

MEETING REPORT

**OPEN SESSION OF THE
STANDING TECHNICAL COMMITTEE**

OF THE

**EUROPEAN COMMISSION FOR THE
CONTROL OF FOOT-AND-MOUTH DISEASE (EuFMD)**

HELD AT

BORGO EGNAZIA (ITALY)

29-31 OCTOBER 2018

**FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED
NATIONS
ROME, 2018**

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FOREWORD

Increasing global security in the supply of effective foot-and-mouth disease (FMD) vaccines: can we really manage the risks and achieve progressive control without it?

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

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

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

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

Global security of supply of FMD vaccines therefore affects all countries – so what can be done about it, and how do we manage the risks without it?

This is a big issue and needs all involved to have an overview of the scale of the problem, and the barriers and constraints to increased security. We must continue to cope with the risks posed by lack of vaccine, or to over-reliance on vaccination.

This issue is as relevant to FMD free countries as those endemically affected. If we are to progress we must do more than listen, we need also to talk, to meet together, and work out new and better ways to get big actions to address this global issue. We urge all those concerned to improve this situation to collaborate, from scientists interested in what limits new vaccines to be used, those work on epidemiology and surveillance to vaccine bank managers through to industry leaders and investors keen to form new alliances to increase supply of vaccines.

Vaccine security would mean the confidence that vaccines are affordable, available and effective and accessible by stakeholders.

Isn't that worth giving our attention, time and effort to?

Dr. Keith Sumption



EuFMD Executive Secretary

European Commission for the Control of Foot-and-Mouth Disease

SUMMARY OF THE OPEN SESSION 2018

The Open Session of the Standing Technical Committee of the European Commission for the Control of Foot-and-Mouth Disease (EuFMD) is held every two years and has become the largest technical and scientific meeting on FMD to be convened on a regular basis. The EuFMD has organized these meetings since the early 1970s, alternating a Closed Session with an Open Session for scientific exchange.

The Open Session 2018 (OS18) meeting was held in Borgo Egnazia, Italy, from 29–31 October. Under the theme of *Increasing Global Security in the supply of effective FMD vaccines*, a total of 23 technical sessions were held in plenary and parallel streams, along with panel discussions, poster sessions and post-meeting workshops. The meeting agenda is provided in **Appendix 1**.

The meeting was attended by 270 participants from around the world, including five members of the Standing Technical Committee and observers from both public and private sectors, scientific institutions, research laboratories and academia. The list of participants is provided in **Appendix 2**. The recording and uploading of presentations to the Open Session Online platform facilitated participation of a broad international audience. An additional 290 participants joined the Open Session Online, which was streamed live during the meeting and can be accessed at <https://eufmdlearning.works/course/view.php?id=178>.

All talks were mentioned using the Twitter handle #OS18 and a number of videos of the talks were shared live using social media. A dedicated mobile application enabled participants to keep track of events, plan their meeting timetables, vote for the posters and provide feedback on the meeting conclusions. Awards were handed out to best presentation for each day, overall, and best poster.

Following the conclusion of the OS18 programme, two post-meeting workshops were held at the same venue on (1) *Field evaluation of novel livestock vaccines*, with support from the International Veterinary Vaccinology Network; and (2) *Pragmatist vaccine antigen prioritization*, in partnership with the Pirbright Institute.

Key messages were captured from meeting presentations, panel discussions and in consultation with participants through the mobile application. It was agreed that quality vaccines are not enough, and that the barriers that prevent their availability must be addressed. The meeting concluded that improving vaccine availability needs urgent attention by both public and private sectors and a new form of partnership is needed to shift the vaccine stewardship paradigm to (1) create an enabling environment for investment in vaccine security; (2) continue to support research and development for innovative technologies and partnerships; and (3) ensure inclusion of all stakeholders in the value chain. The meeting conclusions and recommendations are given below.

CONCLUSIONS AND RECOMMENDATIONS FROM THE OPEN SESSION 2018

The conclusions and recommendations from OS18 combine key messages from the presentations, discussions and feedback directly from participants via the dedicated OS18 App and the section on the opinion of the participants upon the topics below.

CONCLUSIONS

1. Improving vaccine availability needs urgent attention by both the public and private sectors to increase global security in the supply of effective vaccines.
2. Manufacturers are key partners in the network of stakeholders that contribute to FMD control, and engagement with them is valued and necessary to address the issues surrounding Global Vaccine Security.
3. Development of a new form of partnership through a public-private professional network is needed to achieve real change in vaccine supply through:
 - a. creating an enabling environment for investment in vaccine security;
 - b. continuing to support R&D for innovative technologies and partnerships; and
 - c. ensuring inclusion of all stakeholders in the value chain.

4. Quality vaccines are not enough, the barriers that prevent their availability must be addressed. Further work is required to better understand the barriers to vaccine supply, including registration and regulation barriers; and solutions, such as mutual registration.
5. The five key challenges requiring consideration to support manufacturing FMD vaccines are:
 - a. biosecure facilities require a significant capital investment;
 - b. it is difficult and expensive to produce quality FMD vaccine;
 - c. constant research and development into emerging strains is needed;
 - d. the registration process is complex; and
 - e. there is a lack of visibility in the demand and forecasts for FMD vaccine need.
6. Capacity to identify and prepare for future risks relies on the adequacy of viral information for the identification of vaccines for use in regional programmes. Outbreak investigation and sample submission remain core activities to support the continued monitoring of circulating viruses and provision of accurate vaccine matching advice.
7. In endemic settings, livestock keepers should have the right to access effective vaccines to protect their livestock and livelihoods, and inadequate access to effective vaccines in endemic regions is an animal welfare issue.

RECOMMENDS

8. Further work to better understand the drivers for adoption and factors influencing willingness to pay for vaccines, and to quantify the current 'unmet' demand and predicted future growth for vaccines.
9. Recognising that novel vaccine platforms provide opportunities for safe and high potency FMD vaccines and may offer a commercially viable alternative to conventional killed vaccines, further work is recommended to address the many remaining technical and registration challenges, such as addressing criteria for GMO licencing in Europe.
10. Recognising that the traditional vaccine bank model is poorly equipped to respond to trans-pool movements of viruses, innovative approaches to vaccine resources are recommended including:
 - a. exploration of alternatives and/or complementary approaches to traditional vaccine banks; and
 - b. application of new modelling tools to examine the range of policy and operational issues related to vaccine resources
11. Further work is recommended to establish the potential benefits and risks associated with use of attenuated vaccines and the topic of attenuated live FMD vaccines should remain on the agenda for future discussion.
12. The regulatory environment is becoming increasingly demanding, and it is recommended that proposed novel vaccine products should demonstrate significant advantages over existing products to facilitate the licencing process.

OPENING CEREMONY

The OS18 was formally opened by Jean-Luc Angot, President of the EuFMD Executive Committee. Dr Angot reflected that securing adequate volumes of effective vaccines has presented a challenge since the 1950's, when European Commission of Foot and Mouth Disease was established in response to the devastating post-war epizootics of foot and mouth disease in Europe. Through collaborative efforts significant progress in the progressive control of FMD has been made, with almost all of Europe and South America now recognized as officially FMD-free. The role that quality vaccines have played in this progress is acknowledged, and the contributions of pioneers of vaccine technologies must not be forgotten; notably H.S. Frenkel (1891 - 1968) and J.G van Bakkum (1922 – 2018). However, the challenge remains to increase security in the supply of effective FMD vaccines. To move forward, we should embrace public private partnerships and recall the mentality of the 1950s, when progress was made possible through a strong political will.

Dr Silvio Borello, Chief Veterinary Officer for Italy, welcomed participants to Borgo Egnazia. He noted that good progress had been made in the progressive control of FMD since Italy last hosted an Open Session in 2008. However, FMD continues to present a risk to the European region, and Dr Borello congratulated EuFMD for organizing this important multidisciplinary congress, as well as for its continual activity in supporting and enhancing prevention in its 39 European member states, in close collaboration with the European

Commission. The three pillars of EuFMD remain central to reducing the risk posed by FMD. Dr Borello proposed a fourth pillar, solidarity, and offered support on behalf of Italy to address the issues surrounding global FMD vaccine security.

Dr Juan Lubroth, Chief FAO Animal Production and Health, spoke on behalf of FAO, noting the success of the Progressive Control Pathway and the framework it has provided for national capacity and policy development, not only for FMD but for a range of other applications. However, Dr Lubroth reiterated that the global burden of animal diseases remains enormous. FMD is among the most common diseases of ruminants and pigs in endemic regions, with estimates of 250 million cases annually. Considering many of the cases are treated with antibiotics, a holistic approach to address the collective issues of disease burdens is required. Dr Lubroth recognized that almost all resources in the fight against FMD are provided by countries themselves, and increasingly, from the affected livestock producer. To tackle the disease at its source, livestock producers must gain access to the means to protect their animals, through information, early warnings, biosecurity and vaccines. Access to quality vaccines is therefore required to support progressive control. Dr Lubroth recognized the key role of the private sector in developing and delivering vaccines, and identified the need to collaborate to stimulate investment and supply of vaccines. He highlighted FAO's support for creating a dialogue with the private sector to encourage new innovations to support Global Vaccine Security.

Welcoming comments were also provided by Dr Antonio Fasanella (Direttore Generale dell'Istituto Zooprofilattico sperimentale di Puglia e Basilicata), Dr Yu Qiu (Representative, World Organisation for Animal Health), Dr Ewa Camara (Representative, DG-SANTE). Eoin Ryan, Chair, EuFMD Standing Technical Committee, reiterated that OS18 not only facilitates information exchange, but also provides a forum for generation of innovative solutions amongst scientists, policy makers, industry and international non-government organisations. He confirmed that Global Vaccine Security is at the forefront of the Standing Technical Committee's agenda, and declared the OS18 officially open.

SESSION 1: GLOBAL OVERVIEW

A keynote paper presented by Dr Keith Sumption (EuFMD) introduced the concept of Global Vaccine Security and emphasised the importance of access to effective vaccines for the progressive control of FMD. Dr Sumption summarised progress made in the control of FMD over the past 10 years, but highlighted that the burden of FMD remains considerable, particularly in endemic regions of Africa, Asia and the Mid-East. In the ten years following Open Session 2008 in Erice, Italy, a number of key recommendations have been achieved, including implementation and enhancement of the Progressive Control Pathway for FMD (PCP-FMD). However, Dr Sumption reiterated statements made in 2008 that remain relevant today: that FMD control can only be achieved (1) by actions at the field level to break transmission cycles; (2) when vets and animal owners in the front-line are armed with sufficient information and suitable vaccines; and (3) when there is sufficient private and public support. Dr Sumption closed by highlighting that Global Vaccine Security is an issue that affects all countries. He noted that international organisations have a role in supporting the conditions that promote sustained national actions, and that the scale of change needed demands a new approach of global public private partnerships for vaccine security.

Dr Don King (The Pirbright Institute) presented a keynote paper [**Appendix 4**] on the global status report for FMD, with particular focus on tracking the emergence and spread of new viral lineages. Dr King's presentation illustrated the continuing dynamic nature of FMD virus circulation, and the role of the OIE/FAO FMD Laboratory Network in monitoring geographical distribution and spread of transboundary FMD virus lineages to highlight future risks. Capacity to identify and prepare for future risks relies on the adequacy of viral information for the identification of vaccines for use in regional programmes.

SESSION 2: THE SCALE OF THE PROBLEM

Six papers [**Appendix 5, 6, 7, 8, 9 and 10**] were presented to bring attention to recent and ongoing work being undertaken to estimate the scale of demand for: (1) emergency supplies of vaccine in FMD-free regions; (2) vaccine to meet the projected growth in livestock populations and demand for FMD control to meet PCP-programmes; and (3) vaccines for regional crises such as epidemic jumps between virus pools. The drivers for demand were explored, contrasting public financed campaigns with drivers operating at the owner level; for private uptake of vaccines for protection of livestock and to support emerging markets in endemic regions.

The session identified that an extensive population of at-risk livestock exists in endemic regions and demand for vaccines is projected to increase, so a new revolution in supply is needed. A willingness to pay for FMD vaccines appears to exist, but more is needed to quantify this willingness to pay, to understand the drivers for adoption and predict growth in demand.

SESSION 3A: VACCINE SUPPLY

Four papers were presented with the aim of increasing the understanding of the barriers affecting supply and availability of vaccines to private and public buyers, how these barriers may be overcome, and by whom. Aldo Dekker gave a presentation dedicated to the memory of H.S. Frenkel and J.G van Bekkum. He reflected on the 50 years since the death of Frenkel and the passing in 2018 of Professor van Bekkum, both being pioneers in FMD vaccine security in Europe and important contributors to EuFMD. His presentation demonstrated how European vaccine supply issues in the past were met by international partnerships. Ron Bergevoet [Appendix 11] explored the policy issues behind emergency banks as a means to address immediate emergency demands. Pascal Hudelet [Appendix 12] provided valuable insight into the issues for investors in building capacity to upscale supply. Finally, Noel Ainepaln [Appendix 13] introduced how mutual registration processes in East Africa may offer a potential solution to remove barriers of entry to markets and increase access to vaccines.

A panel discussion, chaired by Keith Sumption, followed the session with industry representation from Pascal Hudelet (Boehringer Ingelheim), John Atkinson (MSD Animal Health) and Sacha Seneque (Ceva Global). The panel invited investors to identify priority barriers to address in order to increase supply, and how these might be overcome. Five key challenges were identified by the panel when considering a change to the supply of FMD vaccines:

- (1) biosecure facilities require a significant capital investment;
- (2) it is difficult and expensive to produce quality FMD vaccine;
- (3) constant research and development into emerging strains is needed;
- (4) the registration process is complex; and
- (5) there is a lack of visibility in the demand and forecasts for FMD vaccine need.

The discussion highlighted that reducing unpredictability in demand through enhancing our understanding of future needs would support investment in manufacturing. It was suggested that global capacity is theoretically capable of meeting current demand, but this is not able to be harnessed due to registration complexities and regulations against working with foreign viruses. The panel agreed that mutual registration of vaccines, as is being pioneered in East Africa, would help to address some of the barriers faced. In addition, acceptance of multistrain dosage would support investment, but it is currently not widely accepted outside the EU. A need to increase transparency in the tendering process was also cited. It was concluded that manufacturers are a key partner in the network of stakeholders that contribute to FMD control, and engagement with them is valued and necessary to address the issues surrounding Global Vaccine Security.

