as vaccines in cattle these Mosaic-vaccinated animals are protected from lymphopenia after challenge with virulent FMDV viruses FMDV-O/South Korea/2010 and FMDV-O/Pan Asia/2010. These FMDV-LL3B3D-O Mosaic 2.1 + 2.2.7-vaccinated cows also exhibit robust B and T cell responses to parental FMD O viruses before and after challenge. The ratio of subpopulations of T cells in FMDV-LL3B3D-O Mosaic 2.1 + 2.2.7-vaccinated cattle are also resistant to dysregulation after challenge when compared to un-vaccinated animals. Overall, these data highlight the vast potential of the FMDV-O Mosaic viruses as an effective vaccine platform that could have meaningful impact on the global agricultural industry.

Development of in vitro tests for FMD vaccine quality using ELISA and Lateral Flow Device

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Vaccination remains a cornerstone for controlling and preventing foot-and-mouth disease virus (FMDV) outbreaks. The effectiveness of conventional FMD vaccines depends largely on the presence of the 146S antigen, the intact viral particle responsible for inducing protective immunity. Under improper storage or handling, these particles degrade into 12S subunits, which are less immunogenic and also do not induce a neutralising response.

Monitoring the 146S content is therefore essential to ensure vaccine potency throughout manufacturing, storage, and distribution. While sucrose density gradient ultracentrifugation remains the gold standard for detecting 146S, it is labour intensive and requires specialised equipment, making it impractical for large-scale or routine quality control.

To overcome these limitations, we previously developed a novel ELISA to quantify 146S and total antigen in vaccine samples. The assay uses antibodies targeting VP4 (specific to 146S) and VP2 (present in both 146S and 12S). Here we describe the development of this assay into a kit format using integrin pre-coated/stabilised plates, conjugated detection antibodies and epitope-bearing peptides as internal controls. This ELISA enables high throughput testing in standard lab settings and is compatible with both aqueous and oil-adjuvanted vaccines after appropriate sample preparation. To test the universal nature of this test, a panel of viruses representing the overall genetic diversity in FMDV, was tested for binding to integrin and recognition by the detection antibodies.

Complementing the lab-based ELISA, a lateral flow device (LFD) has been developed for field use.

This portable, easy-to-use test is intended to enable on-the-spot assessment of vaccine integrity, particularly valuable in remote locations or during distribution, where cold chain breaches may compromise vaccine quality. A portable reader or smartphone app can be used to quantify band intensity for rapid interpretation.

Together, the ELISA kit and LFD provide complementary solutions for monitoring vaccine quality from production through to point of use. This could both reduce the reliance on in vivo testing for vaccine quality control and help to ensure animals receive potent, protective doses of vaccine, critical to effective FMD control.

Immunological Features of Nucleoside-modified mRNAs Encoding FMDV VP1

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Despite the widespread use of inactivated vaccines, challenges such as limited stability, insufficient cellular immunity, and frequent boosting requirements highlight the need for alternative FMDV vaccination strategies. mRNA-based vaccines, which have proven effective in recent human applications, offer a promising platform for veterinary use due to their safety, rapid production, and adaptability.
In this study, we designed a nucleoside-modified, in vitro transcribed (IVT) mRNA encoding the VP1 protein of FMDV serotype O (TUR/13/2007 isolate). The construct was verified for in vitro expression in Huh-7 cells using Western blot and ELISA. For delivery and immune enhancement, the mRNA was formulated with PLGA (poly(lactic-co-glycolic acid)) nanoparticles via an emulsion-based method.
BALB/c mice were immunized intramuscularly with the mRNA-PLGA complex. Serum samples collected post-immunization revealed a strong VP1-specific antibody response. Preliminary analyses also suggest potential cellular immune activation, supporting the hypothesis that this platform can elicit a balanced and protective immune profile.
Our findings demonstrate the feasibility of using nucleoside-modified mRNA technology to develop next-generation FMDV vaccines. The combination of structural gene targeting (VP1) and nanoparticle-based delivery may overcome several limitations of traditional vaccines and holds promise for future applications in animal health.
Keywords: FMDV, mRNA vaccine, VP1, PLGA, pseudouridine, immune response

An mRNA nanoparticle vaccine fully protects cattle against virulent FMD challenge

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The efficacy of an mRNA vaccine encoding the FMDV O1 Manisa capsid polyprotein and 3C protease was evaluated in 24 weaner calves. Animals were vaccinated twice either four weeks apart (n=9) with 50 µg of mRNA or one week apart (n=9) with 25 µg. A third group (n=6) served as unvaccinated controls. All animals were challenged intranasopharyngeally with 10^7 TCID50 of FMDV O/FRA/1/2001 two weeks after the second dose.
In the 4-week interdose group, all animals seroconverted strongly to structural proteins (SP, measured by ELISA) prior to challenge, and were fully protected upon virus exposure. No virus shedding, lesions or fever were observed. In contrast, the 1-week interdose group showed weaker SP antibody responses and only partial protection: one animal developed generalized FMD, and three others shed virus transiently. All control animals developed typical FMD lesions with high levels of virus shedding. Viremia data and virus neutralization titers will be presented.
Post-challenge non-structural protein (NSP) seroconversion was observed in all control animals by day 14 but was slow and inconsistent in vaccinated animals. Probable persistent infection (PCR-positive probang samples on days 22 and 25 after challenge) was detected in 4/9 animals in the 4-week interval group, 1/9 in the short-interval group, and in 4/6 controls.
In summary, two doses of the FMD mRNA vaccine given four weeks apart provided complete protection against clinical disease and viral shedding after challenge. A one-week interdose interval with a lower dose of vaccine resulted in reduced immunogenicity and efficacy but still conferred partial protection.

Assessing FMD Vaccine Quality – moving beyond vaccine matching

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Despite widespread use of vaccines to control foot-and-mouth disease, there is no systematic approach to demonstrate the regional relevance of these products. With over two billion doses being administered annually worldwide and the knowledge that some vaccines are sub-optimal, a new algorithm to confirm the suitability of multivalent vaccines is urgently needed.
An approach has been developed to support the purchase of relevant good quality vaccines in East Africa (https://www.wrlfmd.org/fmd-vaccine-quality-control/eastern-africa-foot-and-mouth-disease-virus-fmdv-reference-antigen). This involves the use of a well-characterised antigen panel and virus neutralisation (VN) assays to test post-vaccination responses of formulated vaccines that would be purchased by customers. Such a comparison reveals the range of lineages that a vaccine might provide coverage for. The testing can accommodate different timepoints, booster vaccinations and species. Although there are gaps in our understanding of heterologous protection, and the exact protective titre threshold that should be applied, our results show that poor-quality vaccines induce low levels of neutralising antibodies.
This antigen panel has been expanded to include antigens for all lineages currently circulating. Using VP1 amino acid phylogenetic trees as a guide, forty relevant viral lineages were identified. The Serological Working Group of the FMD Network helped to select representative viruses from each lineage with preference for viruses which had previously been antigenically characterised by PVM studies, vaccine matching and/or PD50/PPG studies. The number of viruses chosen for each lineage was not pre-determined and where possible, isolates were selected to cover multiple pools. This resulted in 50 candidates which are being analysed for their antigenic relatedness by monoclonal antibody ELISA and VN, to highlight viruses that represent the antigenic diversity of virus lineages within and across pools. Once reviewed and approved by the WOAH/FAO FMD Reference Laboratories the panel will be made available to laboratories as a starting point to harmonising results obtained from post-vaccination studies.
The benefit of this simple approach is that testing can be performed independent from the vaccine manufacturer, providing the customer empirical data to confidently select and use FMD vaccines in the field.

Development of a High-Potency Multivalent FMD Vaccine with Broad immunological coverage Across Endemic Pools

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Foot-and-Mouth disease (FMD) remains a major challenge to livestock production and trade worldwide, underscoring the need for effective vaccination strategies. Here, we describe the development and comprehensive evaluation of a high-potency, multivalent FMD vaccine designed to provide broad cross-protection across multiple endemic pools.
The vaccine incorporates well established vaccine strains O1 Campos, A24 Cruzeiro, A2001

Argentina, and the newly developed Asia1 TUR 2015 and SAT2 OMN 2015, formulated in a single water-in-oil emulsion, produced by Biogénesis Bagó (Argentina). The manufacturing process has been thoroughly validated and tightly controlled, in accordance with GMP, ensuring consistency in quality, potency and safety.

Challenge studies conducted at both the WBVR (FAO FMD Reference Laboratory, Lelystad) and The Pirbright Institute (FAO/WOAH FMD World Reference Laboratory), demonstrated a potency of ≥32 PD_{50}/dose for O1 Campos, A24 Cruzeiro and SAT2 OMN 2015 vaccine strains, ≥ 128 PD_{50}/dose for A2001 Argentina and 97 PD_{50}/dose for Asia1 TUR 2015 strain. The vaccine was rigorously assessed for safety, potency and DIVA compatibility. Immunogenicity studies confirmed sustained neutralizing antibody titers for at least six months (mean VNT >2 log_{10} at 180 days post-vaccination) in both cattle (n=16) and sheep (n=8), following intramuscular or subcutaneous immunization.

Vaccine matching studies conducted at The Pirbright Institute revealed robust antigenic coverage against principal FMDV lineages circulating in diverse endemic pools, with heterologous VNT titers ≥1.5 log_{10}. Coverage included key serotype O, A, Asia 1, and SAT2 lineages, encompassing recent field isolates from 2022–2025, such as O/SA-2018 (Germany/Iraq), O/PanAsia-2 (Turkey/Pakistan), O/Ind2001e (Indonesia/Nepal), O/EA-3 (Tunisia), O/EA-2 (Kenya/Uganda), A/Iran-05 (Pakistan), Asia1/Sindh-08 (Pakistan) and SAT2 topotypes XIV (Turkey/Jordan/ Iraq) and V (Algeria).

These results highlight a robust approach to developing high-potency FMD vaccines capable of delivering broad cross-protection and adaptable to evolving epidemiological scenarios in both endemic and high-risk regions.

Early protection conferred in cattle using live attenuated vaccine against foot-and-mouth disease (FMD)

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Foot-and-mouth disease (FMD) remains a significant threat to global livestock industries, necessitating vaccines that provide rapid and robust protection. Vaccination with the inactivated adjuvanted FMDV vaccine continues to be a vital strategy for prevention against the disease. We have previously demonstrated that codon pair deoptimization (CPD) of the P2/P3 coding region of the FMDV A24 Cruzeiro genome resulted in a safe and effective live attenuated vaccine (LAV) strategy in cattle with challenge studies conducted at 28 days post inoculation (dpi) and after a

boost at 14dpi. However, early protection at 4 or 7dpi has not been evaluated yet. In this study we assess the efficacy of this LAV to determine its ability to elicit early protection. Vaccination with 106 pfu of P2/P3 CPD A24 virus 7 days prior to challenge with wildtype virus conferred complete clinical protection in all animals (4/4) without exhibiting viremia or viral shedding determined by virus isolation. Vaccination 4 days prior to challenge conferred partial protection with animals developing mild FMD when compared to control challenged animals. In this group, low level of shedding was detected when compared to WT challenged animals. Vaccination in both groups resulted in a strong serum neutralizing antibody response. These findings demonstrate that FMDV LAV induces a rapid and robust immune response, achieving protective immunity by effectively limiting viral replication and dissemination. This study provides the first evidence that a codon deoptimized P2/P3 FMDV can confer early protection, addressing a critical gap in vaccination strategies. Further optimization of this vaccine with strategies such as adjuvant supplementation, could allow for quicker immune responses leading to earlier protection

EVALUATION OF POTENCY AND DURATION OF IMMUNITY ELICITED BY A MULTIVALENT FMD VACCINE FOR USE IN SOUTH AFRICA

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South Africa experiences sporadic foot and mouth disease (FMD) outbreaks irrespective of routine prophylactic vaccinations of cattle using imported commercial vaccines. The problem could be mitigated by preparation of vaccines from local virus strains which have recently caused outbreaks in its FMD control zone. Five strains were identified and their efficacy as vaccine candidates studied. We therefore demonstrate their respective protective dose, safety, and onset of humoral immunity in naïve cattle. Furthermore, we demonstrate the duration of protective immunity in naïve cattle over 12 months period, when a multivalent vaccine prepared from the five strains is administered as a single dose with or without booster vaccinations. As monovalent vaccines, the five strains were shown to contain a 50 % protective dose between four and 32, elicit humoral immunity with antibody titers ≥ 2.0 log10, from day seven post vaccination and cause no adverse reactions. Meanwhile, vaccination with a multivalent vaccine elicited high antibody titers (≥ 2.0 log10) and clinical protection up to 12 months when one or two booster vaccinations were administered within 6 months of the primary vaccination. Thus, an insignificant difference between the application of one or two booster vaccination was revealed. Owing to the high

potency, we anticipate that the multivalent vaccine could be used successfully for prophylactic and emergency vaccinations without adjustment of the antigen payloads. Furthermore, a prophylactic vaccination regimen comprising primary vaccination of naïve cattle followed by two booster vaccinations 1.5 and 6 months later, could potentially maintain herd immunity over a period of 12 months.

Influence of Physiological and Environmental Factors on FMD Vaccine Effectiveness

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Foot-and-mouth disease (FMD) is a highly contagious and economically significant disease affecting cloven-hoofed animals. Its rapid transmission and substantial impact underscore the need for effective vaccination strategies to control the disease and mitigate its impact. Evaluating FMD vaccines through vaccine matching, efficacy and potency studies in controlled conditions, is critical for informed vaccine selection. However, assessing vaccine effectiveness in field conditions is equally important to evaluate the performance of the whole vaccination program.

A field trial was conducted on 20 pregnant dairy heifers imported from an FMD-free country without vaccination into an endemic country. The animals travelled by air over a distance of ~5,000 km. The heifers were split into two groups: early gestation (± 4 months) and late gestation (± 7 months). A program of three injections of concomitant multivalent (O, A, Asia1, SAT2) and monovalent (SAT1) AFTOVAXPUR vaccines, administered three weeks apart, was assessed during quarantine, starting the day after the animal's arrival.

Homologous and heterologous Virus-Neutralizing Titers (VNT) were measured up to three weeks after the 3rd shot.

Stress factors related to gestation, transportation, and environmental changes induced transient immunosuppression, as evidenced by delayed immunogenic responses in some heifers. Additionally, gestation stage influenced the immunological response, with heifers in early gestation exhibiting greater vaccination uptake compared to those in late gestation.

It is essential to implement measures to facilitate the acclimation of newly imported pregnant heifers, in order to reduce multi-factorial stress. Combined with reinforced biosafety protocols and strict surveillance, alternative acclimation and vaccination protocols could be further investigated to minimize the risk period for the most vulnerable animals and ensure the effectiveness of the primary vaccination for the full herd.

The Challenges of FMD Vaccine Development. How a manufacturer evaluates 'promising vaccine candidates'

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Foot and Mouth Disease (FMD) remains a significant threat to global livestock health and trade, necessitating effective control measures. For over seven decades, inactivated, adjuvanted vaccines have been the cornerstone of FMD prevention, produced at industrial scale and widely utilized. Despite the emergence of innovative vaccine candidates leveraging diverse platform technologies, none have successfully transitioned to industrial-scale production. Promising research often encounters barriers during development or is deemed unfeasible by manufacturers due to various critical factors that make the product non-viable from a technical or an economic point of view.

This presentation explores the key criteria employed by vaccine manufacturers when evaluating new vaccine candidates for FMD. These criteria encompass legal and regulatory requirements, antigen yield, process scalability, and versatility of the technology. A viable vaccine platform must demonstrate robust performance across all FMD strains while meeting or exceeding the efficacy and safety benchmarks established by current inactivated vaccines. Additionally, the platform must align with industrial production capabilities and regulatory frameworks to ensure feasibility. While these challenges are substantial, the pursuit of next-generation FMD vaccines remains essential to address unmet needs and improve global disease control. Understanding of the decision-making process for vaccine candidate selection should help researchers increase the translational potential of novel vaccine technologies, fostering collaboration between academia and industry to drive innovation in FMD vaccine development.