SESSION 3B: VIROLOGY

During this session, five papers [Appendix 14, 15, 16, 17 and 18] were presented to showcase cutting edge research investigating the mechanisms of virus pathogenicity, ecology, evolutionary dynamics and phylogenetics. Findings from studies presented in this session support improved strategies for vaccine strain selection, vaccine implementation and post-vaccination surveillance.

SESSION 4A: BREAKING BARRIERS

Presentations in this session [Appendix 19 and 20] investigated the barriers that prevent end-users from accessing quality vaccines, including the public policy environments affecting who can import, distribute, purchase and deliver FMD vaccines to animals. The private and public sector rights and responsibilities in FMD prevention and control were considered, and discussions explored the historic implications of vaccine safety concerns on vaccine stewardship and the notion of FMD control as a public good and responsibility. Research

gaps were reviewed and the potential of developments in vaccine technologies to reduce biocontainment barriers were explored.

SESSION 4B: IMMUNOPATHOLOGY

During this session, three papers [**Appendix 21, 22 and 23**] were presented on the gene signatures associated with FMD virus infection and persistence, and on transmission from carrier animals via oropharyngeal fluid. The session concluded more work is required to improve our understanding of FMD virus persistence mechanisms, as well as the epidemiological importance of carriers in the transmission of FMD.

SESSION 5A: VACCINE SELECTION

Seven papers [**Appendix 24, 25, 26, 27, 28, 29 and 30**] examined the issues related to vaccine selection. It was suggested that consumer confidence in vaccines is affected by the limited data available on circulating viruses in endemic settings. New surveillance tools, such as environmental and bulk milk testing, may reduce reliance on outbreaks for sample collection data. Discussion highlighted that both public and private consumers have an interest in increasing confidence in vaccine selection, and a role may exist for the public sector to disseminate information to support appropriate vaccine selection.

SESSION 5B: MODELLING FREE AND NON-FREE AREAS

In this session, six papers [**Appendix 31, 32, 33, 34, 35 and 36**] shared approaches and results from recent modelling studies in both FMD and non-free settings. A range of modelling approaches were presented, investigating (1) the application of reinforcement learning and adaptive management approaches for outbreak control; (2) FMD virus transmission dynamics and spread pathways; (3) implications of control strategies; and (4) livestock mobility network analysis. Discussion highlighted the role that modern modelling capabilities might play in advising on policy for (1) progressive control of FMD in non-free areas; and (2) surveillance, preparedness and response in free areas.

SESSION 6A: CONVENTIONAL VACCINES

In this session, papers discussed the existing potential of conventional vaccines, what limits them and how much room for improvement remains with regards to stability, optimization for species and quality of immune response [**Appendix 37**].

In his keynote presentation, Tim Doel highlighted the important role of trivalent vaccination campaigns in supporting FMD control and eradication programmes in Europe and South America. He noted that, in cattle, good quality conventional vaccines confer high levels of protection and some cross-protectivity has been demonstrated between serologically diverse strains of the A serotype. While the instability of conventional FMD vaccines is recognised, Dr Dole clarified that good quality GMP products and proper cold chain maintenance minimises the issues associated with virus and vaccine instability. However, the limitations for good quality conventional vaccines are associated with their use in the field, where suboptimal conditions for storage and use often exist, in addition to complications such as maternal antibodies. A number of challenges associated with bringing a novel vaccine to market were noted, associated with: (1) product development and licencing; (2) manufacturing costs; (3) quality control; (4) quality assurance; and (5) the cost of facilities. In summary, conventional vaccines have a large body of safety and efficacy data to support their continued acceptance by regulators and practitioners. The regulatory environment is becoming increasingly demanding, and proposed novel vaccine products should demonstrate significant advantages over existing products to facilitate the licencing process.

SESSION 6B: MODELLING BUSINESS SECURITY DURING OUTBREAKS

The session showcased recent modelling work and provided a forum to consider the ways in which models can assist with questions of risk, costs and benefits associated with maintaining business continuity during outbreaks in FMD-free settings. Discussions identified the need for well-planned responses that balance the needs for disease control and business continuity in the livestock industries [**Appendix 38**]. As movement restrictions have a dramatic impact on the cost of livestock diseases such as FMD, it was suggested that

movement controls need to be carefully matched to the epidemiological and economic consequences of the disease, as optimal bans can have substantial financial benefits [Appendix 39].

Enhancements to the Australian Animal Disease Spread Model (AADIS) were also presented [Appendix 40], which allow comparisons of post-outbreak management strategies for securing proof-of-freedom from FMD and return to trade. The new modelling capability will support the development and refinement of post-outbreak management policies that facilitate the earliest possible recovery of FMD-free status and return to trade.

SESSION 7: CHAMPIONING NEW VACCINES

Three papers considered the potential for novel vaccine technologies. Vaccine production is currently carried out in high containment manufacturing facilities, resulting in significant production costs and limitations for production capacity. Issues associated with stability and production times for conventional vaccines were also cited. The session discussed progress and limitations faced when seeking to improve vaccine potency, vector stability and route of delivery, while minimising time until protection, number of vaccine doses, cost and side effects. Promising innovations discussed in this session included:

- (1) virus like particles (VLPs) expressed in insect cells using the baculovirus expression system and used to formulate vaccines with an appropriate adjuvant [Appendix 41];
- (2) a recombinant-replication-defective human adenovirus type 5 coding for FMDV capsid proteins and also the 3Cpro that allows for the in vivo assembly of VLPs (Ad5-FMD) [Appendix 42]; and
- (3) a novel, marked FMD-LL3B3D vaccine platform consisting of attenuated virus containing negative markers in the NSPs 3B and 3Dpol [Appendix 43].

It was suggested that novel vaccine platforms provide opportunities for safe and high potency FMD vaccines and may offer a commercially viable alternative to conventional killed vaccines. However, further work is required to address the many remaining technical and registration challenges, such as addressing criteria for GMO licencing in Europe.

SESSION 8A: THE FUTURE OF FMD VACCINES

The two papers in this session considered the future of FMD vaccines, and the potential acceptability of novel technologies for future commercial use. The application of structural design and reverse genetics to enhance FMD vaccines in Africa was discussed [Appendix 44]. It was concluded that applied research into the development of novel FMD vaccines and disease control strategies need to enable a fit-for-purpose approach to FMD control in the geographical context of interest.

Rational design of attenuated FMD vaccines using the Synthetic Attenuated Virus Engineering (SAVE) method was also described [Appendix 45]. It was discussed that these live, attenuated FMD virus strains may: (1) offer potential application for use directly as vaccines; (2) decrease biosecurity risks and costs related to vaccine production; (3) facilitate rapid production of vaccines in response to new strains; (4) be applicable to all serotypes; and (5) enable targeted vaccination of wildlife reservoirs.

During discussion time, it was noted that successful global disease eradication campaigns have all used live attenuated vaccines. The potential benefits and risks associated with use of attenuated vaccines were discussed, and concerns were raised regarding the possibility for recombination and reversion to virulence. Further work is needed, including to understand the mechanism for attenuation, before these methods can be applied in animal trials. There was a general consensus that the topic of attenuated live FMD vaccines should remain on the agenda for future discussion.

SESSION 8B: VACCINE EFFICACY AND EFFECTIVENESS

An introduction to this session was provided by David Paton and Giancarlo Ferrari, who gave an overview of the importance of improved field studies to support vaccine efficacy and effectiveness. A key message was that, regardless of whether a country intends to progress beyond Stage 2 of the PCP-FMD, verification of the effectiveness of a vaccination program is necessary, as is monitoring maintenance of vaccine efficacy between

campaigns. Field observational studies were noted as a very practical approach to monitor this in the endemic setting. Two papers were presented to consider: (1) the application of field trials to estimate the effectiveness of vaccination programmes in the Maghreb region [**Appendix 46**]; and (2) preliminary results from work to model the impact of farming practices on vaccine effectiveness in endemic settings [**Appendix 47**].

SESSION 9A: AFRICA EPI-NET

Ten papers [**appendices 48 - 57**] were presented as part of a discussion to foster greater understanding of viral circulation and FMD epidemiology in Africa, with the objective to promote networking and facilitate ongoing collaboration in surveillance between regional laboratories and academic partners.

SESSION 9B: IMPROVING CONVENTIONAL VACCINES

Six papers [**Appendix 58, 59, 60, 61, 62 and 63**] shared recent research related to improving conventional vaccines, including through development of higher yields, lower doses, duration of immunity and wider coverage. Presentations indicated that classical vaccine production systems remain important for delivery of global vaccine needs. While development of new vaccines is time consuming, it was also shown that established vaccine strains can cover more variants if they are produced to a sufficient quality. Furthermore, addition of immune stimulants to existing vaccines can improve the response and reduce the amount of antigen needed. However, quality control of vaccines is essential and new tools to test antigen integrity are required. The time taken to get new vaccines to market can be reduced if molecular biotechnology is used to improve adaptation to cell culture. New adjuvants as well as new routes of application can improve the quality and thereby the duration of immunity. But for all vaccines it remains important that quality is monitored and validated, not only by producers but also by customers.

SESSION 9C: BREAKOUT SESSIONS

A side workshop focused on managing the risk and achieving progressive control of FMD in North Africa took place on the 29th October 2018 with participants from Algeria, Morocco, and Tunisia. During the event, participants and EuFMD experts presented and discussed: (1) the current FMD situation; (2) the surveillance and control measures implemented and possible improvements; and (3) the vaccine quality, availability and vaccination strategies applied.

A side meeting was organized on the 30th October between participants from Turkey and EuFMD experts to discuss: (1) the status of the West-Eurasia laboratory network; (2) the implementation of immunogenicity studies in the Trans Caucasus with the possible support of the SAP institute of Turkey; and (3) the animal mobility programme.

SESSION 10A: DIAGNOSES AND DIAGNOSTIC TOOLS

Through four papers [**Appendix 64, 65, 66 and 67**], this session provided information on the recent assay validation work for improved diagnosis, and addressed the issue of emergency supply of diagnostic kits. Different options for serological diagnostic banks with their advantages and limitations were presented by a representative of a commercial company, which motivated a short debate and common agreement on the importance for a collaborative, multinational approach to this issue.

SESSION 10B: VACCINE QUALITY ASSURANCE

Three papers considered the interdependence of FMD virus pathogenicity, challenge system and outcome of vaccine studies [**Appendix 68**]; correlation between vaccine match in potency tests and r1-value [**Appendix 69**]; and potency assessment using standardised serological assays [**Appendix 70**]. Key messages from the session included:

- (1) Although tongue inoculation is useful as a standardized approach to FMDV vaccine testing, it is important that intrinsic aspects of FMDV pathogenesis are considered in the interpretation of experimental outcomes.
- (2) The choice of vaccine is often based on r1-value, however the optimal measure for vaccine match would be the heterologous potency of a vaccine divided by the homologous potency of a vaccine. Existing studies suggest a significant correlation between potency ratio and r1-value, but further work is needed as studies are limited and publication bias likely exists.

- (3) When considering correlation between antibody titres and protection, inclusion of a standard serum is a good way to make results between laboratories more comparable.

SESSION 10C: EUFMDIS DEMONSTRATION

The EuFMD outbreak simulation model (EuFMDis) was presented during several talks earlier in the week, as well as demonstrated during coffee breaks. This session provided a formal demonstration of the model capabilities, along with facilitated discussion. Participants demonstrated a strong interest in the model, with around thirty attendees and a lively discussion about opportunities and challenges for disease simulation modelling. The value of a pan-European FMD simulation model was supported.

SESSION 11A: MIDDLE EAST AND ASIA EPI-NET

Six papers [Appendix 71, 72, 73, 74, 75 and 76] were presented on the FMD situation in pools 1, 2 and 3 to facilitate networking and encourage a collaborative approach to surveillance and research within and between the regions.

SESSION 11B: VACCINE PERFORMANCE

This session reiterated the importance of post vaccination monitoring and field studies to validate vaccine performance. Three papers [Appendix 77, 78 and 79] presented findings of recent studies. A need was highlighted for regional collaboration and improved surveillance to support appropriate vaccine selection. Establishment of a vaccine banks was advocated in endemic areas, such as the Maghreb region, to improve timely access to appropriate vaccines.

CLOSING CEREMONY

At the closing ceremony, Corissa Miller presented a summary of the feedback collected through the mobile application, which is outlined in full in **Appendix 80**. Awards were handed out for the following:

- Best presentation of day 1: Jonahnton Arzt - Transmission of FMD from persistently infected carrier cattle to naïve cattle via transfer of oropharyngeal fluid; Pascal Hudelet - The Challenges of FMD Vaccine Production
- Best presentation of day 2: Aurore Romey - Rapid On Site Diagnosis of FMD and Safe and Cost-Effective Shipment of Samples Using Lateral Flow Devices for Laboratory Diagnostics; Emiliana Brocchi - Field Trial to Estimate the Effectiveness of the vaccination program implemented in the Maghreb region
- Best presentation of day 3: Michael Eschboumer - Multiplex Real Time RT-PCR for Detection of FMDV Rift Valley Fever Virus and Bovine Viral Diarrhea Virus in Bulk Tank Milk; Carolina Stenfeldt - The Interdependence of FMDV Pathogenesis Challenge System and Outcome of Vaccine Studies
- Best presentation overall: Martin Ryan - Rational Design of Attenuated FMDV vaccines by elevation of CPG and UPA Dinucleotide Frequencies
- Best poster: Emma Brown-Evaluation of Environmental Sampling as a Low Technology Method for Surveillance of FMD Virus in an Endemic Area
- Lifetime achievement award: Tim Dole, for 38 years of service to improving FMD vaccines.

The meeting was formally closed by Keith Sumption, who thanked the hosts, presenters, participants and staff of EuFMD for their contributions and support to make the OS18 a success.

WORKSHOP 1: PRAGMATIST VACCINE ANTIGEN PRIORITISATION

A workshop was held the day after the Open Session to introduce participants to the *Prioritisation of Antigen Management with International Surveillance Tool* (PRAGMATIST). The tool was developed collaboratively by EuFMD and the World Reference Laboratory to assist risk managers to select which strains are most important to maintain in their FMD vaccine banks. The workshop demonstrated how to apply the transparent, evidence-based framework to evaluate available vaccine antigens and participants practiced adapting the tool to their region-specific risk profiles.

WORKSHOP 2: FIELD EVALUATION OF NOVEL LIVESTOCK VACCINES

A workshop funded by the International Veterinary Vaccine Network (IVVN) on performing vaccine field efficacy trials was conducted the day after the Open Session. It was attended by 30 people, with fully funded scholarships given to attendees from Nigeria, Mongolia and Tunisia. Bursaries were also given for other attendees from countries including Europe, South Africa and Zambia. There were additional attendees from Turkey and the USA. The workshop was an excellent forum for discussion on performing vaccine field trials.

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APPENDIX 2: LIST OF PARTICIPANTS

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APPENDIX 3: POSTER PRESENTATIONS

POSTER

HOUSEHOLD PREFERENCES FOR DIAGNOSTIC TESTING TO VACCINE MATCH IN AN ENDEMIC SETTING

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Introduction

The limited availability of FMD vaccines matched to the circulating virus type in northern Tanzania results in household uncertainty towards vaccine quality and subsequent use of antibiotics in place of vaccines. This uncertainty may be overcome with enhanced, timely information on vaccine quality. To explore this, we surveyed livestock dependent households to investigate their willingness to pay (WTP) for a hypothetical diagnostic test that could tell which vaccine to apply in an emergency situation.

Materials and Methods

We employed a cross-sectional survey on 466 households in northern Tanzania using a double-bounded contingent valuation method with a maximum likelihood estimator to assess preferences for a hypothetical diagnostic test offered as a public good. We determine the potential of the test for vaccine matching by examining the relationship between household WTP for diagnostic testing with household use of other livestock health inputs.

Results

The calculated WTP price for diagnostic testing averages USD 2.90 (95 percent CI: USD 2.00, 3.80) or USD 0.19 per cow compared to USD 2.60 (95 percent CI: USD 2.40, 2.80) for an emergency vaccine per cow. Household adoption of an emergency vaccine does not directly encourage diagnostic testing adoption but WTP is increased through the joint household use of antibiotics and vaccines (USD 1.30, p value 0.03) and use of government veterinarians jointly with antibiotics (USD 1.40, p value 0.02).

Discussion

Diagnostic testing with vaccine matching for FMD has implications for livestock management systems that extend beyond the direct benefit to the household. The test provides information that is necessary for community-wide disease control and can help reduce antibiotic usage through increased vaccination. Our results suggest an opportunity exists to promote effective disease control technologies in northern Tanzania through established veterinary systems and through networks of households that already invest in livestock health inputs.

POSTER

VP1 IS IMPORTANT IN HEPARIN SULPHATE BINDING OF FMDV STRAIN O MANISA

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Introduction

Many FMDV strains are selected that bind to heparan sulphate when adapted to cell culture (Jackson et al. 1996). It was shown that VP3 residues 55 – 60, VP1 residues 195 – 197 and VP2 residues 134 – 138 interact with heparan sulphate (Fry et al. 1999). To analyse whether changes in these amino acids could improve growth on BHK cells we compared growth of wild-type isolate O NET/2001 with cell adapted virus O Manisa and mutants containing different parts of both genomes.

Materials and Methods

The full length genomic region of both O NET/2001 and O Manisa preceded by a T7 promoter site were cloned in pOK12 (Rebel et al. 2000). Mutants of both viruses were produced. The infectious copies were linearized and transfected to BSRT7 cells to produce virus and were passaged in primary lamb kidney cells before testing them for growth on BHK cells.

Results

CPE observed after transfection correlated with the combined presence of Glutamine on position 133 in VP2 and arginine on position 56 in VP3. However, the best virus growth was observed with strains containing mainly a O Manisa backbone, indicating that amino acids of VP1, most likely aspartate at position 197 and glutamine at position 198 (Fry et al. 1999) improved the consistency of the virus growth.

Discussion

Changing only the heparin sulphate recognising amino acids in VP2 and VP3 did not result in consistent growth in BHK cells. The results are a strong indicator that the previously identified amino acid in VP1 are also necessary for adaptation to BHK cells. These results are important for vaccine producers that want to use molecular techniques to improve FMDV vaccine virus growth and yield.

POSTER

REPLICATION DYNAMICS OF MIXED FMD VIRUSES IN VITRO

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Introduction

Foot-and-mouth disease (FMD) is one of the most infectious viral diseases of livestock world-wide. The etiological agent (Aphthovirus, Picornaviridae) is present as seven serotypes with multiple variants. In endemic countries different serotypes and variants of virus often co-circulate with the possibility that animals become infected with multiple viruses. Therefore, simultaneous presence of two viruses in the same sample can occur, making virus isolation (VI) tricky and sometimes misleading.

The aim of this work was to gain insight into the dynamics of replication of two serotypes of FMDV co-infecting various cell lines in vitro.

Materials and Methods

Three cell lines, BHK-21, IBRS-2 and LFBK $\alpha\alpha\beta\beta_6$, were co-infected with two FMDV serotypes (O and A) at different ratios; samples were collected sequentially up to 48 hours after infection and analysed by ELISA and a serotype-specific rRT-PCR, that enabled identification and quantification of the grown viruses. To investigate the possible impact of virus strains, experiments were repeated using two different topotypes per each serotype, namely O/ME-SA/Ind-2001d with A/ASIA/Iran-05 and O/EA-3 with A/AFRICA/G-IV.

Results

The results of both serotype-specific Ag-ELISA and rRT-PCR showed that FMD viruses of serotype A have a better fitness than type O viruses when cultured in BHK-21 and IBRS-2 cell lines, while LFBK $\alpha\alpha\beta\beta_6$ cells allowed replication of the various co-infecting viruses without promoting one specific serotype. In this cell line the selection was only oriented versus one virus when its concentration was 100X compared to the other virus, suggesting that LFBK $\alpha\alpha\beta\beta_6$ cells supports the growth of both serotypes with similar efficiency.

Discussion

Our results corroborate previous observations that LFBK $\alpha\alpha\beta\beta_6$ are the preferable cell line for VI from field suspect samples, thanks to the speed of viral replication and to a wider susceptibility to various FMD viruses, with no predilection for a specific serotype. Conversely, BHK-21 and IBRS-2 cells are more susceptible to FMDV serotype A compared to O.

POSTER

USING HIGH THROUGHPUT SEQUENCING TO CHARACTERISE LOW-FREQUENCY DIVERSITY OF FMDV DURING VACCINE STRAIN ADAPTATION

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Introduction

RNA viruses such as foot-and-mouth disease virus (FMDV) exist as heterogeneous populations, with sequence diversity arising due to the large viral population size, high replicate rate and poor proof-reading ability of the viral RNA-dependent RNA polymerase. High throughput sequencing (HTS) technologies allows for the characterisation of low-frequency variants to investigate their importance in viral evolution. To assess how the population structure of FMDV changes during the adaptation process to culture in Baby Hamster Kidney (BHK) cells, two wild-type FMDV strains (Type A TUR/44/2011 and TUR/05/2012) and a clonally derived virus (O1Kaufbeuren) were sequenced.

Materials and Methods

Viral strains were grown in BHK cells (MOI: 0.01) for over 3 or 4 passage series. Total RNA was extracted using TRIzol (Thermo Fisher Scientific) and FMDV RNA copies were quantified using qRT-PCR. In duplicates, RNA was converted into cDNA and the capsid-encoding region amplified using high-fidelity polymerase enzymes. Amplified products were then sequenced on an Illumina MiSeq using the Nextera XT protocol. Raw data was trimmed by Sickle using a qScore of 30 and a read length of 70bp before being aligned to their respective reference sequences using BWA-MEM. The alignment allowed for the creation of consensus sequences, the prediction of low-frequency variants and the generation of Shannon entropy statistics.

Results

FMDV RNA yields for all samples were between 2.82×10^7 and 2.94×10^4 RNA copies/ μ l. Analysis of the viruses derived from the O1Kaufbeuren clone revealed an expansion of low-frequency non-synonymous variants over the passage series. In contrast, for wildtype derived viruses, the overall low-frequency diversity of TUR/44/2012 was high but remained stable following each passage, whilst the TUR/05/2012 dataset saw a moderate increase in diversity, until a consensus change in passage 3 resulted in stability of diversity in the subsequent passages.

Discussion

These findings will provide important insights into the swarm cloud development during the vaccine strain adaptation process for both wild type and colonially derived FMDV.

POSTER

IDENTIFICATION OF NOVEL ANTIBODY BINDING DETERMINANTS OF SEROTYPE O FMDV

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Introduction

Foot-and-mouth disease virus (FMDV) displays various epitopes on the capsid outer surface. Five neutralising antigenic sites have been identified in serotype O FMDV using murine monoclonal antibodies. In addition, there is evidence of the existence of other, yet unidentified epitopes, which are believed to play a role in antibody-mediated protection. However, the relative importance of different epitopes in FMD vaccine induced-protection has not been ascertained to date in great details. Attempts were made in this study to identify such epitopes using a reverse genetics approach.

Materials and methods

Using reverse genetics technique two recombinant viruses were generated that contained mutations at the five neutralising antigenic sites (5M) and two additional mutations (5M2/5). Serological characterisation of 5M and 5M2/5 viruses revealed 56% and 74% reduction in neutralising antibody titre indicating VP2-74 and VP2-191 having significant impact on the antigenic nature of the virus. Further the 5M2/5 virus was passaged 25 times on RS cells in the presence (5M2/5 P25+sera) or absence (5M2/5 P25) of a post-vaccinal serum with an aim to identify additional epitopes capable of reducing the neutralisation efficiency further.

Results

Capsid sequence analysis of the 5M2/5 P25+sera virus identified several nucleotide changes in the capsid coding region leading to amino acid substitutions at three positions. Serological characterisation of this virus in VN test revealed a further 15% reduction in VN titre which indicates that the virus can completely escape neutralisation if the specific mutations in capsid can be made.

Discussion

This study shows that impairment of the 5 antigenic sites of type O FMDV is not enough to achieve complete escape of virus neutralisation using bovine sera raised against the parent virus. Preliminary work on this resulted in further (~15%) reduction in VN titre indicating complete escape from neutralisation can be achieved which could identify capsid amino acid residues of antigenic significance.

POSTER

EVALUATION OF ENVIRONMENTAL SAMPLING AS A LOW TECHNOLOGY METHOD FOR SURVEILLANCE OF FMDV IN AN ENDEMIC AREA

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Introduction

Environmental sampling enables disease surveillance beyond regular investigation of clinical cases, extending data on the circulation of a pathogen in a specific area. Developing straightforward, low technology methods suitable for use in field conditions is key to the inclusion of such approaches alongside traditional surveillance techniques. Environmental contamination by foot-and-mouth disease virus (FMDV) in excretions and secretions from infected individuals promotes transmission, but also presents an opportunity for non-invasive sample collection, facilitating diagnostic and surveillance purposes.

Materials and Methods

Electrostatic dust cloths were used to collect environmental swabs at sites with reported outbreaks of FMDV, in the Kathmandu Valley, Nepal, which is endemic for FMD. A limited number of aerosol samples were also collected. A total of nine sites were visited and sampled between November 2016 and November 2017. Samples were stored in lysis buffer and transported to The Pirbright Institute, where an rRT-PCR assay was used to detect FMDV RNA.

Results

FMDV RNA was detected in environmental samples from premises with animals at all stages of clinical disease, from uninfected, suspected preclinical, clinical and recovering cattle. Categorising lesion ages as fresh (1-5 days), healing (6-10 days) and old (>10 days), there was a significantly higher proportion of positive samples for households with fresh lesions compared with those with old lesions ($P=0.02$).

Discussion

Development of methods that can reliably detect FMDV RNA in the environment is significant, as this extends the toolbox available for surveillance for this disease. Development of low technology, straightforward surveillance methods such as this can support a robust response to outbreaks. Pairing these methods with existing and novel diagnostic tests will improve capability for the rapid detection of outbreaks and implementation of timely interventions to control outbreaks. In endemic areas, these methods can be implemented to extend surveillance beyond the investigation of clinical cases, providing additional data to assess virus circulation in specific areas.

POSTER

EVALUATING THE EFFICENCY OF ENVIRONMENTAL SAMPLING METHODS FOR THE DETECTION AND QUANTIFICATION FMDV

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Introduction

Foot-and-mouth disease virus (FMDV) can be found in the breath, secretions and excretions from acutely infected animals and can survive outside of the animal host, implicating the environment as a potential transmission route. The objective of this study was to assess the efficiency of environmental sampling methods for the recovery and quantification of FMDV from the environment.

Materials and methods

Environmental swabs: the surfaces of a range of materials including wood, metal, plastic, glass, brick and rope were spiked with a range of concentrations (10¹- 10⁶ TCID₅₀) of FMDV O/UKG/34/2001. The virus was left to dry for ~30 minutes, then the surface was swabbed with an electrostatic dust cloth. Swab samples were then tested using qPCR and virus isolation to assess virus recovery from the surfaces.

Aerosol sampling: stocks of FMDV O/UKG/34/2001, A/TAI/17/2016, and ASIA1/SHAMIR/VV/2001 were aerosolized to validate the collection efficiency of the Coriolis micro air sampler (Bertin Technologies). FMDV was nebulised at concentrations of 10², 10⁴ and 10⁶ TCID₅₀ and at three distances from the sampler: 10cm, 75cm, and 150cm. Samples were tested for FMDV RNA and live virus by qPCR and virus isolation, respectively.

Results

Environmental swabs: FMDV RNA was detected from all surfaces at all concentrations, except for brick and wood where 10¹ was not recovered. Live virus was only recovered from plastic, wood, rope and brick when spiked with higher concentrations of virus (10⁵ and 10⁶ TCID₅₀). *Aerosol sampling:* all serotypes were detected in respective collected aerosol samples. For each serotype the proportion of virus recovered decreased as the distance between the nebuliser and sampler increased. The higher the starting concentration of virus the more efficient the recovery was from sampled aerosols.

Discussion

This study demonstrates that use of electrostatic dust cloths as swabs and the Coriolis air sampler are efficient methods for environmental sampling where FMDV may be present.

POSTER

DEVELOPMENT OF MASTER VACCINE SEEDS FOR FMD CONTROL IN SUB-SAHARAN AFRICA

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Introduction

Many FMDVs, particularly the South African Territories (SAT) serotypes, are thermally unstable and the viral capsid readily dissociates into non-immunogenic pentameric subunits, which can compromise the effectiveness of FMD vaccines. We have carried out stability screens of East African FMDV strains selected from banks of field viruses to identify candidates for new vaccine master seed stocks.