Virology

Evolution of FMDV quasispecies during acute infection through in vivo serial passages in adult mice

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Virulence and replication in RNA viruses are largely shaped by polymerase fidelity and quasispecies diversity. In this study, we examined the outcome of serial in vivo passages on two

FMDV A/Arg/01 variants showing differential degrees of pathogenicity in adult mice: while A01L leads to 100% mortality within 24-48 hours, A01NL causes only mild clinical signs. Viruses were passaged three times in C57BL/6 mice (n = 6/passage), maintaining a constant inoculation dose (1×10^5 $TCID_{50}$/mouse, intraperitoneal route). Clinical signs were monitored at 22 h postinfection (hpi) prior to euthanasia. A significant increase in serum viral titers was detected at 22 hpi for the A01NL variant during passages 2 (6.94 log $TCID_{50}$/ml) and 3 (7.17 log $TCID_{50}$/ml) compared to passage 1 (6.08 log $TCID_{50}$/ml; Tukey, $p < 0.005$). This was accompanied by an increase in the severity of clinical signs. Nevertheless, evolved variant A01NL did not achieve lethality. In turn, the A01L variant proved similar serum titers in consecutive passages (6.84 log $TCID_{50}$/ml, 7.06 log $TCID_{50}$/ml and 7.19 log $TCID_{50}$/ml for passages 1-3, respectively) along with increased progression of clinical severity. The impact of serial passages on the viral genome was assessed through high-throughput sequencing (Illumina). The number of polymorphic sites (SNPs) increased during serial passages for A01NL virus. Conversely, A01L exhibited a progressive decline in the total number of detected SNPs, with significantly fewer sites in passages 2 and 3 compared to passage 1 (Tukey, $p < 0.005$). Of note, the relative frequency of specific genomic positions previously associated with A01L lethality increased during evolution of both A01L and A01NL variants. This is consistent with an evolutionary scenario marked by positive selection of viral haplotypes containing SNPs associated with high replicative fitness, while the final clinical outcome would be modulated by interactions between different variants within the FMDV quasispecies.

Foot-and-mouth disease virus diversity and recombination on dairy farms in Pakistan

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Field studies on foot-and-mouth disease virus have traditionally concentrated on viral isolates obtained from clinical cases. However, FMDV often causes several forms of subclinical infections in ruminants, which contribute to population-level virus maintenance and transmission in endemic areas. The dynamics of these subclinical infections are further complicated by heterologous FMDV coinfections and reinfections. The focus of this presentation concerns the genomic analysis of FMDV isolates obtained from domestic water buffalo (Bubalus bubalis) in Pakistan with no visible signs of disease – subclinical infections. Over a 12-month period,

oropharyngeal fluid was collected at a 3 month interval from buffalo on dairy farms in Islamabad, Pakistan. We used full-genome next-generation sequencing to analyze 68 total FMDV-positive oropharyngeal fluid samples, representing 44 animals across 18 farms. The analysis revealed the circulation of three serotypes – O, A, and Asia-1. Examination of persistent viruses revealed variable within-host evolution, with 0-25 substitutions observed between sampling points. Notably, several animals were infected by recombinant viruses derived from antigenically distinct parental strains. This included at least five different recombinants recovered from one animal, as confirmed through plaque purification of OPF samples. Recent works have elucidated aspects of FMDV recombination in cattle during controlled laboratory studies. Here, we extend these findings to the natural setting, demonstrating that the occurrence and composition of recombinant viruses follow patterns related to transmission and immunology of the host.

Interferon Stimulated Gene MCL-1 Inhibits FMDV Replication by Modulating Mitochondrial Dynamics and Autophagy.

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Foot-and-Mouth disease (FMD) is a viral disease of high economic consequence, as it spreads rapidly in cloven-hoofed animals. Effective countermeasures, including vaccines and biotherapeutics, are urgently needed during an outbreak. Interferon (IFN) based therapies have been previously shown to be successful at counteracting replication of Foot-and-Mouth disease virus (FMDV) in vitro and in vivo. We utilized a high-throughput ISG (IFN stimulated gene) screen to identify the ISG MCL-1 as an antiviral against FMDV replicon. Overexpression of MCL-1 in porcine cells reduced viral titers by approximately 4 logs, confirming its antiviral efficacy. We then explored the underlying mechanisms of MCL-1 antiviral activity, and we found that MCL-1 antiviral effect is not mediated by apoptosis regulation or cell cycle modulation. Instead, MCL-1 overexpression enhanced mitochondrial respiration, ATP production, and coupling efficiency. Infection of porcine cells with FMDV reduces these processes. In addition, MCL-1 overexpression

resulted in elongation of mitochondrial morphology, while FMDV infection caused fragmentation and punctate morphology of the mitochondria. Importantly, these changes in mitochondrial dynamics were independent of MCL-1's regulation of mitochondrial calcium flux. MCL-1 overexpression also suppresses autophagy, a process that is essential for FMDV replication. Our data indicates that MCL-1 is a potent antiviral ISG against FMDV and highlights the critical roles of mitochondrial dynamics and autophagy in FMDV replication. A deeper understanding of these processes may allow for rational design of biotherapeutics and vaccines against FMDV.

New Nomeclature Proposal for FMDV Using a Non-Metric Multidimensional Scaling Based Sequence Space.

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FMD virus nomenclature systems are currently based on combinations of geographic regions and phylogeny. Alternative nomenclature lineage-based systems (for example Pangolin) used for Covid-19, dengue fever, influenza and rabies viruses can be susceptible to discovery order and sampling bias, which can affect their reproducibility. Here, we propose a potentially new nomenclature system based on projection of individual FMDV VP1 genetic sequences into an n-dimensional serotype specific space. Using a Non-Metric Multidimensional Scaling (NMDS) based algorithm, we can quantify and visualise these genetic differences between different sequences within this space. Furthermore, we can fix a set of reference sequences in this space, allowing for the addition of newly discovered sequences. We used a 3D dimensional space due to its ease of interpretation. We can assign each sequence a 3D coordinate and map them to any one of a grid like system of cubes each with its own 'post-code'. Novel sequences with no previous nomenclature classification can be assigned to an existing cube postcode and FMDV topotype depending on their positioning in this space and proximity to currently defined reference sequences. We have developed a user-friendly interface using the Shiny R package to implement this proposed system and allows for the easy addition of newly discovered sequences. This capacity to map sequences into a 3D space based on their genetic relationships provides a robust indication of genetic similarities between strains. We used the Vegan R package functions monoMDS() to construct the 3D space from a dissimilarity matrix of pairwise genetic distance of our aligned reference sequences and MDSaddpoints() to add new sequences to this 3D space. The distortion incurred by this dimensionality reduction can be measured by a stress function value that measures the conservation of the true genetic pairwise distance as dimensions of data is reduced. This value is tracked after the addition of each new sequence to ensure adequate performance of the system. We demonstrate the application of this approach to selected FMDV serotypes. This novel nomenclature method enables a greater understanding of sequence

similarity alongside a faster classification of new sequences and may provide a means to study the correlations between genotype and antigenic phenotype.

Effect of Ruxolitinib on persistent Foot-and–Mouth Disease Virus (FMDV) infection in multilayered cells derived from bovine dorsal soft palate

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The FMD carrier state presents significant challenges for control and eradication. Despite extensive research, the mechanisms underlying FMDV persistence remain largely unknown. Studies suggest that host immune responses play a critical role in maintaining infection.
We have previously established an in vitro model of FMDV persistence in primary bovine dorsal soft palate (DSP) cells cultured as multilayers at the air-liquid interface (ALI). In this model, the analyses of the transcriptional host response during acute and persistent infection highlight a long-lasting stimulation of interferon-stimulated antiviral genes (ISG) that are ultimately ineffective to clear the virus. We hypothesized that the inhibition of the interferon response might modulate the level of replication of persistent FMDV and tested the effect of Ruxolitinib, a Janus kinase inhibitor.
Bovine DSP cells grown as ALI multilayers were inoculated with O/FRA/1/2001/Clone2.2. then monitored until 35 days post-inoculation (dpi). Ruxolitinib was added from 7 dpi, 14 dpi or 21 dpi onwards. Controls were FMDV-infected cells cultured in medium with DMSO (Ruxolitinib diluent) and non-infected cells cultured in medium with Ruxolitinib or DMSO. Samples collected throughout the experiment were analysed by viral isolation, titration, rtRT-PCR, immunohistochemistry and transcriptomic analyses.

Ruxolitinib caused an increase of viral replication and lysis of the persistently infected DSP cells, that became obvious at 7 days after starting treatment and was more marked when the treatment started from 14 or 21 dpi. Furthermore, transcriptomics analyses showed that Ruxolitinib regulates genes related to innate immune system (as expected) but also to extra-cellular matrix.

By showing a modulation of FMDV persistence, these data demonstrate that the inhibition of the

JAK signaling of the type I IFN pathway leads to a disruption of the virus-host cell balance. These new findings give some clues towards a better understanding of FMD persistence mechanisms and can open for further evaluation in vivo.

Poster Presentations – Session 1

Diagnostics

Development of BSL-2 Compatible Neutralization Assays Platforms for FMDV Asia 1 serotype

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Foot-and-Mouth Disease Virus (FMDV) is a highly contagious pathogen affecting cloven-hoofed animals and remains as a major threat to global livestock production. Effective vaccine antibody monitoring relies on accurate neutralization assays, but conventional virus neutralization tests (VNTs) require live virus and BSL-3 containment facility, limiting their scalability and accessibility. To overcome these limitations, we developed two BSL-2-compatible serological platforms based on split-luciferase (NanoBiT) technology for safe and quantitative detection of FMDV-neutralizing antibodies.

We focused on the Asia1 serotype of FMDV as a monitoring virus. The NanoBiT system, composed of a HiBiT tag (1.3 kDa) and a larger LgBiT subunit (18 kDa), enables high-intensity luminescence with minimal background. We engineered Asia1 FMDV to express a HiBiT tag on the VP1 capsid protein and chemically inactivated this virus. In parallel, we generated virus-like particles (VLPs) containing the same HiBiT-tagged VP1 via a baculovirus expression system. Both were applied to LgBiT-expressing LF-BK cells, where luminescence occurred upon virus or VLP entry. Neutralization activity was determined by measuring signal suppression after application with serially diluted sera.

The inactivated-virus based assay produced specific entry-dependent luminescence, but a fixed background limited its quantitative resolution for neutralizing antibody titers. Nevertheless, it was effective for studying virus binding and entry inhibition. The VLP-based assay displayed a 3-log linear detection range and correlated strongly with standard VNT results (Pearson $r \geq 0.95$, $n = 48$). Both assays were completed within 3 hours under BSL-2 conditions and supported high-throughput formats.

These BSL-2-compatible serological platforms for FMDV Asia1 enable safe and scalable alternatives to conventional VNT. The inactivated-virus system is suitable for mechanistic virology

studies, while the VLP-based assay offers a practical tool for vaccine efficacy evaluation and serological surveillance.

A novel approach to titrating serum antibodies against foot and mouth disease virus: Solid phase competitive ELISA using purified mammalian cell line integrin receptors

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Introduction

Conventional SPCE for FMDV antibody detection relies on polyclonal/monoclonal antibodies or recombinant αVβ6. To avoid ethical and logistical challenges, we replaced rabbit anti-FMDV serum with native integrins from LFBK cells as capture ligands.

Methodology and Results

Integrin subunit (αV, α5, β1, β3, β5, β6, β8) expression in LFBK, MDBK, and BHK-21 cells was assessed via qRT-PCR, while FMDV (O/IND/R2/1975) binding was evaluated via ELISA. LFBK cells exhibited the highest integrin expression (β8 > αV > β5 > β6) and strongest ELISA reactivity. α5, β1, and β3 levels were within tenfold of BHK-21. LFBK-derived αV integrins were solubilised with 1 mM Triton-X.

Integrin was affinity-captured using full-length fibronectin, a GRGDSP peptide, and fibronectin type III 9-10 (FNIII) domain expressed in E.coli bacteria, coupled to cyanogen bromide-activated Sepharose 4B. SDS-PAGE and western blotting confirmed αV integrin presence in all eluates. When the 250 mM salt elute was used to capture FMDV antigen in the cell culture supernatant, a reduction in the absorbance was recorded relative to polyclonal sera. However, the absorbance was high in integrin-coated wells compared to uncoated wells, suggesting specific binding. Known positive and negative sera were used to evaluate the integrin capture-SPACE (50 ng/well of integrin fraction in 250 mM eluate), where the absorbance of the antigen control ranged from 0.2-0.3. Nevertheless, positive sera showed dilution-dependent reduction in absorbance and statistical analyses using the Wilcoxon signed test showed that the titer was comparable with that of conventional SPCE ($p=0.324$).

Discussion and Conclusion

Despite the reduced absorbance of native integrin as a capture reagent, the antibody titers could be calculated. The substitution of native integrins didn't have any significant effect on antibody titer in SPCE; hence, the native integrin has the potential as a coating ligand in SPCE.

Validation of a Portable Direct rRT-PCR CMOS Biosensor for Rapid On-Site Detection and Serotyping of Foot-and-Mouth Disease Virus in Clinical Samples

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Rapid and accurate detection and serotyping of foot-and-mouth disease virus (FMDV) are critical for early outbreak control and effective disease surveillance. Although conventional real-time reverse transcription (rRT-PCR) assays are highly sensitive, they require RNA extraction, bulky instrumentation, and centralized laboratory infrastructure—factors that limit their applicability in field settings.

We evaluated a novel, portable biosensor platform that integrates complementary metal-oxide-semiconductor (CMOS) technology with direct rRT-PCR. The system features a nine-well cartridge pre-spotted with primers and probes targeting FMDV serotypes O, A, and Asia1, as well as markers for differentiating vesicular diseases (Swine Vesicular Disease Virus, Seneca Valley Virus). Direct amplification from unprocessed clinical specimens, including saliva, serum, and feces, is enabled by a proprietary buffer, eliminating the need for RNA extraction and simplifying the diagnostic workflow.

Analytical validation demonstrated a detection limit of 10^0–10^2 $TCID_{50}$/mL. During the 2023 FMD outbreak in the Republic of Korea, 101 clinical samples, including saliva, serum, and tissue, were tested using this device and benchmarked against laboratory-based rRT-PCR and VP1 sequencing. We observed that the biosensor had 100% diagnostic sensitivity and specificity, confirming its reliability for field deployment. Additionally, the device can send test results in real-time through a cloud system, allowing others to check and monitor the data remotely.

In this study, we validate that the CMOS-based direct rRT-PCR biosensor could be a robust, field-deployable molecular diagnostic solution for FMDV. Its compact design, multiplexing capability, and cloud-based connectivity support its application in rapid outbreak response, decentralized testing, and high-throughput surveillance, particularly in resource-limited environments. In future studies, we plan to expand the platform to detect FMDV lineages along with serotypes.

Creation of a bank of clinical samples collected from FMDV outbreaks in Anatolia, Türkiye, to evaluate candidate host genes as markers of FMDV persistence in cattle

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The FMD carrier state presents significant challenges for control and eradication. Despite extensive research, the mechanisms underlying FMDV persistence remain largely unknown but it has been suggested that host immune responses may play a critical role in maintaining infection In a previous study, using primary bovine dorsal soft palate cells cultured as multilayers at the air-liquid interface, some highly regulated genes were identified as potential host markers of FMDV persistence. This study aimed to follow alterations in host responses during natural persistent FMDV infection in cattle and evaluate highly regulated genes as potential host markers of FMDV persistence.
In collaboration with field vets in Anatolia (Türkiye), farms were visited 10 days after first clinical signs (~14 days post-infection (dpi)) and probangs, saliva, nasal fluid and serum were collected from sick animals. 20 days later (~35 dpi), the same animals were sampled. In total 100 samples of each type were collected in 8 farms from 50 bovines. Overall 28 probang samples collected from 50 animals were positive, representing 56% of animals persistently FMDV-infected.
In parallel, a multiplex rtRT-PCR was developed at Anses for two candidate persistence markers (LY6E and FBP1) and an epithelial marker as quality control (OCLN). The PCR was further shared with SAP and FLI teams for evaluation of its diagnostic and predictive value of FMDV persistence in cattle. The FLI team tested it on probang samples from experimental infections, but found no differences between carriers and non-carriers. OCLN RNA was unfortunately not detected in any sample. Accordingly, it would be necessary to target a different epithelial marker gene and to test other candidate host markers. Nevertheless, the biobank thus constituted is of great value for further studies.