Materials and methods

The respective stability of each virus was determined using ELISA-based and thermofluor assays. Candidate strains for each serotype were cell adapted on BHK cells until clear CPE was observed. Cattle were vaccinated with inactivated virus prepared from candidate seedstock and the sera was used to perform cross neutralisation assays.

Results

The respective stabilities of 40 East African FMDV strains, belonging to the O, A, SAT1 and SAT2 serotypes, were determined. The least and most stable strains within each serotype were further analysed using accelerated stability assays. Differences of up to 7°C in stability were observed between strains belonging to the same serotype, such that the spectrum of stabilities for each serotype overlapped. Long-term storage (> 12 months) of inactivated viruses confirmed SAT1 strains to be the least stable. In neutralisation assays performed to date, O and SAT2 sera have exhibited lower levels of antigenic recognition to strains from the same serotype in comparison to A and SAT1 sera.

Discussion

This work demonstrates the range of thermal stabilities exhibited by East African FMDV strains belonging to four different serotypes and describes the use of stability analysis as a criterion to include in the selection of candidate FMDV seedstock.

POSTER

STABILIZING FACTORS ASSOCIATED WITH VACCINE ANTIGEN PRODUCTION USING KOREAN LOCAL STRAIN OF FMDV

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Introduction

Outbreak of type O foot-and-mouth disease (FMD) has occurred most frequently all over the world, and there is no exception in South Korea. In the situation of nationwide vaccination in South Korea since 2011, the local isolate, O/SKR/JC/2014, was suggested as a representative strain for domestic vaccine development. Among seven serotypes of foot-and-mouth disease virus (FMDV), types O and SAT2 are notorious for their structural instability. Although it was possible to produce fresh 146S antigens with O/SKR/JC/2014 more than $2\mu\text{g}/\text{ml}$, it was difficult to preserve the intact vaccine antigen for sufficient time. Herein, we aimed to explore several adjustments to produce and maintain intact vaccine antigens using O/SKR/JC/2014.

Materials and methods

Several combinations of media for suspension cell culture and virus propagation were compared in terms of viral titer and the amount of 146S particle. In addition, basal buffers for suspension of concentrated vaccine antigen were also compared. Viral titer was measured by end-point titration to determine the tissue culture infective dose 50 (TCID₅₀) and the amount of 146S particle was calculated by continuous UV spectrophotometry following sucrose gradient centrifugation according to the previous report (Doel et al.1981).

Results

Among combinations of media that resulted in high titers more than 5×10^7 TCID₅₀/ml, three combinations exhibited high stability when the O/SKR/JC/2014 seed viruses were stored at -70°C . In particular, purified 146S particles were the least degraded when they were suspended in potassium-based solution not only for 4°C maintenance, but also for -70°C storage, which should expose vaccine antigen to the degradation due to freeze-thaw cycle.

Discussion

Those exquisite adjustments could be usefully applied to other FMD vaccine strains in case the vaccine antigens are subject to fragility in the process of vaccine production or during storage in experimental temperature.

POSTER

ANTIGENIC PROPERTIES OF STABILIZED VIRUS PARTICLES FOR A FMD VACCINE

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Introduction

Foot-and-mouth disease (FMD) virus is easily inactivated at high temperatures and destroyed in acidic conditions. Hence, it is necessary to increase the stability of FMD virus (FMDV) for its use as an antigen to produce more stable vaccines. This study evaluated whether the single or combined substitution of a single amino acid in VP1 (N17D) or VP2 (H145Y) increases virus stability in seven FMDV serotypes.

Materials and methods

The used an infectious clone carrying the full genome of the O1 Manisa strain, which is the representative vaccine strain of FMD. It is also conducted experiments under acidic conditions to assess the stability of FMDVs carrying seven different P1 genes and the same NSP gene.

Results

The stabilities of the viruses of serotypes O, Asia1 and C, but not A, SAT1, SAT2, or SAT3, were enhanced compared with the parental virus. In the case of serotypes A, SAT1, SAT2, and SAT3, we found that substitution of 2–3 amino acids in the P1 region improves stability under stimulation that induces resistance and cell passage, revealing a novel resistance-related sequence. We injected an experimental vaccine that contained the antigen that persists under acidic conditions into mice, and we confirmed the protective capability of this antigen against challenge infection.

Discussion

To increase the acid resistance of FMDV, replacing specific amino acids may generally improve virus stability.

POSTER

CHIMERIC SAT2 FMDV WITH INCREASED CAPSID THERMOSTABILITY FOR IMPROVED VACCINES

A. Kotecha³, E. Perez-Martin¹, Y. Harvey¹, F. Zhang¹, S. Ilca³, E.E. Fry³, B. Jackson¹, F. Maree², K. Scott², C.W. Hecksel⁵, M.M Harmsen⁴, V. Mioulet¹, B. Wood¹, N. Juleff¹, D.I. Stuart^{3,5}, B. Charleston¹ and J. Seago¹ ¹ The Pirbright Institute, Woking, Surrey, GU24 0NF, United Kingdom; ² Transboundary Animal Disease Programme, ARC-Onderstepoort Veterinary Institute, Private Bag X05, Onderstepoort 0110, South Africa; ³ Division of Structural Biology, Wellcome Trust Centre for Human Genetics, University of Oxford, Roosevelt Drive Oxford OX3 7BN, United Kingdom; ⁴ Wageningen Bioveterinary Research, Division Virology, P.O. Box 65, 8200 AB Lelystad, The Netherlands; ⁵ Diamond Light Source, Harwell Science and Innovation Campus, Didcot OX11 0DE, UK.

Introduction

Many FMDVs, particularly the South African Territories (SAT) serotypes, are thermally unstable and the viral capsid readily dissociates into non-immunogenic pentameric subunits, which can compromise the effectiveness of FMD vaccines. Here we report the construction of a chimeric clone between the SAT2 and O serotypes, designed to have SAT2 antigenicity, but improved stability.

Materials and methods

An established reverse genetics workflow was used to produce recombinant chimeric SAT2 FMDVs. The respective stability of each virus was determined using ELISA-based and thermofluor assays. Cryo-EM analysis of inactivated recombinant FMDVs was performed to investigate capsid structures. Cattle were vaccinated with wild type and stabilised viruses following exposure to an elevated temperature and subsequent VNT assays were performed to determine the levels of neutralising antibodies generated.

Results

Characterisation of the chimeric virus showed growth kinetics equal to that of the wild type SAT2 virus with better thermostability, attributable to changes in the VP4 structural protein. Sequence and structural analyses confirmed that no changes from SAT2 were present elsewhere in the capsid. We show such thermostable SAT2 viruses can induce improved neutralising-antibody responses following the exposure of vaccine to an elevated temperature.

Discussion

This work highlights the potential benefit of vaccines with improved thermal stability and gives an insight into the viral components that influence stability.

POSTER

NEW CAGE-LIKE PARTICLE ADJUVANT INCREASED THE IMMUNOGENICITY AND THE PROTECTION INDUCED BY A VACCINE AGAINST FMDV A/ARG/2001.

J. Bidart^{1,2}, C. Kornuta^{1,2}, M. Gammella¹, C. Langellotti^{1,2}, R. Galarza³, L. Calvino⁴, V. Quattrocchi¹, I. Marcipar^{2,5}, P. Zamorano^{1,2,6}

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Introduction

Foot and Mouth Disease is an acute disease caused by Foot and Mouth Disease Virus (FMDV) which causes important economy losses, this is why it is necessary to obtain a vaccine with new and economic adjuvant that stimulates protective immune response. The murine model used predicts the FMD-vaccines' quality in cattle.

New cage-like particle or Immunostimulant Particle Adjuvant (ISPA) are lipid boxes formulated with dipalmitoyl-phosphatidylcholine, cholesterol, steralamine and QuilA.

Materials and Methods

BALB/c mice were immunized and at 21 dpv challenged with FMDV; cattle were immunized S.C. The methods used were: ELISA; seroneutralizing assay; lymphoproliferation and CD4+/CD8+ stained.

Results

Mice (n=5) were immunized subcutaneous with: inactivated FMDV (iFMDV); iFMDV-ISPA; Commercial vaccine; ISPA or PBS, at 21 dpv animals were challenged with infective FMDV. 100% of mice vaccinated with iFMDV-ISPA or commercial vaccine were protected and only 40% in iFMDV group. At this time, iFMDV-ISPA group presented FMDV-Ab titers: 5.06 ± 0.07 higher than iFMDV (3.8 ± 0.3) ($p < 0.05$) and similar than commercial vaccine (4.62 ± 0.02) group. A significant increase in the levels of Ab α -FMDV IgG1, IgG2a, IgG2b and IgG3 was detected in iFMDV-ISPA group with respect to FMDV group.

At 21 dpv, when splenocytes were stimulated with inactivated virus, iFMDV-ISPA group showed higher proliferation than iFMDV, ISPA or PBS groups ($p < 0.05$), and similar than commercial vaccine group. A slight increase was observed in CD4+/IFN α + population in iFMDV-ISPA and commercial vaccine groups compared to iFMDV.

Calves (n=4) were vaccinated with iFMDV or iFMDV-ISPA, at 30 dpv there was an increase in total Ab α -FMDV (3.7 ± 0.6) in iFMDV-ISPA calves, in comparison with iFMDV group ($p < 0.05$). SNT were 1.8 ± 0.3 (correlated with Percentage Expectation of Protection (PEP), higher than 80%) and 1.02 ± 0.02 in iFMDV-ISPA calves and iFMDV respectively ($p < 0.05$). When Bovine-DCs were incubated with ISPA, an increase of CD40 and IL6 expression was detected (preliminary results).

Discussion

In mice, the inclusion of ISPA in an FMD-vaccine induced an increase in humoral and cellular immunity, and a better protection against viral challenge. In cattle, the antibodies against FMDV are linked to PEP higher than 80%.

POSTER

DEVELOPMENT OF MASTER VACCINE SEEDS FOR FMD CONTROL IN SUB-SAHARAN AFRICA

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Introduction

Many FMDVs, particularly the South African Territories (SAT) serotypes, are thermally unstable and the viral capsid readily dissociates into non-immunogenic pentameric subunits, which can compromise the effectiveness of FMD vaccines. We have carried out stability screens of East African FMDV strains selected from banks of field viruses to identify candidates for new vaccine master seed stocks.

Materials and methods

The respective stability of each virus was determined using ELISA-based and thermofluor assays. Candidate strains for each serotype were cell adapted on BHK cells until clear CPE was observed. Cattle were vaccinated with inactivated virus prepared from candidate seedstock and the sera was used to perform cross neutralisation assays.

Results

The respective stabilities of 40 East African FMDV strains, belonging to the O, A, SAT1 and SAT2 serotypes, were determined. The least and most stable strains within each serotype were further analysed using accelerated stability assays. Differences of up to 7°C in stability were observed between strains belonging to the same serotype, such that the spectrum of stabilities for each serotype overlapped. Long-term storage (> 12 months) of inactivated viruses confirmed SAT1 strains to be the least stable. In neutralisation assays performed to date, O and SAT2 sera have exhibited lower levels of antigenic recognition to strains from the same serotype in comparison to A and SAT1 sera.

Discussion

This work demonstrates the range of thermal stabilities exhibited by East African FMDV strains belonging to four different serotypes and describes the use of stability analysis as a criterion to include in the selection of candidate FMDV seedstock.

POSTER

STABILIZING FACTORS ASSOCIATED WITH VACCINE ANTIGEN PRODUCTION USING KOREAN LOCAL STRAIN OF FMDV

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Introduction

Outbreak of type O foot-and-mouth disease (FMD) has occurred most frequently all over the world, and there is no exception in South Korea. In the situation of nationwide vaccination in South Korea since 2011, the local isolate, O/SKR/JC/2014, was suggested as a representative strain for domestic vaccine development. Among seven serotypes of foot-and-mouth disease virus (FMDV), types O and SAT2 are notorious for their structural instability. Although it was possible to produce fresh 146S antigens with O/SKR/JC/2014 more than $2\mu\text{g/ml}$, it was difficult to preserve the intact vaccine antigen for sufficient time. Herein, we aimed to explore several adjustments to produce and maintain intact vaccine antigens using O/SKR/JC/2014.

Materials and methods

Several combinations of media for suspension cell culture and virus propagation were compared in terms of viral titer and the amount of 146S particle. In addition, basal buffers for suspension of concentrated vaccine antigen were also compared. Viral titer was measured by end-point titration to determine the tissue culture infective dose 50 (TCID₅₀) and the amount of 146S particle was calculated by continuous UV spectrophotometry following sucrose gradient centrifugation according to the previous report (Doel et al.1981).

Results

Among combinations of media that resulted in high titers more than 5×10^7 TCID₅₀/ml, three combinations exhibited high stability when the O/SKR/JC/2014 seed viruses were stored at -70°C . In particular, purified 146S particles was the least degraded when they were suspended in potassium-based solution not only for 4°C maintenance, but also for -70°C storage, which should expose vaccine antigen to the degradation due to freeze-thaw cycle.

Discussion

Those exquisite adjustments could be usefully applied to other FMD vaccine strains in case the vaccine antigens are subject to fragility in the process of vaccine production or during storage in experimental temperature.

POSTER

ANTIGENIC PROPERTIES OF STABILIZED VIRUS PARTICLES FOR A FMD VACCINE

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Introduction

Foot-and-mouth disease (FMD) virus is easily inactivated at high temperatures and destroyed in acidic conditions. Hence, it is necessary to increase the stability of FMD virus (FMDV) for its use as an antigen to produce more stable vaccines. This study evaluated whether the single or combined substitution of a single amino acid in VP1 (N17D) or VP2 (H145Y) increases virus stability in seven FMDV serotypes.

Materials and methods

The used an infectious clone carrying the full genome of the O1 Manisa strain, which is the representative vaccine strain of FMD. It is also conducted experiments under acidic conditions to assess the stability of FMDVs carrying seven different P1 genes and the same NSP gene.

Results

The stabilities of the viruses of serotypes O, Asia1 and C, but not A, SAT1, SAT2, or SAT3, were enhanced compared with the parental virus. In the case of serotypes A, SAT1, SAT2, and SAT3, we found that substitution of 2–3 amino acids in the P1 region improves stability under stimulation that induces resistance and cell passage, revealing a novel resistance-related sequence. We injected an experimental vaccine that contained the antigen that persists under acidic conditions into mice, and we confirmed the protective capability of this antigen against challenge infection.