DEVELOPMENT OF A NEW ISOTHERMAL ASSAY FOR THE ON-FIELD DETECTION OF FOOT-AND-MOUTH DISEASE VIRUS

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Introduction
In the last years, many innovative methods have been used to developed new, fast and reliable Point-of-Care (POC) tests for pathogens detection, based on antigen-antibody reactions or detection of nucleic acids. Molecular methods are usually more sensitive, but they are often difficult to adapt to field situations because they require complex equipment. Among the isothermal reactions, RPA (Recombinase Polymerase Amplification) enables fast (5-20 min) amplification of DNA at low and fixed temperature (37-42°C), advantageous conditions for the design of POC tests. Here we aimed to develop an RT-RPA assay for FMDV detection and to

adapt it to on-field conditions.

Materials and Methods
For reverse transcription of viral RNA, two enzymes were tested: (i) RevertAid-RT (Thermo Fisher Scientific Inc., US) and (ii) MMLV-RT (Takara Bio, Japan). The incubation at 42°C for 20 min was followed by inactivation at 95°C for 5 min. Specific primers 32 nt long, targeting a conserved region of FMDV genome were designed for RPA. The amplification was carried out using TwistAMP® (TwistDxTM Ltd., UK) and first detected with electrophoresis on agarose gel. Using a forward primer conjugated with FAM and CTP conjugated with Biotin enabled the use of HybriDetect LFA Kit (Milenia Biotech GmbH, Germany) to detect the results. The assay was developed using in vitro cultured FMDV strains and then its effectiveness was confirmed using field samples.

Results and Discussion
Method development involved the inclusion of a reverse transcription step and the evaluation of different enzymes, titration of reagents and reaction volumes, evaluation of a different detection system using conjugated primers to replace agarose gel electrophoresis (not on-field friendly). This RT-RPA showed a good analytical specificity, regardless the RT enzyme. In detail, it (i) correctly detected 6/7 FMDV serotypes (serotype C excluded), (ii) proved its efficacy detecting FMDV in field samples and (iii) did not react against other viruses (SVDV, EMDV, SVV, LSDV). Conversely, the analytical sensitivity changed depending on the employed RT enzyme. With RevertAid the detection limit was around 103 TCID50/ml (RNA extracted by cultured virus) and 106 gene copy numbers (synthetic RNA), while adding MMLV-RT the limits decreased to 102 TCID50/ml and 105 gene copy numbers.
The use of LFA for the detection is crucial to adapt the assay to on-field requirements, but it still remained the most ineffectual step, needing of improvements for unclear negative results. Nevertheless, these data suggested that RT-RPA, supported by on-field systems for the RNA extraction and LFA for the detection, is a promising candidate to be carried out as FMDV on-field detection assay.

Monoclonal antibodies against FMDV type SAT 3: preliminary characterization and potential use in diagnostic assays

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In the spectrum of diagnostic tools for Foot-and-Mouth Disease (FMD) the development of ELISAs immunoassays for SAT3 diagnosis has been relatively neglected. Although this serotype is confined to Southern Africa, the risk of escape due to market globalization makes filling this gap a priority. The availability of monoclonal antibodies (MAbs) specific for the serotype SAT3 is strategic for the development of diagnostic ELISAs, both for antibodies detection and for antigen detection, enabling to complete the portfolio of the FMDV diagnosis based on ready to use ELISAs.
For this purpose, a panel of 50 MAbs was obtained from four fusion procedures using splenocytes of four Balb/C mice immunised subcutaneously with 50 ug/each of purified VLPs (strain SAT3/ZIM/3/81) in complete Freund's adjuvant and boosted intraperitoneally, once or twice at 1-month intervals, with the same antigen dose in PBS. Three days after the last boost mice were sacrificed and MAbs were generated according to internal standard procedures. Hybridomas were screened by indirect-trapping ELISA against SAT3 ZIM/3/81 inactivated virus and heterotypic cross-reactivity was subsequently analysed against reference strains of O, A, Asia 1, SAT1 and SAT 2 serotypes, immune-trapped onto ELISA plates by the specific hyperimmune rabbit serum.
Nine SAT3-specific hybridomas were initially selected, cloned and grown at high cellular density using Integra CELLine flasks to obtain high MAbs yield to be used, after immune-purification, as catching and peroxidase-conjugated detector antibody in ELISA assays.
Performances of a sandwich ELISA carried out with MAbs combinations, used as catching (9 MAbs) and conjugated antibody (7 MAbs), were initially evaluated to titrate supernatants of infected cells: dose-response curves of the homologous virus SAT3 ZIM/3/81 and eight further SAT3 isolates collected from 1965 to 2018 were generated.
Preliminary results showed that none combination recognises all the SAT3 strains analysed, however other Mabs are under evaluation.

Operation of a Foot-and-Mouth Disease Serum Bank to Standardize Serological Diagnosis in South Korea

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The Animal and Plant Quarantine Agency (APQA) in South Korea serves as national reference laboratory for Foot-and-Mouth Disease (FMD) and supports FMD research and development. In particular, a FMD serum bank has been operated since 2017 to collect various types of sera to support the standardization and development of reliable serological methods. This study introduces the operation and management procedures of the FMD serum bank and provides information on serum samples collected from 2017 to June 2025 in APQA.
The samples are evaluated using ELISA (NSP, SP), VNT, and EITB. Depending on the priority, these serum samples are either stored at –70°C or freeze-drying. Serological data are incorporated into the serum bank database, including animal species, challenged or infected virus type, vaccination

history, and test results.
A total of 4,064 serum samples is possessed in the FMD serum bank. The majority were field sera (46.9%, n = 1,906) collected from NSP antibody-positive farms and FMD outbreak farms. This is followed by sera from vaccinated animals (24.6%, n = 989), virus-infected animals (10.3%, n = 417), animals that were vaccinated and subsequently challenged (8.4%, n = 341), FMD-free countries (6.7%, n = 274), NSP/SP negative samples (2.7%, n = 111), and national reference sera (0.6%, n = 26).
These collected samples are used for specific purposes, including proficiency testing of regional and private veterinary laboratories, development of diagnostic kits, and validation of commercially available antibody test kits. We are committed to securing reference serum panels that meet recognized international performance standards. Furthermore, we aim to secure various types of sera that have never occurred in South Korea, such as SAT1-3 by collaboration with global institutions.

Epidemiology

Uncovering Surveillance and Vaccine Matching Gaps in Small Ruminants and Domestic Pigs: A 24-Year Review of FMDV in East Africa

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This study investigates the molecular epidemiology of foot-and-mouth disease virus (FMDV) in East Africa over a 24-year period (2000–2024), focusing on the spatial and temporal distribution of serotypes and topotypes in Uganda, Kenya, and Tanzania. Using a systematic review of peer-reviewed publications and virus submissions to the World Reference Laboratory for FMD (WRL-FMD), the study also evaluates the antigenic match between circulating field strains and vaccine strains. The findings highlight a predominant reliance on cattle-derived samples—over 90%—despite the known susceptibility of all cloven-hoofed animals, including small ruminants and domestic pigs, to FMDV.
Serotype O was the most commonly detected across the region, with serotypes A, SAT 1, and SAT 2 increasingly reported in the last decade. Kenya submitted the highest number of samples and underwent the most vaccine-matching evaluations, while Uganda had the lowest submission and testing rates. Key possible challenges in virus recovery include inadequate cold chain management, poor sample types, and untimely sample collection. Antigenic matching results indicated good coverage by O-serotype vaccines in Kenya and Uganda, and better performance of SAT 2 vaccines in Tanzania. However, a major gap remains in understanding the antigenic diversity and vaccine coverage of FMDV strains in small ruminants and pigs, as these species are largely underrepresented in both surveillance data and vaccine evaluation studies.

These findings emphasize the virological complexity of FMDV in endemic settings and reveal critical surveillance and vaccine-matching gaps in small ruminants and pigs—species that are often asymptomatic but may significantly contribute to virus transmission. Addressing these gaps requires targeted sampling, expanded serotype monitoring, and inclusive vaccine matching protocols that account for all susceptible livestock species. Enhanced coordination between researchers, diagnosticians, and policy-makers is essential to improve FMD control strategies across the East African region.

Mathematical Modelling of Foot and Mouth Disease Transmission in Beef herds under Endemic Conditions

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Foot and mouth disease is a highly contagious disease that causes severe economic losses in endemic countries. Understanding the transmission dynamics of FMD virus (FMDV)is important for the implementation of disease control strategies at the country and herd levels to minimize the economic losses associated with FMD outbreaks. Foot and mouth disease virus transmission between herds has been modelled in multiple studies, while few studies investigated the FMDV transmission within the infected herd in endemic countries. Beef herds represent a higher risk for FMDV outbreaks due to the high turnover rate. Medium-sized beef herds (50-500 animals) in Egypt represent more than 60% of the locally produced beef supply in the country. The current study aimed to simulate the FMD transmission in a medium-sized beef herd in Egypt 1) in a vaccinated herd, 2) under different vaccination and replacement introduction scenarios. Data on the FMD outbreak were collected from a medium-sized beef herd (350 animals) vaccinated during 2017. The FMDV transmission in vaccinated herds differs from that in naïve herds and may be affected by vaccine effectiveness and the number of vaccinated cattle. A mathematical model was specified in R software 4.3.3 using the deSolve, reshape2, and ggplot2 packages. Five replacement introduction scenarios were simulated. The best scenario results were displayed in simulated herds with 90% vaccination using a trivalent vaccine and 80% vaccine efficacy, which showed an increase in clinical FMD cases that peaked at week five (2.8%) from the first case, before the outbreak ended at week eight. Meanwhile, the FMD outbreak failed to occur in fully vaccinated herds with 80% vaccine efficacy, while fully vaccinated herds with 60% vaccine efficacy experienced an outbreak and peak of infection at the third week (12%) and the outbreak ended around day 50. Foot and mouth disease outbreak for beef herds receiving all their animals together (all-in-all-out) with unknown vaccination and vaccinating all animals at receiving,

assuming a 21-day lag of immunity, when an infected animal is introduced at receiving the number of clinical cases increased rapidly to peak on days 6 and 7 (36% infected cases), and the outbreak ended at day 20. Introducing replacement beef cattle sourced from live animal markets with unknown vaccination status is a very common practice in Egypt. The number of vaccinated animals and the vaccine efficacy greatly affected the occurrence, duration, and number of infected animals during the FMD outbreak.

Phylogenetic Analysis of O/ME-SA/Ind-2001e lineage Foot-and-Mouth Disease Virus in the 2025 Outbreak in the Republic of Korea

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Foot-and-mouth disease (FMD) is a highly contagious viral disease affecting cloven-hoofed animals, caused by seven distinct serotypes (O, A, C, Asia1, SAT1, SAT2, SAT3). In 2025, FMD re-emerged in the Republic of Korea, two years after the previous outbreak in Chungcheongbuk-do (CB) in 2023. The first case was reported in cattle on 13th March 2025, in Yeongam County, Jeollanam-do (JN), a southwestern province historically free of the disease. After that, serotype O FMDV was detected in a total of 19 farms (cattle and pigs) located in two counties of JN by 14th April 2025. The recent Korean outbreak in 2023 was caused by serotype O, ME-SA/Ind-2001e lineage. Therefore, we conducted a phylogenetic analysis of VP1 sequences to determine whether the 2025 Korean outbreak resulted from domestic circulation or a new transboundary introduction in this study.

Nucleotide sequencing was directly performed using clinical samples collected from the 19 affected farms and the VP1 sequences were successfully obtained in the samples of 15 farms. VP1 sequences were aligned using Clustal W, and a maximum likelihood phylogenetic tree was constructed using MEGA version X. All sequences were clustered within the O/ME-SA/Ind-2001e lineage. Notably, the JN sequences formed a strongly supported monophyletic group (bootstrap value = 100%) with O/MOG/1/2021 and other Mongolian sequences identified between 2021 and 2022. The nucleotide identity of JN sequences with the Mongolian sequences ranged from 97.79% to 98.10%, and it was slightly lower than that with the sequences from the 2023 CB outbreak (97.31%–97.95%). Despite high sequence identity (about 97%) to the 2023 CB viruses, the 2025 JN sequences formed a distinct phylogenetic cluster. Furthermore, the absence of intermediate viral sequences in national surveillance data collected between the 2023 CB and the 2025 JN outbreaks could support a recent external introduction rather than prolonged domestic circulation.

This study provides genetic evidence suggesting that the 2025 JN outbreak could be due to a recent transboundary introduction rather than domestic persistence from the previous 2023 CB

outbreak. Further epidemiological investigations are required to identify potential sources because the exact route and mechanism of viral introduction remain unclear.

ENHANCED ACTIVE SURVEILLANCE AND SEROTYPE PROFYLING FOR FOOT AND MOUTH DISEASE CONTROL IN 6 COUNTIES -KENYA.

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Abstract

Foot and Mouth Disease (FMD) remains a major threat to livestock production in Kenya, especially in the North Rift region, majorly in these counties: Uasin Gishu , Nandi, Elgeiyo-Marakwet, Trans-Nzoia, Turkana, and West Pokot. These counties are characterized by extensive livestock movement during trading, often across their borders, facilitating the rapid spread of FMD. Despite routine vaccination programs, outbreaks persist due to the lack of up-to-date data on circulating serotypes, as a result of poor antigenic match between field strains and vaccines. This proposal outlines an active surveillance program to be led by the National Veterinary Laboratory (NVL) in Eldoret to address these gaps through systematic field data collection and laboratory analysis. The project focus on identifying the prevalent FMD serotypes in the region to support targeted vaccine production, improve disease control strategies, and reduce economic losses caused by movement restrictions and trade bans during outbreaks. The major objectives include assembling epidemiological data, performing laboratory serotyping, enhancing vaccination campaigns, capacity building of staff, and ensuring continuous monitoring systems. Field activities will involve collaboration with County Directors of Veterinary Services (CDVs) for farmer sensitization, case identification, and sample collection (serum, pharyngeal fluids epithelium tissues and blood). Samples will be analyzed in the National FMD laboratory -Embakasi Nairobi, while the results will used to inform immediate responses and long-term planning.

By the end of the project, a clearer understanding of FMD serotype changes will be achieved, enabling the development of effective control and eradication measures, including customized vaccine formulations. This will promote immunity of the herd, lower disease incidence, support food security and improve economic stability in these counties. The project also integrates biosecurity reinforcement, inter-county coordination, and enhance farmer education to address challenges such as unpermitted livestock movement, communal grazing, and interactions with the wildlife. The expected outcome is a significant advancement in FMD control in the North Rift region of Kenya.

Prepared by

Peris Sambili

FMD CHAMPION NATIONAL VETERINARY LABORATORY ELDORET KENYA.

SEROPREVALENCE AND RISK-FACTORS FOR FOOT-AND-MOUTH DISEASE IN GOATS IN UGANDA FOLLOWING THE 2024 WIDESPREAD OUTBREAKS: A RISK-BASED SURVEY

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Background

Foot-and-Mouth Disease (FMD) is a highly contagious viral disease with major economic implications for Uganda's agricultural sector. Most affected small ruminants show no clinical signs but may silently spread the virus. Unlike cattle, the role of small ruminants in the epidemiology of FMD remains unclear. The objective of this study was to investigate the seroprevalence and risk factors associated with FMD seropositivity in goats (with no history of vaccination), in select districts of Uganda following reported outbreaks.

Methods

A cross-sectional study was designed and blood samples collected from sampling 832 goats from 80 farms across five districts (Kasese, Kiboga, Kiruhura, Nakasongola, and Rakai), following the 2024 FMD outbreak crisis that affected over 40/135 districts across the country. A questionnaire was used to collect data on animal and farm-level factors (FMD history, neighboring farms, herd characteristics, management practices). Serum samples were tested using FMDV-NSP ELISA Kits. Descriptive statistics were performed, and binary logistic regression was used to assess associations between putative factors and FMD seropositivity. Thematic analysis was performed for open-ended questions.

Results

Overall, seroprevalence at animal level was 19.8% (165/832), and at farm level was 48.8% (39/80) ranging from 0% to 100%. The highest seroprevalence was observed on farms in Kiruhura district. Farms which had reports of cattle infected with FMDV in the past six months had significantly higher seropositivity in goats on the same farm. The farm level risk factors included proximity to national game parks, FMDV infected neighboring while farms with a recent history of cattle vaccination had a protective effect against FMD infection in goats. Thematic analysis revealed major control challenges, including inadequate vaccine supply, mixing of animals during grazing, and uncontrolled animal movement.