Discussion

To increase the acid resistance of FMDV, replacing specific amino acids may generally improve virus stability.

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Materials and Methods

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Results

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POSTER

POST-VACCINATION MONITORING OF TRIVALENT FMD VACCINE CONTAINING O1 MANISA, O3039, A22 IRAQ TO EVALUATE VACCINE EFFECTIVENESS IN SMALL SCALE FIELD TRIALS

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Introduction

In 2018, Foot-and-Mouth disease viruses of the A/ASIA/SEA-97 lineage emerged in pigs in South Korea. A factor which has contributed to outbreak of this virus in pigs was unvaccinated with serotype A. Because of outbreaks of FMDV, mostly of serotype O, type-O vaccination of pigs is obligatory. Therefore, emergency vaccine containing A22-IRAQ was administered in pigs during and around the time of an outbreak in March, 2018. The aim of this study was to evaluate the immune response of emergency vaccination in field farm against vaccine strains as well as a field strain from A/SKR/5/2018 which was isolated from Gimpo in South Korea to assess vaccine effectiveness in field trials.

Materials and methods

In general, it is useful to test using ELISA Kit for assessing large-scale serological immune response, however the sensitivity of commercial ELISA kit was found to be limited by species. Therefore, small-scale farm (National Institute of Animal Science, NIAS) was purposely selected that had accurate individual vaccine history information to assess vaccine-induced virus neutralization (VN) titres to the vaccine and field strains. 25 pigs vaccinated twice 4 weeks interval bleed every week after prime vaccination up to 8 weeks.

Results

The VN-titres of all pigs from NIAS achieved a sufficiently high level against vaccine virus, A22-IRAQ and field virus, A/SKR/5/2018 respectively in 2 weeks and 4 weeks after prime vaccination, and remained high up to 8 weeks.

Discussion

Previously, vaccine matching studies carried out by the World Reference Laboratory, Pirbright indicated that the r_1 value was 0.43 based on VNT. In this study, we showed that emergency vaccination using inactivated vaccine with high $r_1 (>0.3)$ value with the field strain provided high VN-titers, which may be justifiable as protection in pigs. In addition, monitoring post-vaccination serology is an important component of evaluation for FMD vaccination programs.

POSTER

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

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FMD viruses are usually confined to specific geographical regions and spread to new areas may lead to significant epidemics. In Zambia, the disease is endemic around the three known high risk areas. However, in 2015 an outbreak outside the traditional endemic areas involving Western Zambia attributed to SAT 3 was reported. A quiescence of the disease followed from May 2016 until April 2017. Precipitating factors behind the epidemiology change of disease in this region is unknown. Herein we describe investigations on latest disease foci in Lukulu District which historically has never recorded FMD.

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

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

POSTER

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

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Introduction

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

Materials and methods

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

Results

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

Discussion

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

POSTER

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

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Introduction

Foot-and-mouth disease (FMD) is an endemic transboundary animal disease that affects livestock health across sub-Saharan Africa. Since the first official report of FMD in Nigeria in 1924, serotypes O, A, SAT 1 and SAT 2 have been documented in Nigeria (Fasina et al, 2013; Lazarus et al., 2012; WRLFMD 2010; WRLFMD 2014). These studies were to characterize the FMD viruses circulating in Nigeria from 2007-2014 and to antigenically match the isolates for the development of polyvalent indigenous vaccine, and also to assist policy makers with decisions for effective disease control.

Materials and methods

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

Results

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

Discussion

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

POSTER

FMDV ADSORBED TO GENOTUBE SWABS REMAINS INFECTIOUS AT HIGH TEMPERATURE

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Introduction

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

Materials and Methods

GenoTubes were dipped in FMDV A IRN/8/2015, dried and then incubated for 2 hours in a forced-air oven preheated to 100°C. A second set was kept at room temperature. To assess the time required for heat transfer to the swab, a thermocouple was placed inside a sealed swab tube that was also placed in the oven. After heating, the foam tip of each swab was washed with culture media. The eluate was titrated on LFBK- α V α 6 cells. Cytopathic effect on the titration plates was evaluated microscopically and confirmed by antigen ELISA.

Results

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

Discussion

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

POSTER

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

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Introduction

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

Methods

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

Results

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

Discussion

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

POSTER

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

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Introduction

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

Materials and methods

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

Results

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

Discussion

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

POSTER

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

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Introduction

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

Materials and Methods

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

Results and discussion

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

POSTER

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

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Introduction

South Korea has been suffered foot-and-mouth disease (FMD) epidemics since 2000. One of the major changes in FMD control policy in last decade was introduction of FMD vaccines. All susceptible animals are subjected to FMD vaccination. Thus, both SP and NSP antibody detecting assays are performed as a part of National FMD sero-surveillance program. Here we described the production of swine sera panel to validate FMD serological assays and characterized its reactivity in NSP Ab assays.

Materials and methods

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

Results

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

Conclusion

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

POSTER

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

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Introduction

Vaccination is one of the most important interventions in foot-and-mouth disease virus (FMDV) outbreak prevention and control. Antigenic matching between vaccine and outbreak virus is critical for vaccination effectiveness and is usually determined using serological assays, such as virus neutralisation tests (VNTs). The ratio between the titer of vaccine serum against a field virus and the titer of vaccine serum against the vaccine virus (r_1) is calculated. An r_1 value <0.3 is supposed to be predictive of a vaccine mismatch. However, while the r_1 value is generally used as indicator for protection, its reliability is limited, it has high variance and substantial variation between laboratories exists. Currently, there is no reliable in vitro alternative for r_1 . Therefore, there is a need to optimize the current assay as well as develop novel assays.

Materials and methods

At Boehringer Ingelheim Animal Health an r_1 VNT protocol had been developed using publicly available IBRS-2 cells (FLI), and reagents and disposables which are commercially available to enable assay harmonisation between laboratories. This assay has been validated and the intrinsic test variance was determined. Thereby the effect of repeating the assay and performing the assay in 1D or 2D was examined.

Results

This VNT has a low variance, but r_1 variance is still high. Repeating the assay increased confidence and use of strict cell culture protocols did reduce the assay variance. Performing the assay in 1D instead of 2D gives similar variance, while enabling the performance of repeats.

Discussion

These studies have led to more insight in r_1 variability and to what extent it can be reduced. A VNT protocol for FMDV antigenic matching has been created, enabling assay harmonisation between laboratories. This assay could contribute to more reliable vaccine matching, however, research on novel assays enabling more predictable vaccine matching is still required.

POSTER

USE OF SEROLOGICAL TESTS FOR CHECKING NSP PURITY OF FMD VACCINES

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Introduction

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

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

Materials and Methods

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

Results

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

Discussion

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

POSTER

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

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Introduction

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

Materials and methods

In this study, the protection levels (r_1 value) of four vaccine strains of the serotype A produced by FMD Institute were investigated. The vaccine strains were belonging to A/Asia/GVII lineage (ANep84, ATUR16, ATUR17) and A/Asia/IRN05 (ATUR06). Results were obtained by using in vitro and in vivo methods; VNT for r_1 value and challenge studies, respectively. In challenge studies 42 cattle were used.

Results

There were poor antigenic relationship detected between A/Asia/IRN05ATUR06-A/Asia/GVI-ANep84 and A/Asia/GVIIATUR17-A/Asia/GVIIATUR16 in vitro. A/Asia/GVIIATUR16-A/Asia/GVIIATUR17 r_1 value was detected as almost 0,3 (0,27) and the rest of four tests were showed protection (r_1 -values $\geq 0,3$). After challenge, lesions were occurred on tongue (inoculation site) in all animals. Most severe clinical signs including back hoof were seen in the challenge study with A/Asia/IRN05ATUR06. Lesions on the palate were seen on the A/Asia/GVIIATUR17 challenge study, finally there were no lesions occurred except inoculation sites on the other three challenge studies which A/Asia/GVIANep84 and A/Asia/GVIIATUR16 were used.

Discussion

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

POSTER

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

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Introduction

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

Material and methods

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

Results

An improved system for immediate as well as monthly reporting of the FMD outbreaks in the Transcaucasus and neighbouring territories has been developed through an improved on-line system and mapping tool. A new online system for collection and sharing the monthly reporting of the level of implementation of the vaccination programmes, with improved visualisation mapping tool has been developed;

Countries have agreed to collaborate for the development of a risk mapping system that can utilise national data on live animals values, market activities and known animal movement patterns. Countries have participated in a simulation exercises for testing the emergency preparedness, improved their capacity to monitor effectiveness of vaccine and vaccination programmes (through workshops, e-learning) and agreed to share results on immunogenicity studies and post vaccination serosurvey.

Discussion

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

POSTER

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

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Introduction

In the spring of 2017, a new introduction of FMDV serotype A occurred in the Maghreb region (Algeria and Tunisia). These were the first reports of field outbreaks of FMD due to serotype A in these countries after >25 years. Here, we report the whole genome sequence of viruses recovered during the outbreaks in Algeria and phylogenetic analysis, based on the VP1 coding region, highlighting connections with countries in sub-Saharan Africa.

Methods and Results

A total of six samples originated from three different outbreaks in Algeria were analyzed, FMDV serotype A was detected by Ag-ELISA. Virus isolates from all three outbreaks were obtained after the first passage in LFBK- α V α 6 cells, which showed a better performance for virus isolation compared to BHK-21 and IB-RS2 cells. The phylogenetic analysis of the VP1 coding sequences grouped them within the A/AFRICA/G-IV lineage, most closely related to sequences originating from Nigeria in 2015, sharing more than 98% nt identity; older FMDV sequences (2009-2013) from Nigeria had lower nt identities (86.3-94.2%). FMDV sequences from countries in West Africa (Cameroon, Togo, Mali) and North-East Africa (Egypt, Eritrea, Sudan) were found to be more distantly related with nt identities ranging between 84.2- 90.2% and 82.8-87.8%, respectively. One complete full genome sequence (8119 nt) and two near-complete sequences (7616 and 7627 nt) were obtained from the three isolates by Miseq Illumina platform. Sequences differed at only 27 nt sites, 19/24 located within the polyprotein-coding region were synonymous and five non-synonymous.

Discussion

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

POSTER

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

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Introduction

Foot and Mouth Disease (FMD) causes significant economic loss in Lao PDR and perpetuates the cycle of smallholder poverty through reduction in animal production and limitations to market access for trading in livestock and their products. To determine the role of goats in the epidemiology of FMD in Lao PDR, we used a cross-sectional sero-prevalence study that identified antibodies to the non-structural proteins (NSP), an indication of previous infection, and structural proteins (SP) that could be due to vaccination or infection.

Materials and methods

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

Results

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

Discussion

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

POSTER

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

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Introduction

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

Materials and Methods

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

Results

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

Discussion

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

POSTER

CLINICAL SENSITIVITY OF CATTLE, SHEEP AND GOATS TO DIFFERENT SERO- TYPES OF FMDV IN CENTRAL REGION OF IRAN

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

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

The objective of this study was to investigate the relative occurrence of different FMD sero- types in large and small ruminants. In this period, 3993 outbreaks (3462 bovine and 531 ovine) were reported. There were re- ported 7 major surges in FMD outbreak reporting due to new strains of FMD virus (A Iran 05/ AG-VII and O PanAsia2 Qom-15). 964 outbreaks randomly sampled and there were reported 475 O serotypes, 362 A serotypes and 127 Asia1 serotypes.

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

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

Our data showed that FMD outbreaks due to O serotype occurred in cattle and sheep at the same time in one epidemiological unit but outbreaks due to A and Asia 1 serotypes occurred only in cattle. This study underpins that sheep and goats have a different clinical sensitivity to various FMD serotypes compared with cattle. This finding is important in prevention and control strategy of FMD.

POSTER

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

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Introduction

The West African territories are considered as regions with continuous FMDV circulation where outbreaks of FMDV serotypes O, A, SAT1 and SAT2 have been reported. An early diagnosis of FMD is crucial to implement adequate outbreak management. This study describes the development of a multiplex conventional RT-PCR for both detection and typing of FMD virus circulating in this region, and its evaluation on panels of field samples.

Materials-methods

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

Results

The 6-plex prototype detected all FMDV strains tested and identified the four serotypes of interest (O/A/SAT1/SAT2) without any improper amplification. Using this multiplex protocol, 37 samples from Benin were positive for the 3D target and were correctly serotyped by 6-plex (33) or by 3-plex and simplex RT-PCR (4). 39/40 isolates from Nigeria were properly serotyped using 6-plex (30) or simplex (9). The corresponding field samples as well 39 clinical positive samples from Senegal are under investigation.

Discussion

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

POSTER

A MODIFIED DENDRIMER-RNA VACCINE PLATFORM AGAINST FMD

J.S. Chahal Tiba Biotech LLC

Abstract

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

POSTER

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

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Introduction

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

Material and methods

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

Results

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

Discussion

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

POSTER

GENETIC CHARACTERIZATION OF THE 2018 FMD VIRUSES IN SOUTH KOREA

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Introduction

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

Materials and methods

Viral RNAs were extracted from nasal epithelium and vesicular fluid samples using a Magna- Pure96 system (Roche). The VP1 region was amplified using a one-step RT-PCR kit (Qiagen) and then purified with ExoSAP-IT (USB) and directly sequenced on an ABI3130 genetic analyzer (Applied Biosystems). Phylogenetic tree of the VP1 (639bp) estimated using the neighbor-joining method in MEGA-6. For the complete genomes sequence, we designed pairs of primers to produce 20 overlapping amplicons spanning the entire viral genome. Sequence analyses were performed using SeqMan Pro (DNASTar Lasergene, USA).

Results

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

Discussion

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

POSTER

INVESTIGATING CROSS REACTIVITY OF SEROLOGICAL ENZYME LINKED IMMUNOSORBENT ASSAYS

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Introduction

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

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

Method and Results

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

Discussion

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

POSTER

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

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Introduction

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

Materials and methods

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

Results

This is the first time a recombinant antibody phage display library derived from the immune repertoire of FMD–SAT immune buffalo has been constructed. The total library size was 3.84×10^7 cfu. Virus-specific binders will be selected and characterised and their use in the development of improved diagnostic assays investigated.