Conclusion

The findings highlight the need to integrate small ruminants into FMD control programs. Strategies may include improving vaccine access and implementation of vaccination programs targeting high-risk areas such as around national parks and districts like Kiruhura, and controlled or regulated livestock movement.

Assessment of transmission risk from persistently infected animals with foot-and-mouth disease in Thailand

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Persistently infected (PI) animals with foot-and-mouth disease virus (FMDV) harbor infectious viruses in the pharyngeal region for extended periods. Therefore, PI animals may pose a threat in FMD-endemic countries, although there is limited evidence demonstrating that PI animals serve as an infectious source to other susceptible contact animals. To elucidate the actual risk caused by PI animals in Thailand, we initiated investigations on PI animals in 2024.

Four farms affected by recent FMD outbreaks were selected as study sites: one farm in Nakhon Pathom Province (30 cattle), two farms in Chiang Mai Province (20 cattle each), and one farm in Saraburi Province (20 cattle and 7 small ruminants), with outbreaks occurring in February 2024, February 2024, and January 2025, respectively. Oropharyngeal fluid and blood samples were collected at 2-4 month intervals: six times since June-July 2024 for farms in Nakhon Pathom and Chiang Mai provinces, and three times since February 2025 for Saraburi farm. Oropharyngeal fluid samples were tested for viral RNA detection and infectious virus isolation, while serum samples were tested for antibodies against viral non-structural proteins.

Viral RNA detection showed varying positive rates across farms and time points. Initial positive rates were high (70-100%) but generally declined over time. Two Chiang Mai farms showed no positive animals by April 2025, while the Nakhon Pathom farm maintained detectable levels (33% in May 2025). The Saraburi farm showed a dramatic decrease from 100% to 11% within two months. All samples tested for virus isolation have been negative, and antibody testing is in progress.

Viral RNA persistence without recoverable infectious virus suggests that PI animals may pose limited transmission risk under field conditions. Variation in clearance rates between farms indicates that factors such as management practices, animal genetics, or viral strains may influence PI dynamics and support refined FMD control strategies.

AN UPDATE ON THE EPIDEMIOLOGY OF CIRCULATING FOOT-AND-MOUTH DISEASE VIRUS IN CAMEROON, CENTRAL AFRICAN ENDEMIC REGION

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Foot-and-mouth disease (FMD) is an infectious viral disease of even-toed animals. Cameroon is a major livestock production basin of the central African region, and as a transit zone between West and Central Africa, it is greatly exposed to FMD and its economic consequences. This analytical review paper aims to update on the ecology of circulating FMDV in the endemic central African region from 1990 to 2025 to contribute to its control. An online search was conducted, and relevant published papers (n=26) on FMDV circulation in host species, soil/air, and other ecological drivers were analyzed. From the nationwide FMD cases confirmed between 1990 and 2025, serotypes A, O, SAT 1, and SAT2 were reported in most studies, but one study indicated antibodies reactive to SAT 3 in cattle of the far North region. Environmental studies highlighted the following potential environmental contamination sources: muscids, soils, swabs from shoes, vehicles, and manure around herds with average temperature and relative humidity of >24°C and >75% respectively. Moreover, a complex marketing system, slaughter, marketing, and drinking spots speculatively represented high exposure areas. The complex animal movements across porous borders without strict veterinary service inspection posts explain the ease of introduction and circulation of new strains (example in 2015, SAT 1 topotype X and SAT 2 topotype VII were circulating among Nigeria-Cameroon and Cameroon-Chad cattle, respectively). Furthermore, the southward movement of animals into game reserves, the Guinean savannah, the northwestern grasslands, and the forest ecological zones has been speculated to represent a possible mechanism for FMDV spread, as livestock normally share drinking spots and come into direct contact with these wild beasts. These ecozones generally have high buffalo, antelope, and warthog populations. There is a need for a regional and robust epidemiological study to characterize the circulating FMDV strains in wildlife and livestock areas.

Immunology

In vitro assessment of the cellular and humoral immune response induced by a FMDV SAT2 ERITREA 98 vaccine in cattle against homologous and heterologous FMDV strains

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In 2023, two exotic foot-and-mouth disease virus (FMDV) strains, i.e. SAT2 topotypes V and XIV, were introduced into North Africa and West Asia, respectively. We investigated whether a SAT2

topotype VII ERITREA 98 vaccine available in these regions induces cross-reactive cellular and humoral immune responses against these emerging topotypes.

Ten cattle were vaccinated with a double-oil emulsion FMDV SAT2 ERITREA 98 vaccine (≥3 PD50, kindly provided by Boehringer Ingelheim). As per manufacturers' instructions, a second vaccination was administered 28 days post-vaccination (dpv) to four animals. Blood samples were collected at different time points. Cellular and humoral immune responses were assessed with an Interferon-Gamma Release Assay (IGRA) and a Virus Neutralization Test (VNT). The FMDV strains used for restimulation in the IGRA – both live and heat-inactivated strains – and the VNT were: SAT2/ERI/12/98 (VII, homologous), SAT2/V/ALG/23Z015382/2023 (V), SAT2/XIV/OMN/23Z001434/2023 (XIV) and SAT2/ZIM/11/91 (II). A VNT antibody titer ≥1/45 (corresponding to ≥1.65 log10) was considered positive (WOAH Manual).

All animals tested positive in the IGRA from 7 dpv onwards. High levels of IFN-gamma were observed upon restimulation with homologous and heterologous virus strains from 10 dpv until euthanasia at either 23 (6 animals; vaccinated once) or 51 dpv (4 animals; vaccinated twice). From 10 dpv onwards, all animals tested positive in the homologous VNT for SAT2/ERI/12/98. After the first vaccination, mean VNT titers of 2.32, 1.64, 1.32 and 1.61 log10, were obtained for SAT2/ERI/12/98, SAT2/V/ALG/23Z015382/2023, SAT2/XIV/OMN/23Z001434/2023 and SAT2/ZIM/11/91 at 21 dpv, respectively. Twenty-one days after the second vaccination, the mean VNT titers were 3.10, 2.36, 2.09 and 2.47 log10 for these virus strains, respectively.

In conclusion, the SAT2 ERITREA 98 vaccine (≥3 PD50) induces a strong cross-reactive cellular immune response and high homologous antibody titers after one vaccination. The second vaccination 28 days later significantly increases the heterologous antibody titers to high levels.

Pathogenesis

Effect of therapeutic diet on the modulation of acute phase proteins and cytokines in crossbred calves infected with foot-and-mouth disease virus (FMDV)

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A therapeutic diet (TD) compatible with the oral lesions would increase voluntary feed intake and minimize the inimical effects of FMD infection during acute phase. Accordingly, we evaluated a TD in on soluble circulating immune response indicators. Crossbred bull calves (n=18) with body weight of 123 ± 1.3 kg were experimentally infected with 1.0 × 104 median dose of bovine tongue infectious dose of FMDV by intra-dermo sublingual route. The TD was a total mixed ration enriched with 19% CP and provided 2.90 Mcal ME/kg on dry matter basis. The calves were fed one of the three forms of the TD (n = 6/group) for 6 weeks post-FMDV infection (WPI): (i) TD in mash form (TDM) (ii) TD in cooked form (TDC) and (iii) TDC + customised nutrient supplement

(TDCNS) such as Zn, Cu, Cr, Mn, and Se. Four calves, which served as uninfected control, were fed TDM. Blood samples collected on day 0, 3, 5, 12, 19, 26, 33 and 42 post-infection (dpi) were assayed for serum concentrations of haptoglobin, serum Amyloid-A, TNF-α, IL 1β, IL-6, IL4 and IL-10 using ELISA kits. The results indicated that the serum concentrations of acute phase proteins and pro-inflammatory cytokines elevated significantly in the infected TD fed groups by dpi 3-5 as compared to uninfected TD fed group ($P<0.05$). The concentration of the inflammatory mediators among the groups were comparable ($P>0.05$) by dpi 12-19. It was concluded that TDC with or without CNS moderated the inflammatory response following FMD infection.

Socioeconomics of Disease Control

Intersection of Foot and Mouth Disease and Xenotransplantation: A bioethical exposition of contemporary issues.

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The world is facing a shortage of human organ donations of gargantuan proportions. In 2017, about 140,000 solid organ transplants were performed worldwide—but this covered only 10% of the global need. The acute need has birthed a market for illicit organ trade. The World Health Organization estimates 5%–10% of transplants happen through illicit organ trade, highlighting both the unmet need and dangerous underground markets. Organ scarcity means countless preventable deaths and life-threatening delays every year. Xenotransplantation offers a possible solution to alleviate the shortage of organ donations thereby undercutting the illicit organ trade. Xenotransplantation research is making rapid strides, especially with pig-to-human transplants entering clinical territory. The intersection of Foot-and-Mouth Disease (FMD) research and xenotransplantation raises a complex web of ethical concerns involving animal welfare, biosecurity, public health, and global equity. It is important to be ahead of the curve and grapple with these issues as the science develops. This paper articulates contemporary bioethical issues at the intersection of xenotransplantation research and Foot and Mouth Disease research. It seeks to stimulate debate and discourse on the issue as well as inform policy formulation. The ethical issues articulated include: biosafety and pathogen spillover concerns, balancing advancing life-saving transplant technologies while minimizing global risk of introducing or reintroducing a catastrophic livestock disease, the ethical appropriateness of raising and genetically manipulating large populations of pigs—possibly under strict confinement and unnatural conditions—for human health goals given that both FMD research and xenotransplantation rely on intensive use of genetically modified animals, particularly pigs; transparency and public trust; dual use research and biocontainment; global health equity and whether nations suffering from FMD outbreaks also bear the burden of producing pigs for xenotransplant research.

Vaccines

Exploring Foot and Mouth Disease Virus Proteins to Design Subunit Vaccine Using Immunoinformatics Approach

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Foot and Mouth Disease (FMD) is an economically important transboundary disease of cloven-hoofed animals like cattle, buffalo, sheep, goats, and pigs, etc caused by Foot and Mouth Disease Virus (FMDV). Millions of animals are affected by this primary animal disease which continues to be the main sanitary barrier to both domestic and foreign trade in livestock & livestock products. The most successful FMDV prevention strategy currently available is inactivated vaccination. The inactivated vaccines for foot and mouth disease are aluminum-based, oil-emulsion-based, and have monovalent, bivalent, and multivalent structures. To combat this deadly disease, chemically inactivated FMDV vaccinations are frequently employed in endemic areas. Moreover, because of antigenic differences, traditional vaccinations are unable to protect against heterologous strains. Coupled with this, traditional vaccines have other drawbacks such as the necessity for refrigeration for storage, the requirement for routine booster vaccinations, and the challenge of telling diseased from immunized animals.

A novel method for creating multi-epitope DNA vaccines uses epitopes in the building process. Vaccines based on epitopes are created for the initiation of specific antibody production versus structural epitopic antigen. This approach might provide a way to produce a vaccine for the pathogen from which commercialized vaccinations were unable to immunize the livestock. In this study, we have created a development policy for the "Common FMD Vaccine". These many FMD viral antigen determinants could aid in the animals' defense against heterologous FMDV strains. Novel vaccines have been researched for more than thirty years in the hopes that new biotechnology will produce effective novel vaccines. Throughout this research, we developed a subunit vaccine against FMDV using an immunoinformatics strategy. Before they can be authorized and introduced to the market, they must, however, complete the research and development stage and additional tests.

Keywords: FMDV; immunoinformatics; inactivated vaccine; non-structural protein; subunit vaccine; FMDV proteins

Foot and Mouth Disease virus-like particle produced in E.coli expression system is a potential antigens for novel vaccine

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Foot-and-mouth disease virus (FMDV) continues to pose a significant threat to livestock health and the global agricultural economy, particularly in endemic regions of Asia, Africa, and the Middle East. Current vaccines based on chemically inactivated FMDV present several challenges, including biosafety risks, high production costs, and limited effectiveness against emerging viral variants. To overcome these limitations, we developed virus like particle (VLP) vaccines targeting FMDV serotypes O, A, and Asia1 using an Escherichia coli expression system. The resulting VLPs self-assembled into ~30 nm particles with native virus like morphology and antigenic properties, as confirmed by transmission electron microscopy, SDS-PAGE, and Western blot analysis. Immunization studies in a murine model demonstrated that the VLP vaccines elicited strong serotype specific humoral immune responses and conferred effective protection against viral challenge. Notably, the vaccines induced robust immune responses even at lower antigen doses, suggesting the feasibility of dose sparing formulations. These findings demonstrate that FMDV VLPs produced in E. coli are highly immunogenic and capable of eliciting protective immunity, highlighting their promise as safe, scalable, and cost effective alternatives to conventional inactivated FMD vaccines.

Evaluation of FMDV type O field strains of lineages SEA-Mya98, ME-SA Ind 2001 and ME-SA Panasia, during 2019-2024 in Vietnam and its vaccine matching assessment with O1 Campos monovalent vaccine strain.

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Foot-and-mouth disease (FMD) is a highly infectious disease with potential for rapid spread and severe economic impact. The use of vaccines that contain strains that match antigenically with the FMD strains circulating in the field is one of the main tools, together with a good epidemiology

plan for its eradication. Supplying information in relation to characterization of circulating strains, is useful to decide eventual changes in antigen banks in order to be prepared to attend eventual incursions. Here we present actualized information of viral circulating strains collected from type O outbreaks in Vietnam between 2019 and 2024.

Materials and Methods

Baby Hamster kidney cell line (BHK-21) was used for a Bi dimensional Virusneutralization test (VNT) against sera of 28 days post vaccination with a monovalent O1 Campos commercial vaccines (Bioaftogen). Heterologous titers above 1.5 log10 are considered crossreactive. For r1 determination assays the sera must be tested at least 3 times (WOAH, 2022 recommendation) in independent assays for antibody titers against the homologous FMD vaccine strain and the heterologous field isolates. Sera that are part of this pool are medium to high titer sera previously tested against the homologous virus type. The relationship between strains was according to the r1 value (r1: reciprocal serum titer against heterologous virus/reciprocal serum titer against homologous virus). The interpretation of the results was as described (Rweyemamu M., 1984): r1 values greater than 0.3 indicate that the field isolate is sufficiently similar to the vaccine strain and that the use of the vaccine is likely to confer protection against challenge with the field isolate.

Results and Conclusion

The results for individual neutralizing titers for antigenic characterization showed good crossreactivity for type O strains and r1 values over 0.3, when assayed using a swine sera pool vaccinated with a monovalent vaccine containing O1 Campos collected at 28 days post vaccination (dpv). Results indicate that the vaccines tested could be potentially used for controlling these type O lineage outbreaks in regions where the lineages studied circulate.

THERMAL STABILITY EVALUATION OF CDVac AFTOSA BIVALENT VACCINE IN CATTLE

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Introduction: FMD vaccines have been an important component of disease control and eradication strategies. However, factors like the diversity of FMD serotypes, logistical hurdles, and limited resources can hinder effective vaccination campaigns. the development of high-quality vaccines that show great efficacy, long lasting immunity and with thermostable capacity that secure potency under eventual temperature excursions above the conservation specifications are essential to ensure disease control. In this report, we explore the protective capacity of CDVac Aftosa bivalent vaccine against FMD in cattle after being expose to 37°C for 72 hours.

Methodology: The Bivalent oil-in-water emulsion vaccine, CDVac Aftosa Bivalent (O1campos; A24 cruzeiro) was manufactured at CDV (Centro Diagnóstico Veterinario, Argentina). Two bottles were preserved at temperatures between 2-8°C (35-46°F), and two bottles were expose to 37°C (98.6°F) for 72 hours and then preserved between 2-8°C (35-46°F) until vaccination. Two groups of seventeen naïve cattle were vaccinated with a 2 ml dose intramuscularly (IM) with each of the

vaccines to be tested. 2 naïve cattle were leaves unvaccinated as a control group. Sera was analyzed by ELISA-LP at different time points up to 90 days post-vaccination to determine the onset and immunity duration. ELISA titres from both groups were compared to determine if there were significant differences. Results: 90 DPV no significant difference was observed between the group vaccinated with the vaccines preserved at 2-8 °c and the vaccine with the thermal excursion. Both groups show an EPP above 75 %. Conclusions: The bivalent vaccine demonstrated immunogenic stability up to 90 days post vaccination. No significant differences were observed in the potency of the two groups of vaccinated animals, demonstrating the thermostability of the CDVac Bivalent Foot and Mouth Disease vaccine, ensuring its efficacy against potential deviations in its storage for up to 72 hours at temperatures up to 37°C.