Discussion

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

POSTER

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

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Introduction

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

Materials and methods

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

Results

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

Conclusion

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

POSTER

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

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Introduction

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

Methods

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

Results

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

Discussion

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

POSTER

CHARACTERISATION OF BOVINE DENDRITIC CELLS FOLLOWING FMDV INFECTION

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Introduction

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

Material and methods

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

Results

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

Discussion

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

POSTER

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

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Introduction

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

Materials and Methods

The European Commission for the Control of Foot-and-Mouth Disease (EuFMD) has implemented an innovative needs-based training program which targets both FMD free and non-free countries. The program has been designed following an ongoing needs assessment process and has involved

APPENDIX 4: GLOBAL STATUS REPORT FOR FMD: TRACKING THE EMERGENCE AND SPREAD OF NEW VIRAL LINEAGES

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Introduction

Training has been delivered through a variety of methodologies including online courses, workshops and innovative field based training courses. Online training has reached over 5500 participants from over 50 countries and in nine languages to date.

Discussion

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

Data from the OIE/FAO FMD Laboratory Network (www.foot-and-mouth.org/) are used to monitor the transboundary movements of FMDV, and to provide recommendations about the suitability of vaccine strains that can be used to control outbreaks. In the past twelve months, particular attention has focussed on the emergence of new FMDV lineages into a number of countries in the European neighbourhood. Recent outbreaks in Algeria have been caused by two different FMDV topotypes (A/AFRICA/G-IV in 2017 and O/EA-3 in 2018). In contrast to previous cases in North Africa due to the O/ME-SA/Ind-2001 lineage originating from South Asia, phylogenetic analyses place West Africa as the source of both of lineages (closest viral sequences are from Nigeria); however, without obvious direct epidemiological connections, we should be cautious in attributing specific sources since there are many countries in West and Central Africa that do not submit samples for analyses. New emerging FMD lineages have also been detected in Israel and Palestine, where O/EA-3 has also been detected, as well as cases in Israel due to the A/ASIA/G-VII lineage (previously found in Iran, Turkey, Saudi Arabia and Armenia). Elsewhere, key epidemiological events highlighted during 2017-18 by the Network include (i) detection of a new FMDV lineage (within the O/ME-SA topotype) causing outbreaks in Bashkortostan, Russia, (ii) tracking of the spread of the A/ASIA/Sea-97 and O/ME-SA/Ind-2001 lineages in East Asian countries (including South Korea), and (iii) characterisation of outbreaks due to serotype Asia 1 in Myanmar; the first cases due to this serotype anywhere in Southeast Asia since 2008.

The emergence and circulation of novel strains within endemic settings inevitably heightens the risk to Europe via global trade and movement of people. Together, these unexpected events highlight the ease by which FMDV can cross international boundaries and emphasize the importance of the work undertaken by OIE/FAO FMD Laboratory Network to continuously monitor the global epidemiology of FMD.

APPENDIX 5: MODELLING FMD VACCINE REQUIREMENTS FOR MULTI-COUNTRY FMD OUTBREAKS IN EUROPE

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Introduction

Disease models are increasingly being used to support disease planning and management in many countries. With globalization, growing trade and increased people movements between countries, there is an increasing focus on studying disease control at a regional scale. This is especially important for Europe where there is the relatively free movement between Europe Union member states. EuFMD is supporting a European multi-country modelling project that is developing a decision support tool (the European FMD Spread – EuFMDiS – model) to simulate spread and control of FMD within and between participating countries. Given increasing interest in vaccination as a primary control tool for FMD in previously free countries, a key application of EuFMDiS will be to support vaccination policy development.

Materials and methods

EuFMDiS is based on a hybrid modelling approach as used in the Australian FMD model - AADIS (Bradhurst et al. 2015). FMD transmission within herds is simulated using equation- based modelling (EBM) and transmission between herds is simulated using agent based modelling (ABM). Disease control is based on the measures described in the European FMD directive (2003). Initial development of EuFMDiS has involved seven central European countries (Austria, Bulgaria, Croatia, Italy, Hungary, Romania and Slovenia).

Results

In this presentation we will show how EuFMDiS can be used to compare different approaches to FMD control and quantify vaccine requirements at both national and regional scales.

Discussion

Vaccination is increasingly being recognized as an important tool to assist in containing and eradicating FMD outbreaks. However, there is considerable uncertainty about how and when vaccination should be used. Of particular concern to European disease managers is whether current emergency vaccination arrangements would provide access to sufficient doses of vaccine in a multi-country outbreak.

References

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APPENDIX 6: EVALUATING VACCINATION STRATEGIES TO CONTROL FMD: A COUNTRY COMPARISON STUDY

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Introduction

Vaccination is increasingly being recognised as a potential tool to supplement ‘stamping out’ for controlling foot-and-mouth disease (FMD) outbreaks in non-endemic countries. Infectious disease simulation models provide the opportunity to determine how vaccination might be used in the face of an FMD outbreak. Previously, consistent relative benefits of specific vaccination strategies across different FMD simulation modelling platforms have been demonstrated, using a United Kingdom FMD outbreak scenario. We extended this work to assess the relative effectiveness of selected vaccination strategies in five countries: Australia, New Zealand, the United States, the United Kingdom and Canada.

Materials and Methods

A comparable, but not identical, FMD outbreak scenario was developed for each country with initial seeding of Pan Asia type O FMD virus into an area with a relatively high density of livestock farms. A series of vaccination strategies (in addition to stamping out) were selected to evaluate key areas of interest from a disease response perspective, including: timing of vaccination, species considerations (e.g. vaccination of only those farms with cattle), risk area vaccination, and resources available for vaccination.

Results

The study found that vaccination used with stamping out was effective in reducing epidemic size and duration in a severe outbreak situation. Early vaccination and unconstrained resources for vaccination consistently outperformed other strategies. Vaccination of only those farms with cattle produced comparable results, with some countries demonstrating that this could be as effective as all species vaccination. Restriction of vaccination to higher risk areas was less effective than other strategies.

Discussion

This study demonstrated consistency in the relative effectiveness of selected vaccination strategies under different start up conditions. We conclude that the preferred approach to FMD control depends on clearly defining outbreak management objectives, while having a good understanding of logistic requirements, and the socio-economic implications of different measures.

APPENDIX 7: UNDERSTANDING VACCINE DEMAND IN THE ENDEMIC SETTING

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Introduction

Vaccination is an essential tool for reducing the incidence of FMD in endemic settings. As global FMD control progresses and export markets between endemic countries emerge, a major challenge is securing access to adequate quantities of effective vaccines to meet routine and emergency demand. A theoretical market exists for FMD control, but numerous supply and demand barriers prevent this market from functioning. Understanding the scale of this unmet demand in the endemic setting is an essential step towards addressing these barriers and achieving global vaccine security.

Materials and Methods

A review of the drivers for vaccine demand and uptake at the household level was undertaken, and the scale of demand for FMD vaccines was estimated through a semi-quantitative analysis incorporating national livestock data, expert elicitation and the forecast progress of countries through the Progressive Control Pathway for FMD (PCP-FMD).

Results

The demand for FMD vaccination in Africa, Mid-East and Asia was estimated under different scenarios of progression in the PCP-Pathway, and under different scenarios for access to vaccines under public and private purchase, and relating to willingness to pay for vaccination by different sectors. The demand for FMD vaccine is projected to rise sharply in the coming years if countries are to meet their forecast progression through stages of the PCP-FMD, and meeting this demand is likely to occur only if there is a similar growth in production capacities for FMD vaccines.

Discussion

In many settings, national veterinary services have very limited budgets for control of epizootic diseases and these budgets are increasingly focused upon public health concerns. In contrast livestock owners, particularly of cattle, may have the economic means to afford vaccination and represent a large potential market. Barriers to the functioning of this market include public policies on vaccine delivery by the private sector and availability of effective, registered vaccines. Lack of global FMD vaccine security affects everyone, and addressing this issue requires innovative thinking to create an enabling environment between public and private sectors to increase access to quality vaccines. A shift in the vaccine stewardship paradigm is required, from the traditional top-down public sector oversight of vaccine stocks to one of public-private collaboration for enhanced end-user access to vaccines.

APPENDIX 8: HOUSEHOLD PERCEPTIONS OF RISK AS DRIVERS FOR ADOPTION OF FMD VACCINATION

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Introduction

FMD is endemic in northern Tanzania and the use of vaccines is limited creating uncertainty towards the benefits of vaccination relative to potential risks. Identifying how perceptions of risk affect potential adoption of FMD vaccination is important to evaluating how to increase vaccine uptake in the presence of uncertainty.

Materials and Methods

We employed a cross-sectional survey on 489 households in northern Tanzania using a double-bounded contingent valuation method with a maximum likelihood estimator to assess willingness to pay and adoption of two vaccination scenarios, a routine vaccination applied biannually, and an emergency applied in reaction to a nearby outbreak. Uncertainty and sensitivity to changes in risk are measured by randomly assigning a vaccine efficacy (50 or 100 percent) to households and conditioning the emergency vaccination on outbreak distance. We then compared perceived changes in risk between the two scenarios for response consistency.

Results

Households place a higher value on vaccination as perceived risk and household income increase, but the immediacy of FMD in an emergency scenario increases decision uncertainty (emergency 95 percent CI: USD 2.35-2.80; routine 95 percent CI: USD 1.70-2.04). Male head of households that received the vaccine of 50 percent efficacy would pay less than other head of households for both scenarios. An outbreak with a neighbour compared to an outbreak at the village level presented no difference in the value of vaccination.

Discussion

Households understand the risk of FMD and accurately valued vaccination relative to risk. However, concerns regarding the performance of the vaccine underlie decisions for both routine and emergency vaccination indicating a need for improved vaccine information. Increased perceptions of risk can enhance the value of vaccination but concerns for the performance of vaccines will continue to undermine uptake unless resolved with improved vaccine information.

APPENDIX 9: MASS FMD VACCINATION IN CENTRAL MYANMAR, 2015-2016

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Introduction

Central Myanmar is considered as a major source of large ruminants being traded across mainland Southeast Asia. To reduce FMD prevalence in the key supply market and the following risk of disease transmission during animal movements, a large-scale vaccination project was implemented in Central Myanmar since 2015 under the support of the OIE SEACFMD Campaign.

Materials and Methods

18 townships in Central Myanmar were included in the mass vaccination campaign, based on the susceptible animal estimates, animal movement information, and the socioeconomic importance. Three rounds of vaccination were carried out in February and March of 2015, and February 2016, respectively, using a high potency ($\geq 6\text{PD}_{50}$) vaccine comprising O1/Manisa and O/3039. Serum samples were collected from a cohort of initially FMD naïve cattle at 0, 30 and 180 days post the 2nd vaccination and tested by LP ELISA to evaluate the magnitude and longevity of the vaccine-induced immunity.

Results

Approximately 210,000 animals in up to 1,100 villages, belonging to more than 54,000 owners, have received 3 injections of FMD vaccine during the 2015-2016 vaccination campaign in Central Myanmar. A vaccination coverage rate of 78% in the project areas was estimated through a participatory approach.

The PVM study shows that at 30 days post the 2nd vaccination, 86% of cattle had protective antibodies (defined as titer $>1:100$) against a viral strain that is antigenically equivalent to O/3039. The protective percentage declined to 44% at 180 days post the 2nd vaccination. Field surveillance shows that despite extensive outbreaks occurred in Myanmar from August to October of 2015, few outbreaks occurred in the vaccinated villages.

Discussion

Vaccination programmes for resident livestock populations can provide protection against FMD but it consumes significant resources. To improve the cost-benefit of the vaccination campaign, development and application of animal movement protocols based on agreed sanitary standards should be an essential complement to the vaccination programme.

APPENDIX 10: ASSESSMENT OF THE RISK OF INCURSION OF EXOTIC FMD VIRUSES INTO SOUTHEAST ASIA

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Introduction

The South-East Asia and China Food and Mouth Disease (SEACFMD) campaign recognised that foot and mouth disease viruses (FMDVs) circulating in other regions could pose serious risks to its members. This study assessed the risk of incursion of exotic FMDVs into Southeast Asia (SEA).

Materials and methods

A qualitative risk assessment was conducted according to the World Organisation for Animal Health (OIE) framework. The outcome of interest was the exposure of susceptible livestock to exotic FMDV. Data were gathered from site visits, published studies, grey literature and expert opinion. The findings were validated at a regional workshop.

Results

Ten release and six exposure pathways were characterized. Overall, the likelihood of future incursions was assessed as high. The pathways involving imports of live animals and animal products from FMD-endemic countries in neighbouring regions had the highest likelihood. Surprisingly, even FMD-free countries allow these types of imports.

An incursion would likely have a negative impact on animal health and welfare and, in some cases, valuable trading markets would be jeopardised. An exotic FMDV would likely spread extensively within SEA due to intense intra-regional livestock trade, weak surveillance and lack of well-integrated and risk-based national FMD strategies.

Discussion

Our study indicates that further incursions of exotic FMDV to SEA is not a matter of 'if' but 'when'. A risk-based approach involving public and private stakeholders at regional and national levels is recommended to reduce this risk.

Key words

Risk assessment, exotic FMD viruses, risk pathways

Acknowledgements

Southeast Asian sub-regional office of the World Organisation for Animal Health and Welfare, Australia's Department of Foreign Affairs and Trade, Australia's Department of Agriculture and Water Resources.

APPENDIX 11: VACCINE BANKS: POLICY OPTIONS EVALUATED USING THE EU EVALUATION FRAMEWORK

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Introduction

To ensure access to suitable vaccines vaccine banks are implemented both at EU level as well in a number of individual Member States within the EU. Policy makers are confronted with the decisions which option to choose: 1) participate in the EU vaccine bank or 2) besides participating in the EU vaccine bank also establish a national vaccine bank.

The objective of this paper is to discuss socio-economic aspects related to that decision and to present an approach that can support the decision making process.

Methods

The intervention logic which defines its general objective, specific objectives, inputs, outputs or results, and desired impacts is presented. It indicates how specific inputs are expected to contribute to specific outputs, which in turn create impacts and leading to the achievement of general and specific objectives.