Evaluation of Live-Attenuated Foot-and-Mouth Disease Vaccine Strains in Susceptible Animals

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Foot and mouth disease (FMD) is a highly contagious viral disease affecting cloven hoofed animals, animals such as cattle, pigs, and goats causing severe economic losses in the livestock industry worldwide. Despite ext ensive control measures with inactivated vaccine , outbreaks continue to occur, highlighting the need for more effective vaccines. The development of live attenuated vaccines for FMD presents several challenges. The primary difficulty lies in achieving a balance between attenuation and immunogenicity; the virus must be attenuated sufficiently to prevent disease but still capable of el iciting a strong immune response. Developing new attenuated strains of the FMD virus is crucial for effective vaccination. We designed that these vaccine strains were modified to provide broad protection against virus challenge and be safe for pig, goat an d cattle. The genetic engineering using the three methods (mutation, replacement and deoptimization) are key to designing viruses that meet these criteria, reducing the risk of reversion to virulence and enhancing immunogenicity.
These innovations are exp
ected to produce safer and more effective vaccines, potentially
leading to the eradication of FMD. Live attenuated vaccines could significantly enhance global FMD control programs, reducing economic losses and improving animal health and welfare.

Novel SAT 1/I Foot-and-Mouth Disease Vaccine Strain Confers Broad Cross- Immunity Against emergent Middle Eastern and African Isolates

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Introduction
The recent emergence of SAT1 Foot-and-Mouth Disease Virus (FMDV) in the Middle East, an unusual event for this region, poses a serious threat to livestock populations with little or no prior exposure to this serotype. In this context, the development of a relevant SAT1/I vaccine strain is a timely and strategic intervention. This study presents the immunological evaluation of a novel vaccine, demonstrating its capacity to induce potent immune responses against multiple SAT1 topotypes.
Materials and Methods
The SAT1 2020 vaccine strain was derived from a SAT1/Topotype I field isolate and adapted for growth in BHK cells to ensure high antigen yield and industrial scalability. The monovalent vaccine was produced by Biogénesis Bagó (Argentina) in accordance with GMP as a high potency water-in-oil single emulsion containing purified antigen. Immunogenicity was assessed in FMD-seronegative cattle following a single 2 ml dose in a controlled trial supervised by SENASA, the WOAH Foot-and-Mouth Disease Reference Laboratory in Argentina. Cross-reactivity was evaluated by virus neutralization tests (VNT) at both, WRLFMD (The Pirbright Institute) and SENASA using sera from vaccinated cattle tested against heterologous SAT1 strains.
Results
A single vaccine dose induced a robust neutralizing antibody response in cattle by 30 days post-vaccination, indicating strong immunogenicity. Cross-neutralization assays confirmed broad reactivity against diverse field strains, with high heterologous titers. VNT titer obtained with recent isolates such as QTR/7/2023 (2.23 $\log_{10}$), BAR/50/2025 (1.93 $\log_{10}$) and IRQ/11/2025 (1.89 $\log_{10}$) were satisfactory, supporting the strain cross-protection potential.
Conclusion
The SAT1 2020 vaccine strain shows strong immunogenicity and broad cross-reactivity against recent SAT1 field isolates from the Middle East and Africa. Its broad antigenic spectrum and suitability for industrial production supports its strategic role in regional and global FMD control programs.

Virology

Using Non-Metric Multidimensional Scaling applied to VP1 sequences to classify Foot and Mouth Disease Virus.

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FMD virus nomenclature systems are currently based on combinations of geographic regions and phylogeny. Alternative nomenclature lineage-based systems (for example Pangolin) used for Covid-19, dengue fever, influenza and rabies viruses can be susceptible to discovery order and sampling bias, which can affect their reproducibility. Here, we propose a potentially new nomenclature system based on projection of individual FMDV VP1 genetic sequences into an n-dimensional serotype specific space. Using a Non-Metric Multidimensional Scaling (NMDS) based algorithm, we can quantify and visualise these genetic differences between different sequences within this space. Furthermore, we can fix a set of reference sequences in this space, allowing for the addition of newly discovered sequences. We used a 3D dimensional space due to its ease of interpretation. We can assign each sequence a 3D coordinate and map them to any one of a grid like system of cubes each with its own 'post-code'. Novel sequences with no previous nomenclature classification can be assigned to an existing cube postcode and FMDV topotype depending on their positioning in this space and proximity to currently defined reference sequences. We have developed a user-friendly interface using the Shiny R package to implement this system that allows for the easy addition of newly discovered sequences. This capacity to map sequences into a 3D space based on their genetic relationships provides a robust indication of genetic similarities between strains. We used the Vegan R package functions monoMDS() to construct the 3D space from a dissimilarity matrix of pairwise genetic distance of our aligned reference sequences and MDSaddpoints() to add new sequences to this 3D space. The distortion incurred by this dimensionality reduction can be measured by a stress function value that measures the conservation of the true genetic pairwise distance as dimensions of data is reduced. This value is tracked after the addition of each new sequence to ensure adequate performance of the system. We demonstrate the application of this approach to selected FMDV serotypes. This novel nomenclature method enables a greater understanding of sequence similarity alongside a faster classification of new sequences and may provide a means to study the correlations between genotype and antigenic phenotype.

Use of microthreads for studying foot-and-mouth disease virus survival in aerosols

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Foot-and-mouth disease virus (FMDV) is primarily transmitted through direct contact between infected and susceptible hosts. However, long-distance spread can also occur via aerosols or contaminated fomites. Understanding FMDV aerosol survival is essential for effective disease control and improving outbreak surveillance strategies.
Studying the behaviour of pathogens in aerosols is challenging, particularly high-risk pathogens.

Here, we describe the use of microthreads to capture and study simulated aerosols of foot-and-mouth disease virus (FMDV) within a high-containment laboratory environment. In this approach, spider silk is wound around frames and exposed to aerosolized FMDV in a controlled aerosol chamber. Unevaporated droplets are intercepted by the ultrafine threads, and this setup allows us to determine the duration for which the virus remains viable in the environment and to assess the effects of environmental factors such as temperature, relative humidity (RH) or ultra-violet radiation.

Preliminary findings show that under indoor conditions with high relative humidity (80%) at room temperature (22°C), the virus half-life differs amongst strains, with estimates of approximately 29, 22 and 44 minutes for strains of serotype A, Asia 1 and SAT1, respectively. These are shorter than SARS-CoV-2 or measles virus (~60 and 90 minutes, respectively) but significantly longer than that of influenza A H1N1 (~3 minutes).

This methodology yields valuable data on the environmental conditions that support aerosol transmission and highlights strain-specific differences. The work will be extended to consider other strains, temperatures and relative humidities.

Analysis of differential replication of FMDV variants in cell cultures using subgenomic reporter replicons

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Two subtypes of FMDV serotype A were identified during the 2000-2002 epizootic in Argentina: A/Arg/00, which caused mild clinical signs in infected animals, and A/Arg/01, which was highly virulent. We previously demonstrated that the differential virulence between FMDV A/Arg/00 and A/Arg/01 was linked to the internal ribosome entry site (IRES) element. Precisely, viruses carrying the A/Arg/00 IRES exhibited delayed viral protein synthesis and smaller lytic plaques compared to those with the A/Arg/01 IRES.

To establish an in vitro system for studying FMDV replication, a subgenomic replicon of A/Arg/01 (pRepA01) was constructed by replacing the structural protein-coding sequence with the gene for green fluorescent protein (ptGFP) from Ptilosarcus gurneyi. The subgenomic RNA was transfected into i) BHK-21 cells and ii) an ovine kidney cell line via electroporation. ptGFP fluorescence was assessed over 24 hours using fluorometry and fluorescence microscopy. In cells electroporated with pRepA01, ptGFP expression was detectable at 4 hours post-transfection (hpt), reaching its

maximum level at 10-12 hpt. No fluorescence was observed in cells electroporated with a mutated pRepA01 lacking viral RNA polymerase activity.
Next, a replicon derived from FMDV A/Arg/00 was generated (pRepA01_IRES-00) by replacing the IRES element of A/Arg/01 with the homologous A/Arg/00 IRES sequence. The ptGFP expression kinetics of pRepA01_IRES-00 differed from pRepA01 between 4 and 12 hpt. These results are consistent with those obtained with infectious viral particles and derived clones in cell cultures and suggest the IRES element interacts with trans-acting factors influencing viral replication in a variant-specific manner. Ongoing proteomic analyses aim to characterize these interactions.

Efficiency of FMDV packaging correlates with genetic distance between genome and capsid

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RNA encapsidation or packaging is a crucial step in the picornavirus life cycle and is facilitated by packaging signals dispersed throughout the viral genome. Packaging signals contain RNA secondary structure which implement interactions between the genome and the capsid. In addition to facilitating encapsidation, such interactions are also thought to increase virion stability. Understanding packaging signals and RNA encapsidation into capsids may therefore also contribute to the design of more stable vaccines.

In this study, we investigated the conservation of predicted packaging signals in multiple serotypes of foot-and-mouth disease virus (FMDV) and tested RNA packaging using a trans-encapsidation assay. We assessed the efficiency of packaging of sub-genomic replicon RNA into capsids of different FMDV serotypes and the capsid of bovine enterovirus (BEV), a picornavirus from a different genus.

Our findings suggest that the RNA structures of some FMDV packaging signals are conserved across serotypes, thereby enabling trans-encapsidation of replicon RNA based on O1K FMDV into capsids from different FMDV serotypes. However, not all packaging signals are conserved between serotypes, consistent with the observation that the level of packaging in this system decreased as the trans-encapsidating capsid became more distantly related from the O1K replicon RNA. In contrast, the FMDV replicon RNA could not be packaged at all into BEV capsids, indicating that FMDV packaging signals are incompatible with the BEV capsid.

These results suggest that some FMDV packaging signals may be serotype specific while others are more conserved and may have a more universal function. These findings could have implications for approaches used to package RNA into VLPs for enhanced vaccine stability.

Seeking evidence of FMDV Recombination in Pigs

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Multiple serotypes, topotypes and lineages of FMDV co-circulate in endemic regions, and recombination between strains, even across serotypes, is increasingly recognized as a key driver of FMDV evolution. A deeper understanding of recombination mechanisms is essential for the development and deployment of effective control strategies and vaccines.
Previous research has demonstrated that superinfection of persistently infected cattle with a different serotype gives rise to recombinant FMDV in the upper respiratory tract. Pigs cannot become persistently infected, but due to their central role in FMD epidemiology in many parts of the world, it is critical to investigate whether they enable viral recombination by another mechanism.
We present preliminary results from a series of animal experiments in which pigs were infected intra-oropharyngeally with A24 Cruzeiro and O1 Manisa, both simultaneously and in sequence (A followed by O and O followed by A). While high-resolution sequence analyses are still in progress, we report clinical outcomes, infection kinetics and viral replication patterns as assessed by serotype-specific RT-qPCR.
These data offer new insights into the replication dynamics of FMDV during coinfection in pigs and will complement the recently characterized recombination processes in cattle. Our findings may ultimately contribute to a more nuanced understanding of pigs' potential role as contributors to the genetic diversity and evolution of FMDV.

Poster Presentations – Session 2

Diagnostics

Full set of new solutions for a rapid and reliable Foot and Mouth Virus detection: Double Antibody Sandwich ELISA, freeze dried RT-qPCR and Lateral flow device for point-of-care diagnosis

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Introduction:
Foot and mouth disease (FMD) is one of the most contagious viral diseases in ruminants, causing

epizootic diseases in a few weeks and with devastating economic consequences. Rapid and specific identification of the agent is of utmost importance. Innovative Diagnostics has developed a new set of diagnostic solutions to detect all known serotypes of FMDV: a lateral flow device, the ID Rapid® FMD Antigen, providing result in the field in under 15 minutes ; a freeze-dried RT-qPCR, the ID Gene LyoTM FMDV Triplex, which includes an ultra-rapid amplification program giving results in 40 minutes ; a double antibody sandwich (DAS) ELISA, the ID Screen® FMDV pan-serotype Antigen Capture, which enable a first economic screening so that downstream serotyping is only performed on positive samples. This study presents validation of these new solutions.

Methods:
Inclusivity, as well as diagnostic sensitivity of the 3 tests was evaluated on a panel of 10 inactivated strains from all 7 serotypes and panels provided by different Reference Laboratories. Diagnostic specificity were assessed for the LFD and the DAS-ELISA on 60 negative bovine tongue epitheliums from a non-infected area (France) and for the RT-qPCR on : 160 bovine samples, 50 swine whole blood samples and 100 goat milk from the same area. Exclusivity of each test with respect to closely related viruses (Swine Vesicular Disease Disease (SVV) and vesicular stomatitis (SVV) viruses) was tested.

Results:
The 3 kits correctly detected all FMDV tested strains, including SAT strains, showing 100% inclusivity. The LFD's, RT-qPCR's and DAS-ELISA's measured sensitivities were 100% (95%CI [97.3-100], n=113) ; 100% (95%CI [51-100], n=3) ; 100% (95%CI [97.3-100], n=113). For all kits, all tested negative samples gave negative results and the measured specificity was 100%. SVV and VSV were not detected.

Conclusions:
The ID Gene LYO™ FMDV Triplex, the ID Screen® FMDV pan-serotype Antigen Capture and the ID Rapid® FMD Antigen exclusively detects all FMDV known serotypes. While the ID rapid® kit provides results in the field in under 15 minutes, the DAS-ELISA and the RT-qPCR's performance enable a reliable confirmation of cases or an efficient first line screening of susceptible animals.

Development of a primary lamb kidney cell line towards propagation of foot and mouth disease.

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Background
Foot-and-mouth disease (FMD) has significant socio-economic impact in sub-Saharan Africa. FMD diagnosis depends on propagation of foot and mouth disease virus (FMDV) in cells. Primary lamb kidney cells have been shown to have good sensitivity to the Southern African Territories (SAT) serotypes of FMDV which are prevalent in sub-Saharan Africa, but the utility of primary cells is not ideal due to batch-to-batch variability in performance which can compromise diagnostic reliability.
Methods
Primary lamb kidney cells were transduced using a recombinant human telomerase reverse transcriptase (hTERT) lentiviral supernatant (Cellomics - CMV, GFP, PURO) in the presence of a cationic transduction reagent. Over-expression of TERT was confirmed using real-time PCR and the comparative ΔΔ Ct method. The morphological and growth properties of both primary and transduced cells were monitored, and their diagnostic performance was evaluated.
Results
Three percent of primary cells were successfully transduced but their TERT expression levels decreased with successive passaging. Transduced cells maintained good growth kinetics and confluency up to passage 18, while primary cells did not grow beyond passage 7. No noticeable differences in morphology were observed. During determination of the presence of SAT viruses and SAT virus replication, TCID50/mL assays and virus isolation tests did not show any significant difference between the performance of primary and transduced cells.
Conclusion
A cell resource that offers genetic homogeneity and diagnostic reproducibility for SAT serotypes of FMDV was developed. This cell line, although requiring further validation, is invaluable towards effective and timeous diagnosis of FMD in the sub-Saharan region.

Thermostable virus-like particles (VLP) of foot and mouth disease virus (FMDV) serotype O/IND/R2/1975: An alternate antigen in liquid phase blocking ELISA (LPBE) for titration of antibodies

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Introduction
Incomplete inactivation and thermolability of the capsid limit the use of inactivated FMDV-based LPBE under basic laboratory settings. Prior research has demonstrated that specific mutations can augment the thermostability of FMDV VLPs. Consequently, this investigation aimed to substitute the inactivated antigen of FMDV serotype O with thermostable VLPs on the performance of the

LPBE in titrating anti-structural protein antibodies against the Indian FMDV vaccine strain (serotype O).

Methods and Results

Recombinant baculovirus clone containing the capsid coding region of FMDV (O/IND/R2/1975) with a specific mutation in the VP2 region was employed to produce thermostable virus-like particles (VLPs). Following infection of Tn5 cells with the recombinant baculovirus, cells were harvested on day 4, and the cell lysate containing the expressed VLPs was extracted and stored at 4-8°C. The antigen was titrated by sandwich ELISA, and the optimal dilution for LPBE application was determined. The VLP-based LPBE was applied to titrate antibodies in known positive post-vaccination cattle sera (n=155) and known serotype O negative sera (n=217, comprising 102 cattle, 40 sheep, 71 goats, and 4 buffaloes). Bland-Altman method comparison with conventional LPBE indicated a minimal bias of -0.06 log10. The performance of both LPBE was compared with that of VNT, and receiver operating characteristic curve analysis showed an optimal cut-off titer of 0.972 log10 for classification.

Discussion and Conclusion

The study revealed a relative sensitivity (79% and 83%) and specificity (100% and 98%), and the test exhibited minimal variation between the inactivated FMDV-based and thermostable VLP-based LPBEs for titration of antibodies against FMDV serotype O. These findings suggest that the thermostable VLP-based LPBE possesses the potential to serve as a viable replacement for the inactivated FMDV-based LPBE.

Solid phase competitive ELISA (SPCE) for screening anti-capsid antibodies to FMDV serotype O Indian vaccine strain: Comparison between polyclonal and monoclonal antibody assays

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Introduction

The virus neutralisation test (VNT) is frequently utilised to determine the antibody titers, indicating immune status for selecting calves for potency testing and evaluating vaccine quality. For routine post-vaccination sero-surveillance, enzyme-linked immunosorbent assays (ELISAs) in liquid phase blocking /solid phase competition ELISA (LPBE/SPCE) formats are preferred over VNT. This study explored the application of SPCE for titrating antibodies against O/IND/R2/1975 vaccine strain, employing FMDV O serotype-specific guinea pig polyclonal (pAb) and monoclonal antibodies (mAb) for selection of calves in FMD vaccine quality testing.

Methods and Results

Eight mAbs targeting distinct antigenic sites were assessed for their neutralising capabilities and reactivity against a panel of FMDV isolates, encompassing vaccine strains, O-PanAsia I, II, and Ind2001 viruses. A mAb, namely 11F1E10, demonstrated wide reactivity against various strains of

FMDV serotype O, and was subsequently chosen for the optimisation of mAb-SPCE. A cohort of known positive (n=362) and negative (n=52) sera, categorised based on vaccination and infection history, was subjected to VNT, LPBE, pAb-SPCE, and mAb-SPCE. The diagnostic specificity (DSp) of both pAb-SPCE and mAb-SPCE exceeded 96%, a value demonstrably higher than that observed for VNT (88.5%) and LPBE (90.5%). Conversely, the diagnostic sensitivity (DSn) of pAb-SPCE (76.8%) was inferior to that of other assays (89.5%, 93.9%, and 83.2% for VNT, LPBE, and mAb-SPCE, respectively). Also, the negative predictive value was found to be comparatively lower for heterologous infected cattle sera, requiring cautious interpretation of results.

Discussion and Conclusion

Though the reported SPCEs demonstrated a positive correlation and strong agreement with VNT and LPBE, their negative predictive values were relatively low, indicating their potential application as population assays rather than for individual animal screening.

Epidemiology

Integrating Epidemiological and Sequencing Data for Epidemic Intelligence: Co-designing and Trialling a Hands-on, Capacity-building Workshop to Enhance Preparedness and Response to Transboundary Animal Diseases (TADs) in Endemic Settings

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Collecting and analysing epidemiological data is the foundation for outbreak preparedness and response. Many TAD-endemic countries have valuable epidemiological data documenting disease outbreaks, livestock movements, and extensive historical sample collections. However, data-driven disease intelligence is hampered by limited expertise in conducting advanced epidemiological analyses, including the ability to generate and analyse high-resolution genomic sequence data. These limitations reinforce cycles of external dependence and prevent local animal health services from fully maximising the value of their samples and data.

Developing the capacity to tackle epidemics in endemic settings extends beyond improving

laboratory infrastructure or introducing digital technologies. To address this, the University of Edinburgh, in collaboration with experts from the Pirbright Institute, University of Glasgow, and University of Ghana, co-designed and trialled an intensive four-day workshop addressing these critical issues focused on two streams:

a. Analysis of Epidemiological Data in R – Basic programming skills with applications for data cleaning, visualisation, survey design, network analysis, and mathematical modelling.

b. Nanopore Sequencing and Bioinformatics – Training on WGS using a portable Oxford Nanopore MinION protocol, bioinformatics pipeline and output interpretation, using FMDv as an example.

We trialled this approach in Ghana with 20 participants, including district veterinarians, animal health officials, laboratory technicians, managers, and academics, depending on the workshop. The country-level tailored training used biobanked local samples and simulated data that mimicked the structure of local epidemiological data whenever possible. We noted the importance of introducing data analysts to programming languages like R. Using locally relevant data and samples facilitated participant engagement with the content.

The workshops delivered key competencies mapped to the stages of the PCP-FMD, complementing FAO and EuFMD efforts through MOOCs and practical field courses. This initiative is a significant asset in advancing the GF-TADs strategy. The first iteration of the workshops provided valuable lessons for further development of context-relevant material to enhance data-driven epidemic intelligence on TADs.

CONTINUED CIRCULATION OF FOOT-AND-MOUTH DISEASE VIRUS TOPOTYPES O/EA-3, A/AFRICA/G-IV AND SAT2/VII IN NIGERIA

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Foot-and-mouth disease (FMD) is endemic in Nigeria. In the past decade, investigations have shown clinical cases caused by FMD virus (FMDV) topotypes O/EA-3, O/WA, A/AFRICA/G-IV, SAT1/X and SAT2/VII.

Forty-three epithelium samples from suspect clinical cases of FMD in cattle in Nigeria were

collected from 14 outbreaks in 6 Northern States in 2021 and 2023.
After primary characterization at NVRI the samples were send to Sciensano for confirmatory analysis, VP1 sequencing and phylogenetic characterization.

All 43 samples tested positive on RT-qPCR. Virus was isolated from 21 samples from 12 outbreaks and further characterized by antigen ELISA. Seventeen samples from 10 outbreaks in 2021-2023 were serotype O, 2 samples from one outbreak in 2023 were serotype A and 2 samples from another outbreak in 2023 were serotype SAT2. Serotypes were confirmed by VP1 sequencing and the two remaining outbreaks were also characterized as serotype O by VP1 sequencing.
The serotype O VP1 sequences were topotyped as O/EA-3. The sequences from 2021 and 2023 clustered separately in the phylogenetic trees with 91% VP1 nucleotide (nt) identity. The sequences were most closely related to sequences from Nigeria 2014 (2021) and Nigeria 2017 (2023) with 96% and 95% nt identity, respectively.
The serotype A VP1 sequences from 2023 were topotyped as A/AFRICA/G-IV and were most closely related to sequences from Nigeria 2017 with 94% nt identity.
The SAT2 VP1 sequences from 2023 were topotyped as SAT2/VII and were most closely related to sequences from Nigeria 2011 and 2020 with 98% nt identity, respectively.
Further phylogenetic analysis is ongoing to assign a more accurate origin of each of the FMDV strains.

It was concluded that FMDV topotypes O/EA-3, A/Africa/G-IV and SAT2/VII continue to circulate in Nigeria with most clinical cases caused by different sub-lineages of O/EA-3. This is in line with previous observations.

Foot-and-Mouth Disease Vaccination and Post-vaccination monitoring in Korea, 2024

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A mandatory nationwide FMD vaccination for all cattle, pigs, and goats was initiated to control the FMD, after experiencing a devasting FMD outbreak in 2010, in South Korea. Along with the implementation of FMD vaccines, a massive year-round serosurveillance has been launched in 2011. In 2017, a comprehensive biannual vaccination program for cattle and goats and post-vaccination monitoring, in addition to year-round routine serosurveillance, were introduced. At present, three FMD vaccines, oil-adjuvanted and containing inactivated serotype O and A antigens, following a prime and boost inoculation schedule, for immunization in the field. In this study, we aimed to evaluate the current K-FMD vaccination regimens and campaign by analyzing vaccine-induced herd immunity at the population and various subpopulation levels.
In 2024, national FMD vaccination was carried out, targeting all cattle and goats in April and October. A total of 50,065 serum samples at 28 days post-vaccination from the farms selected

randomly, for monitoring post-vaccination immunity. In particular, the design of PVM was targeted at the risk factors, considering the livestock group, specific area, herd size and husbandary system. Serological tests were performed to assess vaccine induced antibodies and identify the FMDV infection, by the 46 regional veterinary laboratories in cities and provinces using commercially available ELISAs under the supervision of the central laboratories, APQA. The overall population immunity from April to November in 2024 was consistently higher than 80%, and the seroprevalence monitored after the vaccination in fall was higher than the data in spring in all the targeted species. There was no difference in seroprevalence between provinces except the seroprevalence of goats in Jeollanam-do (87.0%) in spring, Gyeongsangnam-do (84.3%) and Incheon-si (86.7%) in fall, where the seroprevalence was relatively low compared to that of goat population in other provinces (90.5~91.8%). The seroprevalence of young calves was lower(94.8~94.9%) than that of cattle(98.9~99.0%) in the course of time. There was no NSP sero-positive reactors in 2024, newly. In conclusion, the FMD vaccination campaign has been successfully implemented in Korea, and the PVM can be a supplementary program for massive routine surveillance.

ENHANCED ACTIVE SURVEILLANCE AND SEROTYPE PROFYLING FOR FOOT AND MOUTH DISEASE CONTROL IN 6 COUNTIES IN KENYA

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2.AFFILIATE:DR.NAULINE CHEPNGENO

Abstract

Foot-and-mouth disease (FMD) remains a major threat to livestock production in Kenya, especially in the North Rift region, primarily in the following counties: Uasin Gishu, Nandi, Elgeiyo-Marakwet, Trans-Nzoia, Turkana, and West Pokot. These Counties are characterized by extensive livestock movement during trading, often across their borders, facilitating the rapid spread of FMD. Despite routine vaccination programs, outbreaks persist due to the lack of up-to-date data on circulating serotypes, as a result of poor antigenic match between field strains and vaccines. This proposal outlines an active surveillance program to be led by the National Veterinary Investigation Laboratory (NVL) in Eldoret to address these gaps through systematic field data collection and laboratory analysis.

The project focuses on identifying the prevalent FMD serotypes in the region to support targeted vaccine production, improve disease control strategies, and reduce economic losses caused by movement restrictions and trade bans during outbreaks. The major objectives include assembling epidemiological data, performing laboratory serotyping, enhancing vaccination campaigns, capacity building of staff, and ensuring continuous monitoring systems. Field activities will involve collaboration with County Directors of Veterinary Services (CDVs) for farmer sensitization, case identification, and sample collection (serum, pharyngeal fluids epithelium tissues and blood). Samples will be analyzed in the National FMD laboratory -Embakasi Nairobi, while the results will

used to inform immediate responses and long-term planning.
By the end of the project, a clearer understanding of FMD serotype changes will be achieved, enabling the development of effective control and eradication measures, including customized vaccine formulations. This will promote immunity of the herd, lower disease incidence, support food security and improve economic stability in these counties. The project also integrates biosecurity reinforcement, inter-county coordination, and enhanced farmer education to address challenges such as unpermitted livestock movement, communal grazing, and interactions with wildlife. The expected outcome is a significant advancement in FMD control in the North Rift region of Kenya.

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Recent incursions of FMDV topotype O/EA-3 in Libya (2019-2025)

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Libya occupies a large central portion of North Africa and is a strategic transit point for the movement of people, goods and animals. However, its geographical position and vast livestock resources make it vulnerable to transboundary diseases, such as FMD. Since its first appearance in 1959, the epidemiological patterns of FMD have been particularly dynamic. The aim of this study was to investigate the most recent outbreaks that occurred in Libya between 2019 and 2025. During this period, epithelium, swabs and blood samples were collected from a total of 62 animals (52 cattle and 10 sheep) presenting with FMD clinical signs from different regions of the country. Most samples consisted of material spotted onto Flinders Technology Associates (FTA) cards while epithelium homogenates were also submitted (n=12). These samples were conferred to the WOAH FMD reference laboratory in Brescia, Italy (IZSLER) and after nucleic acid extraction were screened by a Pan-FMDV real-time RT-PCR targeting the 3D-encoding region. Samples from 41 animals were FMDV positive. Phylogenetic analysis revealed circulation of the FMDV topotype O/EA-3 and supported the hypothesis of at least three different virus incursions during this period. Sequences from 2019 showed high identity with those isolated in the Maghreb during

2018–2019, whereas sequences from samples collected in 2023, 2024, and 2025 differed and clustered with sequences from Egypt and Ethiopia for samples collected during 2017–2018. These findings suggest that FMD has been introduced and spread from countries to the southeast of Libya. Moreover, while the sequences from 2023 and 2025 formed a monophyletic clade, the sequence from 2024 clustered separately, indicating a distinct incursion.
These findings underscore the continued risk of multiple FMDV incursions into Libya from different regions of Africa, highlighting the need for enhanced surveillance and coordinated control measures to prevent further spread across North Africa.

Epidemiological Survey of Foot and Mouth Disease (FMD) in Georgia

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Background and Objectives: Every year the number of countries around the world face the risk of spread of infectious diseases that cause the significant ecological and social-economic damage and develop serious epizootic situation. Therefore, accurate, rapid test diagnostics, study of animal diseases such as Foot-and-mouth disease (FMD) are important for international and local institutions. FMD is a highly contagious disease and outbreaks increase significant economic impact worldwide. The goal of the study was to investigate samples of different animal species on FMD to have a comprehensive picture to handle epizootic stability in Georgia.
Materials and Methods: SLA investigated samples of cattle, sheep, goats and pigs from different regions of Georgia. SLA utilized molecular biology RT-PCR and serological NSP ELISA-Ab assay to apply existing and already certified methods more widely and intensively regarding the FMD.
Results: for this study, in 2024, field samples were collected. In total, n=3298 blood and serum samples from the same animals have been tested at SLA. Samples were collected by National Food Agency (NFA) from different regions of Georgia. All investigated samples were negative on FMD by RT-PCR. Out of 1649 serum samples 55 samples were NSP sero-positive by ELISA-Ab, out of them 30 (3.4%) Large Ruminants (LR) and 25 (4.2%) Small Ruminants (SR). There were no clinical manifestations observed in the animals. Comprehensive study is necessary to understand exact prevalence of NSP in high risk regions and seasonal migration animals to control and evaluate the level of NSP in the country.
Conclusion: The active and NSP sero-survey of the disease will allow the country to carry out complex measures for controlling FMD that has a vital importance to national economy, food security and trade. The research project supported the disease surveillance system in Georgia and significantly improved the country's surveillance and research capabilities on FMD.

Comparative Multi-Omics Analysis of the Open Reading Frames of Foot-and-Mouth Disease Virus Serotypes: Insights into Genetic Heterogeneity, Evolution and Implications for Vaccine Development in Africa

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Background: Despite control measures, the high genetic variability/quasi-species nature of foot-and-mouth disease virus (FMDV, Aphthovirus vesiculae) complicate eradication efforts. This study aimed to characterize the population genetic structure of FMDV serotypes circulating in Africa and assess the conservancy of VP1 as a putative cross-protective vaccine target.

Material/Methods: Near-full- or full-length Open Reading Frames (ORF) of African FMDV isolates (1934-2022) [N = 167: serotypes A (26), O (45), C (3), SAT1 (32), SAT2 (35), and SAT3 (26)], were retrieved from NCBI Virus database, curated and analyzed between January and March, 2025. Serotype-specific VP1 consensus sequences were also generated. High-throughput bioinformatics pipelines/tools were used to comparatively evaluate sequence homologies, nucleotide diversities, recombination events, phylodynamics, and VP1 conservancy.

Results: FMDV Serotypes A and C showed highest genomic and protein identities (67.3% and 97.5%) respectively, and SAT2 had the least identities (38.6% and 69.3%). Whereas, SAT3 had the fewest singleton sites (n = 1) and least genomic polymorphism (4,229) ($P>0.10$), SAT2 had the most parsimony-informative sites (n = 6,795) ($P<0.05$), and widest genomic polymorphism (6,835). Nucleotide diversity (Pi) ranged from 0.2707 (serotype A) to 0.5812 (SAT2), with a mean inter-serotype diversity of 0.4494. InDel haplotypes ranged from 1 (SAT3, C) to 17 (O), with varied haplotype diversities (0.077–0.889), and serotypes O and SAT1 had the least (11.1%) and highest (53.1%) recombination events in their ORFs, respectively. Notably, VP1 conservancy was highest in SAT1 (99.6%) and lowest in serotype A (69.6%), suggesting serotype-dependent variability in potential vaccine targets.