The evaluation will use five evaluation criteria specified for evaluation of EU-funded programmes:

- *Relevance: The extent to which an intervention's objectives are pertinent to needs, problems and issues.*
- *Value added: The value resulting from applying policy measures at the different levels, the value that would have resulted from applying similar measures at regional or national level or EU by public authorities or the private sector.*
- *Effectiveness: The extent to which objectives pursued by an intervention are achieved.*
- *Efficiency: Best relationship between resources employed and results achieved in pursuing a given objective through an intervention.*
- *Coherence: The extent to which the intervention does have synergy and does not contradict other interventions with similar objectives.*

Results and discussion

The framework presented can help decision makers in a systematic evaluation of socio-economic aspects related to establishing EU and national vaccine banks.

APPENDIX 12: THE CHALLENGES OF FMD VACCINE PRODUCTION

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Producing potent and safe FMD vaccine in a reliable manner at industrial scale is complex and challenging, for several reasons: *First*, production needs to take place in a dedicated, high-containment factory. *Second*, FMD vaccine is notoriously difficult to produce and the manufacturer needs to master the necessary know-how. Variable antigen yields, inherent fragility of viral capsids drive up the cost of vaccine. Quality control is also difficult, since there is no standardized international standard for batch potency testing. Quality relies on balance between several factors: antigenic payloads, raw materials, manufacturing process and adjuvant. As a result there is a wide range of vaccine qualities on the market. *Third*, there are specific R&D challenges all along the pathway of development of FMD vaccines: sourcing of isolates, variability of yields, differences between strains, very limited access to animal facilities, unpredictability of vaccine matching profile, and virus evolution in the field that force manufacturers to maintain the capacity to develop new strains regularly. *Fourth*, registration of FMD vaccines is complicated by the multiplicity of strains and combinations thereof. As of today, the EU multistrain approach is not accepted widely beyond European borders. *Finally*, supply chain of FMD vaccines is also specific, because of constant competition between regular, predictable demand and sudden surges due to unpredictable outbreaks. Sales forecasts are often unreliable in a market mostly based on tenders, while countries most at need of vaccine lack the necessary funding for their vaccination programs.

All these reasons explain why the costs to produce high quality FMD vaccines are high and why there are so few global manufacturers. The investment for a newcomer would be uncertain and prohibitive.

Despite all these barriers Boehringer-Ingelheim, a large multinational company, has recently decided to invest more than 200 million € in a new FMD vaccine plant. The decision process was facilitated by the company's commitment to veterinary public health, decades of experience accumulated in the field and processes that were already in place to address all of the above challenges.

APPENDIX 13: MUTUAL REGISTRATION OF VACCINES IN EAST AFRICA: PROGRESS AND ISSUES FOR BETTER ACCESS TO EFFECTIVE FMD VACCINES?

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Most countries in the world have a system of assessment and approval of medicines to ensure that they meet high standards of safety, quality and efficacy before they granted a Marketing Authorisation (MA) by a national regulatory authority (NRA) and are authorized for sale. A mutual recognition procedure (MRP) was developed in the East African Community (EAC) for this purpose.

The first step in developing an MRP is to ensure that each one of a group of countries is working to the same standards. This can be achieved by developing harmonized guidelines, which each of the countries agrees to follow. In 2012 an EAC Technical Working Group (TWG) was inaugurated and it has successfully developed a harmonized process for the registration of veterinary vaccines.

The outcome was a series of technical documents including: A guideline explaining the information that should be included in the dossier; a guideline explaining the structure of the registration dossier; Templates for the details to be included on the packaging of the product; and harmonized application form for applicants to complete.

The MRP could be used for both veterinary immunological and veterinary pharmaceuticals. However, as only veterinary immunological have been harmonized to date, the registration requirements for veterinary pharmaceutical products will need to be harmonized between the relevant regulatory authorities before such products could undergo MRP in the EAC.

The first product to be assessed under this initiative was issued with an MA in June 2018. Two more products are currently being assessed. This MRP has helped reduce duplication of assessments and site inspections for the same medicinal product throughout the regional economic community. It has also contributed towards building experience, confidence and trust between the regulators in each Partner State in the EAC.

APPENDIX 14: IDENTIFICATION OF GENES INVOLVED IN PATHOGENICITY OF FMD VIRUS USING TWO STRAINS ISOLATED IN JAPAN WITH DIFFERENT VIRAL FEATURES

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Introduction

In 2000 and 2010, FMD outbreaks occurred in Japan and spread to 4 and 292 farms, respectively. Causative FMDV strains, O/JPN/2000 and O/JPN/2010, were isolated from affected cattle during each outbreak. In experimental infections, O/JPN/2000 showed only mild symptoms in cattle and goats, while O/JPN/2010 showed typical clinical signs in these animals. The difference in pathogenicity might cause the difference in severity of the two outbreaks; however, the molecular mechanisms underlying the pathogenicity of the virus are not well understood. In the present study, to identify genes involved in pathogenicity of the virus, we constructed chimeric recombinants of O/JPN/2000 and O/JPN/2010 and characterized the pathogenicity of those recombinants by animal experiments.

Materials and methods

The chimeric infectious cDNA clones were prepared by replacing the genetic regions of the full-length cDNA of O/JPN/2010 with corresponding PCR fragments amplified from O/JPN/2000. The recombined cDNA was introduced to a mammalian cell line, Cos-7, and chimeric recombinant viruses were recovered by using ZZR-127 cell cultures. These recombinant viruses, together with parental viruses, were intraperitoneally inoculated to suckling BALB/c mice (more than five mice per group) at the titer of 10 TCID₅₀/head, and mortality rates in 7 days were observed.

Results

Totally 8 recombinant viruses were successfully recovered. Mortality rates of suckling mice which were inoculated with parental viruses, O/JPN/2000 and O/JPN/2010, were 0% and 100%, respectively. Strikingly, recombinant viruses of which one of VP1 or 3D was derived from O/JPN/2000 showed 0% mortality in suckling mice, on the other hand, other recombinant viruses showed 100% mortality.

Discussion

VP1 is outermost of virus particle and presumably responsible for interacting with cellular receptor or immune factors. 3D is RNA polymerase required for virus replication. Our results indicate that VP1 and 3D are individually involved in the pathogenesis of O/JPN/2010 in infected animals.

APPENDIX 15: COMPLETE GENOME SEQUENCE ANALYSIS OF OVER 140 FMD VIRUSES ISOLATED FROM FREE-LIVING AFRICAN BUFFALO (SYNCERUS CAFFER) IN ZIMBABWE

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Introduction

Foot-and-mouth disease virus (FMDV) causes an acute vesicular disease in domestic cloven-hoofed animals. However, in the African buffalo (*Syncerus caffer*) clinical disease is rarely observed and following infection virus is persistently carried in the oesophageal-pharyngeal area of the upper respiratory tract. During the 1990s oesophageal-pharyngeal scrapings were collected from free-living African buffalo in multiple herds in six different geographic areas of Zimbabwe. Virus isolation on primary bovine thyroid cells and typing by ELISA resulted in the identification of 158 FMD viruses each belonging to one of the Southern African Territories serotypes.

Materials and methods

Virus isolates were sequenced using the Illumina MiSeq platform (Logan et al., 2014). After trimming adaptors and merging overlapping read pairs, the viral genomes were assembled in parallel with host contaminants using a novel in-house pipeline with two main components. The first one enables detection and assembly of sequence, irrespective of whether viral or host, even when coverage is low. The second stage scaffolds viral contigs obtained during the previous stage using a reference sequence solely as a guide, without incorporating any of the reference sequence in the final assembly. Phylogenetic analyses were performed using Maximum Likelihood and time-resolved Bayesian methods.

Results

The genome sequences of 143 FMD viruses were assembled. For phylogenetic analyses, the polyprotein-coding region sequences were split into four parts, L, P1, P2 and P3. In the P1 region sequences clustered together by serotype and then by buffalo herd/geographic location, whereas in the L, P2 and P3 regions sequences clustered by buffalo herd/geographic region irrespective of serotype.

Conclusions

Phylogenetic analyses of the different genome regions demonstrated the virus clustering by buffalo herd. The lack of clustering by serotype in non-capsid regions suggests that extensive recombination has taken place between the serotypes. The close relationship between some within-herd viruses suggested the possibility of acute infection epidemics.

APPENDIX 16: EVOLUTION AND COMPETITION OF SAT STRAINS DURING BUFFALO TRANSMISSION IN A CONTROLLED CHALLENGE EXPERIMENT

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Introduction

In Africa, buffalo appear to be the primary FMDV maintenance host without obvious clinical symptoms. Previously, buffaloes isolated for 24 years showed that FMDV can perpetuate long-term without re-introduction. However, experimental studies using defined challenge in an isolation facility showed virus recovery decreases and is cleared over 15-months. Ecological and evolutionary mechanisms contributing to FMDV transmission and persistence in buffalo are unknown. The objective of the study was to investigate antibody and evolutionary dynamics in buffalo during transmission events.

Material and methods

A challenge experiment consisted of three groups of African buffalo, each containing 4 animals/group, were experimentally infected with SAT1 KNP/196/91, SAT2 KNP/19/89 or SAT3 KNP/1/08 FMDV isolates, respectively. Forty-five days later, buffalo were screened for the presence of FMDV and divided into 2 identical groups, each group containing 2 FMDV persistently infected buffalo per serotype, totalling 6 buffalo/group. Six new naive animals were introduced into each group and transmission from carrier to naive evaluated over five months. Total antibodies were quantified using LPBE for the SAT types and antibody kinetics determined. Deep sequencing was performed on selected samples to determine evolutionary patterns during transmission.

Results

The data generated snapshots of the evolving viral population structures within different animals during the sequential transmission events. Analyses of the mutation spectrum of each animal showed polymorphisms of different frequencies across the genome. Bottlenecks occurred between transmission events. There were changes in antibody dynamics through the 260-day experiment.

Discussion

The data shows that viral population complexity is determined by small intra-host bottlenecks and more importantly by inter-host bottlenecks. There were differences in the competition of SAT serotypes measured by differences in population dynamics over time.

These results provide useful information into the evolution of FMDV in buffalo by sequential transmission, which can be used to quantify the risk of new sequence variants transmission to livestock surrounding the Kruger National Park.

APPENDIX 17: FMDV EVOLUTIONARY DYNAMICS WITHIN INFECTED BUFFALOES AND ITS LARGE-SCALE CONSEQUENCES

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Introduction

FMDV infections are known to harbour a rich intra-host dynamics. This originates from the high mutation and recombination rates of the virus, as well as the selective interplay of different variants in the swarm. However, there is only limited information on the evolutionary within-host dynamics during persistent infections.

Materials and methods

Several buffaloes were experimentally infected with a mixed SAT1,2,3 inoculum. The evolution of the SAT1 component was studied through either deep or Sanger sequencing of a number of samples (inoculum, laser micro-dissections of different oropharyngeal tissues of animals culled at different times post infection, and tonsil swabs/probangs). Finally, the results were compared with deep sequencing of a collection of samples of buffalo infections from the WRLFMD at Pirbright.

Results

The SAT1 inoculum showed a complex population structure constituted by multiple quasi-species and their recombinants. This structure is found in many other samples from buffaloes. After infection, we observe systematic changes in the frequency of the quasi-species driven by within-host viral fitness, as well as high rates of recombination. Within-host patterns of recombination are different from phylogenetic ones and are affected by beneficial combinations of co-evolved mutations in quasi-species. Viral replication proceeds at low rates during the persistent phase in oropharyngeal tissues. An exception is the virus found in swabs, which shows a high rate of substitutions but almost no internal variability.

Discussion

Strong within-host recombination, co-infections by multiple quasi-species and epistatic interactions within the FMDV capsid drive intra-host evolution in buffaloes. They also likely have consequences for large-scale evolution and dissemination of SAT viruses, increasing their genetic variability and the potential for genetic exchanges while determining capsid differentiation into serotypes.

APPENDIX 18: ANTIBODY RESPONSES TO THE MAJOR ANTIGENIC SITES OF FMD VIRUS SEROTYPE O AFTER PRIMO-VACCINATION, RE-VACCINATION AND AFTER NATURAL EXPOSURE

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Introduction

Out of the three prevalent serotypes (O, A and Asia1) of FMDV, serotype O is the most common cause of FMDV outbreaks in India. Five neutralizing sites have been identified on the capsid protein of FMDV serotype O through monoclonal-antibody resistant mutant analysis. In this study, the relative dominance of the known neutralizing sites in eliciting antibody response in the polyclonal serum collected from un-infected vaccinated (both primo and re-vaccinated) and naturally infected cattle populations were determined through the reverse genetics approach.

Materials and methods

The known critical amino acid residues present on the five antigenic sites of FMDV serotype O in-use vaccine strain O IND R2/1975 were mutated through the site-directed mutagenesis approach on the full-length infectious cDNA clone. The mutant viruses were rescued in cell-culture and analysed to determine the percentage drop in virus-neutralizing antibody titre using the polyclonal serum samples collected from primo-vaccinated, re-vaccinated and naturally infected cattle population.

Results

From the analysis it was found that, in the serum samples from primo-vaccinated animals, most antibodies directed towards the antigenic site 2, followed by antigenic site 1. While in serum samples from re-vaccinated animals, both the antigenic sites 1 and 2 were equally dominant. In case of naturally infected animals, similar levels of antibodies to all the antigenic sites (site 1 to-5) have been detected.

Discussion

The findings from this study extend our knowledge on the relative dominance of the antigenic epitopes of FMD virus in multiply vaccinated and infected cattle, and will improve our strategies for vaccine strain selection and rational vaccine design.

APPENDIX 19: EXPLORING PRIVATE AND PUBLIC SECTOR RIGHTS AND RESPONSIBILITIES IN PREVENTION AND CONTROL OF FMD: THE CASE OF RIGHT TO ACCESS VACCINES BY LIVESTOCK KEEPERS

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Economics is often defined as the science of allocation of scarce resources to achieve social goals. The aim of economic agents is considered to be improving their welfare given the level of resources available to them. Assuming an equitable distribution of property rights, an efficient allocation of society's scarce resources could maximise the welfare of economic agents and the society as a whole. However, often this is not the case because distorting circumstances such as monopolistic behaviours, asymmetric information and externalities lead to market failure. The mentioned behaviours have a pivotal role to play in the macro and micro epidemiology of contagious livestock diseases such as foot-and-mouth disease (FMD). Behaviours are governed by institutions established in law or practice governed by rules founded for economic, social, religious, educational or professional purposes. Public sector generally takes a societal perspective and aims to define rights and responsibilities to minimise total social costs (i.e. private and public), to provide public goods e.g. surveillance and diagnostics, and to share risk and costs with private sector. Private sector represented by livestock keepers, however, often tend to ignore social costs if allowed, maximising their productivity or profit and exercising certain level of private responsibility subject to behaviours of other producers and neighbours. Public and private sectors' rights with respect to prevention and control of FMD determine ownership, legitimacy of behaviours and liability. These rights are determined by national and international legislations defining property rights. Property rights to FMD-free status depend on who has invested in measures such as vaccination programmes and also the beneficiaries. Property right enforcement is complex when behaviours are difficult to observe and the speed and quality of actions/ inactions are crucial. This paper explores rights of livestock keepers to access FMD vaccine in light of recent developments in diagnostics and vaccine production.