Conclusions: This study reveals extensive intra-serotype genetic diversity across FMDV serotypes in Africa and identifies VP1 as a promising target for cross-serotype vaccine development against FMD. Our findings provide critical insights for regional FMD control strategies and represent the first comprehensive population genetic analysis of African FMDV serotypes.

Keywords: FMDV, ORF, Diversity, VP1, Vaccine, Africa

Foot-and-Mouth disease is back in Europe in 2025: a focus on the cases in Hungary and Slovakia

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The presence of Foot-and-Mouth disease (FMD) in FMD-free countries can be dramatic, with important economic losses (embargos, loss of production, depopulation…).
The European Reference Laboratory (EURL) for FMD has a role into the preparedness of EU laboratories, for quick and reliable detection and identification of any FMD case within the EU. The EURL also assist EU member states to confirm any FMD suspicion.
While Europe had been free of the disease for several years, Bulgaria was faced with an epidemic of FMD in 2011. In January 2025, Germany reported an FMD outbreak. At the beginning of March, a group of heifers on a dairy farm in Hungary (at the border with Slovakia) showed clinical symptoms compatible with FMD. The Hungarian NRL for FMD confirmed the FMD outbreak and the virus identified was belonging to sublineage O/ME-SA/PanAsia-2/ANT-10 (different from the virus that emerged in Germany). This virus was phylogenetically close to strains identified in Pakistan in 2017 and Türkiye in 2024. Slovakia subsequently reported outbreaks of FMD on the Hungarian border, with the same virus identified. By the end of April 2025, six outbreaks were reported in Slovakia and five in Hungary, all of which led to massive depopulation measures (9312 cattle and 9888 pigs were depopulated in Hungary). Even if vaccination is forbidden within the EU since 1991, an emergency suppressive vaccination was applied in both countries (4 farms in Hungary and 5 farms in Slovakia).
EU was free from FMD for a long time, but two different incursions of FMDV occurred in three months. The outbreaks have been contained, thanks to a global effort from the countries and different stakeholders, including the NRLs and EURL. These outbreaks remind us that FMD still represent a threat to FMD-free countries and that preparedness is essential.

Spatial and network-based risk factors for foot-and-mouth disease in Uganda: A decade of outbreak data 2016-2025

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Foot-and-Mouth Disease (FMD) remains a major transboundary animal disease in Uganda, affecting livestock productivity and trade. Understanding the spatial and network-related drivers of FMD outbreaks can inform targeted surveillance and control strategies.
We analyzed FMD outbreak data from the National Animal Disease Diagnostics and Epidemiology Centre (NADDEC) spanning 2016–2025, during which 63 confirmed outbreaks were reported, of which 35 in the dry season and 28 in the wet season. To assess the role of animal movement in disease spread, we utilised a network analysis results from historical livestock movement data using centrality measures (indegree, betweenness, and outdegree) for each district. These network metrics were incorporated into a Poisson regression model alongside ecological and demographic covariates, including livestock density (cattle, goats, sheep, pigs), human population density, proximity to international borders, and adjacency to national parks.
Districts located within or adjacent to national parks had a 42% higher risk of FMD outbreaks (IRR = 1.42), suggesting a potential role of wildlife–livestock interface in disease transmission. Indegree centrality which is a proxy for exposure to infected districts was significantly associated with outbreak risk, where each unit increase led to a 20% rise in outbreak incidence (IRR = 1.2). In contrast, higher sheep density was modestly protective, with a 2.2% decrease in outbreaks per unit increase (IRR = 0.978). Other variables, including betweenness centrality and livestock species densities, had weaker or non-significant effects.
These findings pronounce the importance of network exposure (indegree) and proximity to wildlife habitats in shaping the risk of FMD outbreaks in Uganda given that some wild ungulates could be reservoirs of the disease. We therefore recommend targeted interventions in high-risk districts, particularly those near national parks and with high incoming livestock traffic.

Epidemiology and effectiveness of interventions for Foot and Mouth Disease in Africa: A systematic review and meta-analysis

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Foot and mouth disease remains endemic in most African countries despite several interventions that have been instituted for its control. This systematic review and meta-analysis sought to elucidate the epidemiology of FMD and evaluate the effectiveness of interventions for its control in Africa.
We performed a systematic review and meta-analysis to generate evidence on the epidemiology and effectiveness of interventions for the control of foot and mouth disease in Africa. 113 articles were included in the review.
The overall pooled seroprevalence of FMD in Africa was 16% at 95% CI (4% − 30%). The subgroup analysis showed the following pooled seroprevalence at region: Central Africa at 38%, 95% CI (33% − 43%); Northern Africa at 31%, 95% CI (8% − 57%); Western Africa at 30%, 95% CI (11% − 50%); Eastern Africa at 22% 95% CI (9% − 37%); and Southern Africa at 2%, 95% CI (0% − 9%). Species level: buffaloes at 71%, 95% CI (8% − 100%); goats at 30%, 95% CI (4% − 61%); sheep at 23%, 95% CI (8% − 40%); cattle at 15%, 95%CI (2% − 31%); pigs at 9%, 95% CI (0% − 26%); and other wildlife at 2% (0% − 36%). Diagnostic level: ELISA at 15%, 95% CI (4% − 29%); several tests at 16%, 95% CI (0% − 44%); BTVIA at 29%, 95% CI (14% − 24%); and PCR at 44%, 95% CI (6% − 84%). LFK index of 4.83 indicated publication bias and a high level of heterogeneity. Quarantine and vaccination are the most used control interventions for FMD.
FMD is prevalent in most Africa in buffaloes and goats, it is mostly diagnosed by ELISA. The findings will guide the control of the disease and the use of the PCPFMD. Further research is recommended on the effectiveness of interventions for control.

Immunology

Capsid Integrity and TLR7 Activation: Essential for Effective VLP-Based Foot-and-Mouth Disease Vaccines

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Vaccination against foot-and-mouth disease virus (FMDV) is the primary strategy for controlling the disease. However, producing vaccines based on live virus poses significant biosafety concerns. Consequently, research is shifting toward next-generation vaccines using non-infectious antigens, enabling safer and more cost-effective production. Virus-like particles (VLPs, 75S) are promising

candidates, as they retain the native capsid structure and antigenic sites of FMDV but lack viral RNA, eliminating risks associated with handling infectious material.
In this study, 146S and 75S particles naturally produced by FMDV were purified from the same inactivated viral suspension and used as a model system. Previous immunization studies showed similar production of IgG1 levels between groups; nevertheless, basal levels of IgG2a were detected in 75S-vaccinated mice, whereas those receiving the 146S formulation exhibited a stronger IgG2a response. To determine whether these immunological differences stemmed from intrinsic particle properties rather than alterations introduced during processing, both particle types were subjected to thermal and mechanical stress to mimic conditions encountered during vaccine formulation.
Both particle types remained stable under mechanical agitation. Unexpectedly, 75S particles also retained stability after thermal challenge, reaching the same gradient fractions as untreated 75S particles after sucrose gradient sedimentation, indicating no appreciable shift in sedimentation coefficient. Nonetheless, mice immunized with thermally treated 75S particles exhibited only basal levels of immune response. Notably, despite unchanged sedimentation behavior, subtle conformational changes may have occurred, potentially altering key epitopes in the capsid. These structural modifications, undetectable by standard biophysical methods, may interfere with antigen recognition mechanisms involved in the adaptive response.
These findings underscore the importance of TLR7 stimulation in eliciting robust immune responses and suggest that factors beyond RNA content, such as conformational properties of the capsid, contribute to immunogenicity. This knowledge supports the rational design of safer FMDV vaccines that can elicit strong, protective immunity in livestock.

Pathogenesis

Acute-phase dynamic of Senecavirus A infection in pigs inoculated with a Taiwanese isolate

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Senecavirus A (SVA) is an emerging vesicular disease virus in swine, belonging to the Picornaviridae family. SVA infection is difficult to differentiate from other vesicular diseases such as foot and mouth disease, making timely differential diagnosis essential for outbreak control. Although sporadic cases have been reported in Taiwan since 2012, limited experimental studies have characterized the dynamics of SVA acute-phase infection. In this study, we explore the acute-phase pathogenicity of a Taiwanese SVA isolate in specific-pathogen-free pigs. Six pigs were inoculated with the isolate and monitored for clinical signs and sampled for 14 days. All pigs developed vesicular lesions on the coronary at 2-3 dpi, with complete resolution of vesicle lesions at 14 dpi. No lesions were observed on the oral mucosa, and systemic signs such as fever, lameness, or inappetence were absent. Viral RNA was first detectable at 2 dpi in oral swabs, rectal swabs, and blood. Oral swabs showed the highest detection rate and remained positive in 2 pigs at 14 dpi, while rectal and blood samples turned negative. These results highlight oral swabs as

the most effective sample type for early detection. The sampling timeline and lesion profile documented here offer useful guidance for the design of future studies on SVA pathogenesis, diagnostics, and vaccine evaluation.

First case of Foot-and-Mouth Disease virus (FMDV) in an Elephant from Cambodia

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Foot-and-mouth disease (FMD) is a highly contagious, acute viral disease of livestock caused by the foot-and-mouth disease virus (FMDV), affecting cloven-hoofed animals, including cattle, pigs, sheep, and goats. Infection in other species is extremely rare; however, sporadic FMD cases have been reported in atypical hosts (e.g., elephants, hedgehogs, rodents, bears, dogs, kangaroos) since 2000. In this study, we performed FMD diagnosis and analysis using clinical samples collected from elephants with FMD clinical signs in Cambodia in 2024. The Asian elephants (Elephas maximus) were residing in a zoo and had clinical symptoms of FMD, including lameness, mild fever, and vesicular lesions on the trunk and feet. Real-time reverse transcription polymerase chain reaction (rRT-PCR) targeting the FMDV 3D/IRES region using the tissue and saliva sample, and it was determined to be FMDV positive. To determine the species origin of the samples, the PCR was performed targeting elephant mitochondrial DNA D-loop and Y chromosome SRY genes. Therefore, we confirmed that the FMDV-positive samples originated from a male elephant. The virus was isolated in porcine kidney (LFBK)-αVβ6 cells and followed by whole-genome sequencing (next-generation sequencing) using a nanopore platform. Sequencing analysis revealed that the isolated strain belongs to the O type of FMDV within the Middle East-South Asia (ME-SA) topotype, specifically identified as the Ind-2001e lineage. The VP1 nucleotide sequence homology showed 95.73% to 99.68% with O/ME-SA/Ind-2001e isolates circulating in Cambodia in 2024. Therefore, we suggest that the virus might directly transmit from local livestock to the elephant during outbreaks. In conclusion, this is the first FMD case in elephants in Cambodia, and these findings emphasize the possibility of FMDV infection of wildlife, including captive and domestic wild animals. Furthermore, FMD surveillance and control programs on non-traditional hosts could also be important to manage potential transmission risks at the wildlife-livestock interface.

Vaccines

Continuous Post-Vaccination Evaluation in Industrial Dairy Farms

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Background/Introduction:
Foot-and-mouth disease (FMD) remains one of the most important diseases of ruminants. Despite routine vaccination programs in dairy farms, clinical signs may still appear due to failures in achieving protective immunity. This study emphasizes the importance of continuous post-vaccination monitoring (PVM) in evaluating herd immunity and informing appropriate vaccination strategies.
Material and methods
In this study, 430 serum samples were collected from 11 industrial dairy farms using the AFTOVAC VETAL ANIMAL HEALTH vaccine. Sampling was conducted from spring 2023 to spring 2025. Two farms participated in five sampling rounds due to consistent health management, while the others were sampled once. The antibody titers against FMD serotypes A, O, and Asia1 were measured using the IDVET ELISA kit. This approach helped assess the effectiveness of current vaccination protocols and guide necessary adjustments. Antibody titer evaluation against the A, O & Asia1 serotypes was monitored by IDVET ELISA kit.

Results and Discussion
Results indicated that 90%, 86.5%, and 82% of animals had protective antibody levels against serotypes A, Asia1, and O, respectively. The findings suggest that consistent vaccination, colostrum management, and monitoring of maternal antibody levels are essential for effective immunization. Proper timing of calf vaccination, especially between 6 and 9 months of age, played a critical role in establishing uniform herd immunity. Strategies such as vaccinating calves monthly from 2.5 months of age without considering maternal antibodies are not efficient or cost-effective.
Keywords: Foot-and-mouth disease, antibody titer, vaccination strategy, industrial dairy farm, maternal antibodies

Evaluation of a Tetravalent FMD Vaccine Produced for Eastern Africa

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Foot-and-mouth disease is one of the most contagious diseases of cloven-hoofed animals causing significant economical losses. There are 7 virus pools of FMD which are distinct from each other. Three of these pools are located in Africa, namely Pool 4 East Africa, Pool 5 West Africa and Pool 6 Southern Africa. Vaccination of all disease-sensitive animals is one of the most important control measures to be applied in FMD endemic countries. FMD vaccines for each pool has to

contain carefully selected vaccine strains to provide high level of protection against these circulating viruses.
An antigen panel to assess the regional relevance of foot and mouth disease vaccines has been developed by The Pirbright Institute, Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia and AU PANVAC. This panel consists of 16 FMDV's, 4 viruses from each of types O, A, SAT-1 and SAT-2.
Dollvet has developed a tetravalent FMD vaccine suitable for East Africa with the vaccine strains O Manisa, A/Iran/17, SAT-1/BOT/1/1977 and SAT-2/ERI/12/1998. A group 7 cattle were vaccinated twice with an interval of 21 days and sera samples obtained 10 days post second vaccination. These sera were tested against the East Africa antigen panel. Heterologous antigenic titers were determined against each virus and r1 values were calculated in order to assess the antigenic relationship of vaccine strains and the viruses included in the panel.
The results showed that the tetravalent vaccine of Dollvet was able to provide protection against all viruses according to heterologous titers. Additionally, r1 values proved existence of a good antigenic relationship between vaccine strains and viruses in the East Africa antigen panel.

Evaluation of different foot-and-mouth disease vaccination protocols in cattle in the Zambezi region of Namibia

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Cattle (n = 131) were randomly allocated into four groups. Group 1 (n = 40), Group 2 (n = 29), Group 3 (n = 39) and Group C (n = 23). Groups 1,2 and 3 consisted of animals that were previously vaccinated as opposed to Group C animals which were naïve animals. Groups 1,2 and 3 animals were quarantined for 30 days whilst Group C animals were quarantined for 60 days at the Kopano quarantine located in Katima Mulilo, Zambezi region of Namibia.
FMDV non-structural and structural protein antibody titres in cattle were determined at the start and end of the 30-day quarantine period. Cattle that tested positive for FMDV NSP antibodies at the start of quarantine were excluded from Groups 1 and 2 and placed in Group 3. FMDV structural protein antibodies were determined with a liquid-phase blocking ELISA (LPBE) and a solid-phase competitive ELISA (SPCE). Cattle were considered protected against FMD if LPBE results were ≥ 1.6 log10 or SPCE results were > 50%.
There was a significant difference in the proportion of cattle with protective levels of antibodies

for FMDV SAT-2 and significant differences in the median titres for FMDV SAT-2 and SAT-3 in field-vaccinated cattle compared to cattle vaccinated in the quarantine camp. There were differences in the immunogenicity of the FMDV strains in the vaccine, with serotype O being the most immunogenic and SAT-2 the least immunogenic.
Ultimately, field vaccination is not equivalent to first-time vaccination in the quarantine camp. Enhancing the immunity of cattle herds before their arrival, coupled with widespread vaccination using a very effective vaccine upon arrival, may ensure a reduced risk of FMDV incursion both in the quarantine facility and the Zambezi region.
Keywords: non-structural protein, DIVA, commodity-based trade, endemic, SAT 1, SAT 2, SAT 3, serotype O

NEW-GENERATION VACCINES AGAINST FOOT-AND-MOUTH DISEASE VIRUS: STRATEGIES TO ENHANCE THE IMMUNOGENICITY OF RECOMBINANT EMPTY CAPSIDS

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Current vaccines are based on inactivated foot-and-mouth disease virus (FMDV) but have significant limitations, including the need to produce large amounts of live virus. Virus-like particles (VLPs) are a safe alternative with strong antigenic properties; however, they typically require adjuvants due to low immunogenicity.
This study explored biotechnological strategies for rationally designing VLP-based vaccines. These strategies included immunomodulation with baculovirus, the complementation of cytotoxic cellular responses through recombinant baculoviruses carrying FMDV-derived antigens (Ag) in their nucleocapsids, and Ag-edited capsids to enhance cross-protection against different serotypes. Recombinant baculoviruses AcVP1cap, AcP1-2A-3Cfs, and AcP1Aless-2A-3Cfs were constructed for these purposes. VLPs were produced in Rachiplusia nu pupae, purified by sucrose gradient ultracentrifugation, and confirmed by Western blot, ELISA, TEM, and DLS.
Results: VLPs were produced in pupa biofactories with a yield of 1.19 µg/g. Vaccine formulations containing VLPs or inactivated virus combined with baculovirus as an adjuvant induced high levels of FMDV-specific antibodies and conferred protection ranging from 30% to 85%.
Complementation with AcVP1cap did not enhance protection at the evaluated challenge times. Further studies will assess neutralizing antibodies in pre-challenge samples and optimize protective responses by increasing antigen and adjuvant doses.

COMPARISON OF POTENCY CONTROL METHODS (IN-VIVO/IN-VITRO) OF FOOT-AND-MOUTH DISEASE TRIVALENT VACCINE IN SWINE

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COMPARISON OF POTENCY CONTROL METHODS (IN-VIVO/IN-VITRO) OF FOOT-AND-MOUTH DISEASE TRIVALENT VACCINE IN SWINE

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Introduction: Vaccination is the main strategic tool for controlling FMD infections. The need for potency tests that can be performed quickly and are effective is extremely useful, considering the high number of outbreaks worldwide. In this report, we compare DP50 potency control test (in vivo) with an Elisa assay, and a Sero neutralization assay (in vitro). Methodology: The trivalent oil-in-water emulsion vaccine, CDVac AFTOSA TRIVALENT (O1campos; A Argentina 2001; A24 cruzeiro) was manufactured at CDV (Centro Diagnóstico Veterinario, Argentina). Three groups of 6 naïve 2-month-old piglets were vaccinated intramuscularly (IM) with doses of 2 ml, 0.5 ml and 0.125 ml. A fourth group of 2 naïve piglets was left unvaccinated as control group; all groups were infected with 100.000 ID of O1 campos strain at 29 dpv. Serum samples were analyzed by VNT and ELISA-LP at different time points up to 37 days post-vaccination (8DPI) to determine the potency of vaccine, these results were compared to the DP50 test. Results: After the viral challenge in pigs (8 dpi), animals in the vaccinated groups showed no lesions consistent with foot-and-mouth disease (FMD). At 27dpv, the groups vaccinated with 2 mL and 0.5 mL exhibited PEP greater than 77% for both ELISA-LP and VNT. Following the viral challenge (8 dpi), there was an increase in antibody titres in all groups, with an average protection level above 97% for both ELISA-LP and VNT. Conclusions: At 27 dpv significant differences were observed between the vaccinated groups, having association with de applied doses. Comparing the serologic tests significant differences were also observed ($p<0.05$). 8 DPI all groups exhibited PEP values above 97% for Elisa-LP and VNT, having this correlation with the DP50 results (32 DP50), where none of the vaccinated animals show lesions consistent with FMD.

Effective Early Immunization in Calves Achieved with a Commercial FMD Vaccine That Surpasses Maternal Immunity

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Introduction

Foot-and-mouth disease (FMD) is a major threat to livestock, emphasizing the importance of effective vaccination in endemic regions. This study evaluated the influence of maternally derived antibodies (MDA) on the antibody response to a commercial FMD vaccine in calves, aiming to determine the optimal age for immunization in offspring of vaccinated dams.

Materials and Methods

Two groups of 20 calves, aged 60 (G60) and 90 days (G90), born from multivaccinated dams, received a tetravalent oil-adjuvanted vaccine (Bioaftogen, Biogénesis Bagó: O1 Campos, A24 Cruzeiro, A2001 Argentina, C3 Indaial) at day 0, followed by revaccination at 180 days post-vaccination (dpv). 20 calves of the same ages remained unvaccinated (Controls G60C and G90C) to monitor MDA decline and were vaccinated for the first time at 240 and 270 days of age (180 dpv). Serum samples were collected on day 0, monthly up to 180 dpv, and again up to 60 days post-revaccination.Antibody titres were measured using liquid-phase ELISA

Results

Before vaccination a high level of specific antibodies both at 60 (mean titre log 10=2,69-2,89) and 90 days of age (mean titre log 10=2.47-2.64) was shown. In both vaccinated groups, a single dose induced protective antibody levels, even in the presence of MDA, with significant differences from controls starting at 60 dpv. Notably, in both groups, protective titres were maintained up to 180 dpv. In unvaccinated controls protective levels of MDA were detected up to 90-120 days of age. Revaccination at 180 dpv significantly boosted the immune response compared to controls. No adverse effects were observed, supporting the vaccine safety

Conclusion

Bioaftogen vaccine successfully overcame the colostral antibody barrier, allowing effective immunization with long-lasting protective antibody response in the presence of high MDA levels. This highlights the importance of proper vaccination timing to ensure early and lasting protection against FMD in young calves.

Initial validation of a small animal model for potency assessment of FMD vaccines for batch release

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Cattle serology is an accepted alternative to FMDV challenge for the batch release of FMD vaccines. However, the costs and limited availability of sero-negative calves in India due to extensive vaccination necessitates the need for an alternative methodology. Accordingly, we evaluated the potential of a guinea pig serology-based regression model to predict the cattle serological responses to provide data for vaccine batch release.

Oil-adjuvanted FMD vaccine batches containing FMDV O, A and Asia1 antigens of Indian vaccine strains (n=16 batches from 3 manufacturers) were administered simultaneously to calves (n=5-16/batch) and guinea pigs (n=10/batch). Neutralizing antibody titers were determined by virus neutralization test (VNT) for each serotype using 28 days post-vaccination sera, and the mean titer for each batch was used to construct serotype-specific linear regression models. Model-based guinea pig cut-off titers were tailored for each serotype using the cattle results and the serological cut-offs defined in the Indian tender document. Using the results from cattle serology as the pass/fail criteria, the ability of guinea pig serology to predict these outcomes was cross-tabulated. The accuracy (%) of the guinea-pig serology was 69, 88, and 100 for serotypes O, A, and Asia1, respectively, despite a moderate R^2 (0.35-0.42) and positive correlation [Pearson's r, 0.58-0.64; ($p<0.05$)].

The pilot study, showed that guinea pig data for serotypes A and Asia1 were able to accurately predict cattle responses, although data for serotype O had comparatively less predictive power, likely due to the closeness of the observed mean cattle batch titers (1.58 log10VN50) to the tender cut-off for this serotype (1.65 log10VN50), and the inherent variability of VNT. Testing more batches is anticipated to improve the prediction for serotype O. These data provide encouragement to initiatives to develop a small animal model for FMD vaccine batch release to replace the cattle serology-based batch release assay.

FIELD EVALUATION OF IMMUNE RESPONSE AGAINST A 0,5 ML FOOT-AND-MOUTH DISEASE VACCINE THROUGH A NEEDLE-FREE INJECTION DEVICE OR CONVENTIONAL NEEDLE

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Introduction

Foot-and-mouth disease (FMD) is a highly contagious viral disease affecting swine and other livestock, with major economic consequences. Effective vaccination strategies are essential. Recent studies show that reduced volume (0.5 mL) ultraconcentrated vaccines maintain protective responses in pigs. Needle-free (NF) also provide operational advantages, improving safety, animal welfare, and ease of use during large-scale vaccination.

This work reports the immunogenicity of this reduced volume vaccine applied by subcutaneous injection or needle-free Free.

Materials and Methods

Sixteen FMD-seronegative piglets (8 weeks old) were divided into two groups (n=8). Both groups received the same vaccination protocol of two doses 30 days apart of newly developed Bioaftogen 0,5 ml vaccine containing O1 Campos, A24 Cruzeiro, and A2001 Argentina strains

(Biogenesis Bago, Argentina). Group 1 received vaccination with a commercial NF device and Group 2 through conventional needle (SC). All animals were blooded at day 0, 30 dpv and 26 days after revaccination. A commercial Liquid Phase Blocking ELISA was used. Titers were analyzed by Student's t-test.

Results and Discussion

All pigs were seronegative for FMD before vaccination. On the other hand, all animals developed protective titers by 30 dpv. Booster vaccination significantly increased responses for all serotypes. No significant differences were observed between NF and SC at 30 dpv. After boost, A24 Cruzeiro SC titers were higher ($p<0.05$); for O1 Campos and A24 Cruzeiro no significant differences were observed. In general, both administration routes showed effective immune responses. These findings confirm the immunogenicity of the newly developed Bioaftogen 0.5 mL FMD vaccine, regardless of delivery method, and the practical advantage of using NF.

Conclusion

The Bioaftogen 0.5 mL FMD vaccine induced protective immune responses via both SC and NF delivery. The comparable efficacy, combined with the logistical and welfare advantages of NF systems, supports its use in modern swine vaccination programs.

Virology

Epitope tags in FMDV 3A reveal microtubule-dependent merging of sites of replication and cell-type dependent superinfection exclusion.

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Foot-and-mouth disease virus (FMDV) replication occurs at distinct sites within the cytoplasm of infected cells and is mediated by virally encoded non-structural proteins. One such protein is 3A, which can be used as a marker of replication sites. Here we have used reverse genetics to generate viable viruses carrying either a FLAG or HA epitope tag in the 3A replication protein, but otherwise identical. The rescued viruses were utilised in subsequent co-infection experiments.

To investigate replication dynamics by confocal microscopy, cells were co-infected at high MOI with two viruses encoding either FLAG-tagged or HA-tagged 3A and the cells were labelled using antibodies directed against the epitope tags. At early time points, puncta within co-infected cells contained either HA-tagged 3A (red) or FLAG-tagged 3A (green) but not both, whereas at later time points 3A puncta were larger and contained both forms of 3A. These data indicated that early sites of replication were derived from individual incoming viruses, and that these early sites subsequently merged. In support of this hypothesis, when cells were coinfected in the presence of nocodazole, which disassembles microtubules, the signals for HA-tagged 3A and FLAG-tagged 3A accumulated to high levels but remained entirely separate. This suggests that the merging of initial sites of replication results from microtubule dependent transport.

In addition, we have utilised our tagged viruses to investigate superinfection exclusion. Cells were first infected with the FLAG-tagged virus and then the HA-tagged virus. A degree of superinfection exclusion was observed in PK-15 but not IBRS-2 cells.

Taken together our co-infection data indicates that sites of replication are initiated from single FMDV genomes, and these sites subsequently merge in a microtubule-dependent manner. Our superinfection exclusion data indicate that this phenomenon is cell-type dependent, perhaps mediated by cellular antiviral responses active in some cell lines, but defective in others.

Exploring the guinea pig model in the putative persistent phase of infection with foot-and-mouth disease virus

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Guinea pigs (GP) are a suitable alternative (biosafety-wise, financially and logistically) for the preliminar evaluation of antiviral compounds and vaccines against foot-and-mouth disease virus (FMDV). This study investigates the potential of GP to study infection in the putative persistent phase.

GP were intradermally inoculated in the hind footpads with either FMDV O/FRA/1/2001 – a strain known to establish persistence in cattle and in primary bovine cell models – or guinea-pig-adapted FMDV O1/MAN/TUR/69 – known to cause secondary vesicular lesions. GP were examined daily for clinical symptoms and evolution of body weight. Real time RT-PCR targeting the viral polymerase (3D) was performed on various lymphoid and non-lymphoid tissues.

In addition to virus replication in the inoculated hind footpads and – in some GP – in the tongue and front footpads, Ct values of 28.5±2.2 were observed in the spleen and liver at 2 to 3 days post inoculation (dpi) in half of the inoculated GP (O/FRA: 4/8; O1/MAN: 1/2), suggesting virus replication in these non-epithelial tissues. At that time, viral RNA was consistently detected in the popliteal lymph nodes (ln.) draining the inoculated footpads of all 10 inoculated GP, Ct 31.8±1.9. Viral RNA levels gradually decreased to undetectable in most tissues over time, while the popliteal ln. generally remained positive until 28 dpi. At 14 dpi (O/FRA: 6/6 ; O1/MAN: 6/6) and 28 dpi (O/FRA: 4/6 ; O1/MAN: 6/6), Ct values were 30.5±4.5 and 36.2±3.2, respectively.

The observed retention of FMDV RNA in ln. draining replication sites in GP aligns with data showing that FMDV (RNA) can persist in the spleen in mice (Gordon et al. 2022) and in lymphoid

organs draining lesion sites in pigs (Stenfeldt et al. 2014) until at least 28 dpi. Further research is ongoing to detect live virus or viral protein in the popliteal ln. of GP inoculated with FMDV.

Competitive interactions within mixtures of infectious clones A01Lc and A01NLc: impact on FMDV lethality in mice

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Despite lacking their natural quasispecies, the viruses rescued from the infectious clones A01Lc and A01NLc reproduce the lethal and attenuated phenotypes in adult mice of their parental strains, FMDV A/Arg/01 variants A01L and A01NL, respectively. In this work, we (1) generated mixtures of A01Lc and A01NLc clones, (2) evaluated the lethality and clinical signs produced in mice over 7 days (n = 6), and (3) assessed viremia levels and FMDV genetic composition by Illumina at 24 hpi (n = 3/condition). The assay involved 6 groups: (a) 100% A01Lc; (b) 10% A01Lc; (c) 10% A01NLc | 90% A01Lc; (d) 10% A01Lc | 90% A01NLc; (e) 100% A01NLc and (f) a negative control group. Mice were inoculated intraperitoneally with $1x10^5$ $TCID_{50}$/ml or PBS. Compared to the control group, 100% lethality was observed in groups a, b, and c between days 2 and 3 post-infection. Group d showed 50% lethality by day 5 and moderate clinical signs, whereas group e proved 100% survival and mild-to-moderate signs of disease (Mantel–Cox, $p < 0.0001$). A significant difference was found only between groups a and c compared to group e (Tukey, $p < 0.05$). Quasispecies in sera from mice belonging to groups a, c, and d exhibited positive selection of the lethal haplotype (A01Lc) (Tukey, $p < 0.05$). In particular, in group d the lethal variant accounted for only 10% in the inoculum and evolved to 90% at 24 hpi, suggesting that virus-virus interactions within the quasispecies can modify lethality, clinical severity, and viral replication in vivo. Taken together, our results provide an initial approach for the in vivo study of viral mixtures and a novel perspective for analyzing competition among minority haplotypes in quasispecies and their impact on virulence.

Porcine tonsil slices as a tool to study early FMDV-host interaction

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Epithelial cells covering porcine tonsils are considered a potential site of primary FMDV replication, for example following experimental infection via the intraoropharyngeal route. A detailed investigation of these early events may provide important insights into the unfolding innate immune response and help understand differential outcomes of FMDV infection across strains and species.

To expand the available toolbox for such studies, we began investigating precision-cut tissue slices from porcine soft palate tonsils. Slices of 200 µm thickness were prepared using a Krumdieck tissue slicer. Following a 30-minute stirring step at 37 °C to remove agarose used during slicing, the tissues were transferred to 24-well plates for an overnight resting phase. The next day, slices were infected with different dilutions of recombinant FMDV derived from an O1Kaufbeuren infectious clone whose capsid proteins had been swapped for those from the O/UKG/35/2001 field isolate (O/UKG-WTcap). Slices were incubated with virus for 4 h, 8 h, or overnight. Samples were then embedded in OCT, sectioned using a cryostat, and analysed by laser scanning confocal microscopy. FMDV 3A protein was detected using monoclonal antibody 2C2, and epithelial cells were visualised with a pan-cytokeratin antibody.
Preliminary results show 3A-positive signals in cytokeratin-labelled epithelial cells after overnight incubation, consistent with viral replication in the slice cultures. No 3A-positive cells were detected in subepithelial, lymphocyte-rich areas. Ongoing work focuses on further optimisation and characterisation of the system, including detection of FMDV structural proteins to better understand the relationship between virus distribution and replication.

Once fully established, this tissue slice model may become a powerful tool for investigating innate immune responses at cellular and subcellular resolution.