APPENDIX 20: FMD RESEARCH GAP ANALYSIS WORKSHOP 2018

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Introduction

During June 2018, the US Dept. of Agriculture, together with the National Institute of Agricultural Technology (INTA, Argentina) and the Global Foot and Mouth Disease Research Alliance (GFRA) organized the “FMD Research Gap Analysis Workshop” in Buenos Aires, Argentina. This was the third edition of this workshop held with the purpose of bringing together FMD experts worldwide to assess gaps in the scientific information and veterinary medical countermeasures needed to control FMD on a global scale. The workshop was two- and-a-half days long, and it was organized into thematic blocks including vaccines, immunity, diagnosis, epidemiology, virology and pathogenesis. Due to the dynamics of the meeting, the number of participants was limited to approximately thirty with a broad expertise representation, including researchers from different areas of knowledge, representatives of national and international sanitary and regulatory bodies, as well as representatives from vaccine manufacturing industry worldwide. Each thematic block (2 hours each) was coordinated by two experts who introduced the state of knowledge, as well as directed the discussion with the rest of the attendees to assess where gaps remain. The final day of the meeting, the participants were divided into smaller groups to prepare a brief summary by thematic blocks. For this presentation, we will briefly share some of the gaps identified, making special focus in the FMD vaccines and immunity sections.

APPENDIX 21: GENE SIGNATURES ASSOCIATED WITH FMDV INFECTION AND PERSISTENCE PART I: PERSISTENT FMDV INFECTION IN AN AIR-LIQUID INTERFACE MODEL OF BOVINE SOFT PALATE

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Introduction

Persistent infection with foot-and-mouth disease virus (FMDV) delays the process to recover a FMDV-free status. In carrier cattle, which harbour FMDV >28 days post infection (DPI), replicating virus has been detected in epithelial cells in the nasopharynx and soft palate (SP). To induce viral persistence, FMDV likely suppresses immune responses or changes to escape these responses. The aim of this study was the development of a model to be able to characterise gene signatures within FMDV and its target cells. The overall goal was to identify factors that can be used to prevent persistent infections or to improve diagnostics. An air-liquid interface multilayer model of bovine SP cells was developed and characterised by immunostaining and electron microscopy. After five weeks of culture without further passage, the cells were infected with FMDV O/FRA/1/2001 at an MOI of 0.01 or 1, respectively. The infection was monitored until 28 DPI by virus isolation in cell culture, RT-qPCR, immunofluorescence, and immunohistochemistry.

Results

At the time of infection, approximately 20% of the cells had a polygonal morphology and displayed tight junctions, as observed in stratified squamous epithelia. Cells with similar morphology expressed cytokeratin. A limited cytopathic effect was induced, restricted to the upper cell layers. FMDV antigen, FMDV RNA and live FMDV were detected through day 1 to 28, with peaks at day 1 and 2. At day 28, FMDV antigen was detected in sparse cells.

Discussion

The air-liquid interface model allowed long-term culture of SP cells in multilayers, without disruption. Epithelial cell characteristics such as cytokeratin expression and tight junctions were preserved in a subset of cells during 9 weeks of culture. The detection of FMDV until 28 DPI opens unique possibilities to investigate FMDV persistence in a controlled manner. Transcriptional data will be presented in a joint communication.

APPENDIX 22: GENE SIGNATURES ASSOCIATED WITH FMDV INFECTION AND PERSISTENCE PART II: TRANSCRIPTOMIC ANALYSIS OF ACUTE AND PERSISTENT FMDV INFECTION IN BOVINE SOFT PALATE

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Introduction

The transcriptional alterations of the nasopharynx and soft palate (SP) during FMDV infection in cattle are not well understood. Therefore, an air-liquid interface multilayer model of bovine SP cells was developed and is presented in a joint communication. By using a transcriptomic approach, this system was used to identify host gene signatures during acute (24 hours post infection - HPI) and persistent infection (28 days post infection - DPI) in order to determine mechanisms and potential molecular targets that enable persistent infection. This knowledge may help to prevent establishment of persistent infection.

Materials and methods

The infection experiment was performed twice with different donor animals and two biological replicates per animal and time point (0, 24 HPI and 28 DPI). Whole-transcriptome libraries were sequenced with the Ion S5XL (~24 million reads/replicate) and gene expression was analyzed using Salmon and DESeq2. Expression levels of selected genes were confirmed by RT-qPCR and protein mass spectrometry.

Results

Principal component analysis demonstrated the robustness of RNA sequencing, showing strong influence of donor animal, infection and time. 315 and 73 genes were differentially expressed at 24 HPI and 28 DPI, respectively. The majority of these genes were up-regulated and related to the immune system (MX1, OAS2, IFIH1). Interestingly, 10 genes were only differentially expressed during persistent infection (ANKRD1, NCAM1, collagens). Most of these were down-regulated and related to the development of the extracellular matrix or keratinocyte differentiation.

Discussion

The results indicate time-dependent gene signatures during FMDV infection that include the activation of the innate immune system, particularly interferon and cytokine signaling. However, the overall number of regulated genes and their expression was reduced at 28 DPI, in comparison to acute infection. Furthermore, the down-regulation of a few unique genes at 28 DPI indicates a modulation of epithelial maturation during persistent FMDV infection. These genes represent interesting candidates for future experiments.

APPENDIX 23: TRANSMISSION OF FMD FROM PERSISTENTLY INFECTED CARRIER CATTLE TO NAÏVE CATTLE VIA TRANSFER OF OROPHARYNGEAL FLUID

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Although the FMDV carrier state in cattle has been characterized in detail under both natural and laboratory conditions, the crucial issue of whether carrier cattle pose a significant risk of contagion remains unresolved. Specifically, despite a lack of experimental evidence of transmission from FMDV carrier cattle, the FMDV carrier state has had a profound impact upon the regulation of global trade in animal products. The objective of this study was to investigate the potential risk of disease transmission from oropharyngeal fluid and nasopharyngeal tissues harvested from persistently infected FMDV carrier cattle under controlled experimental conditions.

Materials and Methods

Oropharyngeal fluid (OPF) and nasopharyngeal tissues were harvested at 30 days post infection from seven cattle that had been infected with FMDV A24. Eight naïve cattle were challenged by intra-nasopharyngeal deposition of the untreated OPF. Additionally, one group of 5 pigs were challenged by intra-oropharyngeal deposition of the same OPF, and another 5 pigs were fed macerated nasopharyngeal tissues from the same cohort of FMDV carriers.

Results

All cattle challenged with OPF (challenge dose determined to 102 TCID₅₀ on LFBK_{αvβ6} cells) developed clinical FMD of similar severity as animals that had been infected using a high-titer inoculum. In contrast, pigs exposed via intra-oropharyngeal inoculation of OPF, or by ingestion of nasopharyngeal tissues, did not develop FMD.

Discussion

The successful transmission under these experimental conditions supports the perceived risk of contagion associated with persistently infected FMDV carrier cattle. However, the probability that a sufficient quantity of FMDV from a carrier animal would reach susceptible cells within the nasopharynx of a naïve animal under natural conditions is likely very small. Nonetheless, these data demonstrated that FMDV infection of susceptible cattle can be seeded by exposure to very low levels of virus from carrier animals, despite the presence of secreted anti-FMDV antibodies.

APPENDIX 24: RAPID, ON SITE, DIAGNOSIS OF FMD AND SAFE AND COST-EFFECTIVE SHIPMENT OF SAMPLES USING LATERAL FLOW DEVICES FOR LABORATORY DIAGNOSTICS

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Introduction

Identification of circulating strains is an essential step towards the global eradication of FMD. However, the cost of sending FMD samples is an obstacle to submission of samples to Reference Laboratories due to shipping conditions. A cost-effective and safe method for shipment of samples from FMD-suspected cases, based on the inactivation of FMDV on lateral flow devices (LFDs) has been developed and validated in the laboratory using reference strains and archival samples. This method allows subsequent detection and typing of FMDV by RT-PCR and virus rescue using RNA transfection (Romey et al. 2017). The present study aims to further evaluate this protocol on freshly collected clinical samples through collaboration with field veterinarians in endemic countries in order to test the performance and safety of the entire process directly in the field.

Materials-methods

Epithelium or vesicular fluid samples will be collected from suspect clinical cases of FMD in Nigeria, Turkey and Pakistan and will be tested in the field using LFDs. The selected positive inactivated (or not) LFDs will be analyzed firstly by the national laboratory (Nigeria & Turkey) to ensure that the inactivation process is effective. Then, the duplicated sample will be submitted to European reference laboratories (France, Denmark) for molecular detection and virus rescue by transfection of viral genome.

Results

Sample collection and inactivation on LFDs are in progress. Transfections are currently being optimized to ensure virus rescue from RNA genomes recovered from inactivated LFDs.

Discussion

This study will contribute to demonstrate that using LFDs is a safe way for room-temperature, dry-transport of inactivated FMDV samples from endemic areas. It may substantially decrease the shipping cost thus increasing field sample submission.

APPENDIX 25: THE UTILITY OF POOLED MILK FOR FMD SURVEILLANCE IN NAKURU COUNTY, KENYA

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Introduction

Milk is a non-invasive sample type routinely collected from dairy farms, which could be useful for herd-level foot-and-mouth disease (FMD) surveillance. Using this alternative sample could address some of the potential biases of traditional surveillance methods with under-reporting or sub-clinical infection. Previous studies have demonstrated that FMD virus (FMDV) can be detected in milk samples from experimentally infected cows by real-time reverse transcription polymerase chain reaction (rRT-PCR), before, during and after the development of clinical signs (up to 28 days post contact).

Materials and Methods

This study aimed to investigate the potential of pooled milk as a sample type for FMD surveillance using rRT-PCR, compared with reports of disease incidence in Nakuru County, Kenya. There are typically several FMD outbreaks per year, and many smallholder dairy farmers sell milk to co-operatives who pool milk for onward sales. This study collected weekly, pooled milk samples from six dairy co-operatives alongside periodic cross-sectional surveys of small-holder farmers to gain information on clinical disease, milk production and trends in milk sales.

Results

FMDV RNA was detected in 11/264 milk samples, and SAT 1 serotype was also identified using a type specific rRT-PCR, concurrent with confirmed outbreaks in the study area. FMDV RNA was detected when the FMD incidence in the study area was $\geq 2.5\%$, i.e. at least one farmer reported having experienced FMD on their farm. This indicates that the pooled milk surveillance system can detect a threshold FMD farm level incidence of 2.5%, when up to 26% of smallholder farmers were contributing milk to pooling facilities.

Discussion

This pilot study identifies potential for using pooled milk for FMD surveillance in endemic regions, although further optimisation is required to maximise the sensitivity of the system, for example through investigating the collection of samples at different levels of the milk supply chain.

APPENDIX 26: EVALUATION OF ENVIRONMENTAL SAMPLING AS A LOW TECHNOLOGY METHOD FOR SURVEILLANCE OF FMDV IN AN ENDEMIC AREA

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Introduction

Environmental sampling enables disease surveillance beyond regular investigation of clinical cases, extending data on the circulation of a pathogen in a specific area. Developing straightforward, low technology methods suitable for use in field conditions is key to the inclusion of such approaches alongside traditional surveillance techniques. Environmental contamination by foot-and-mouth disease virus (FMDV) in excretions and secretions from infected individuals promotes transmission, but also presents an opportunity for non-invasive sample collection, facilitating diagnostic and surveillance purposes.

Materials and Methods

Electrostatic dust cloths were used to collect environmental swabs at sites with reported outbreaks of FMDV, in the Kathmandu Valley, Nepal, which is endemic for FMD. A limited number of aerosol samples were also collected. A total of nine sites were visited and sampled between November 2016 and November 2017. Samples were stored in lysis buffer and transported to The Pirbright Institute, where an rRT-PCR assay was used to detect FMDV RNA.

Results

FMDV RNA was detected in environmental samples from premises with animals at all stages of clinical disease, from uninfected, suspected preclinical, clinical and recovering cattle. Categorising lesion ages as fresh (1-5 days), healing (6-10 days) and old (>10 days), there was a significantly higher proportion of positive samples for households with fresh lesions compared with those with old lesions (P=0.02).

Discussion

Development of methods that can reliably detect FMDV RNA in the environment is significant, as this extends the toolbox available for surveillance for this disease.

Development of low technology, straightforward surveillance methods such as this can support a robust response to outbreaks. Pairing these methods with existing and novel diagnostic tests will improve capability for the rapid detection of outbreaks and implementation of timely interventions to control outbreaks. In endemic areas, these methods can be implemented to extend surveillance beyond the investigation of clinical cases, providing additional data to assess virus circulation in specific areas.

APPENDIX 27: ENVIRONMENTAL SAMPLING: A SURVEILLANCE TOOL FOR FMDV IN MARKETPLACES

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Introduction

Live animal markets bring infected and non-infected animals in close contact providing the potential for direct and indirect transmission, facilitating wide onward spread of disease. Foot-and-mouth disease (FMD) is currently endemic in Nepal where commercialised goat markets are in operation. The Khasibazar market, Kathmandu is a large goat market with an estimated monthly turnover of 30,000 goats. We present data on the use of environmental sampling to detect FMD in this market.

Materials and methods

Four visits were made to the market during 2016/17 (one visit in November 2016, one in April 2017 and two in November 2017). Environmental swabs were taken from the holding pens, from surfaces deemed most likely to have come into contact with secretions and excretions of goats, including; rope ties, mesh fences, floor, walls, feed buckets and weighing scales. The geographical origin of the goats and amount of time spent at the market was recorded for each pen. In total 185 samples were collected and tested for FMD viral RNA by rRT-PCR.

Results

FMDV RNA was detected in five samples from three visits; one from 32 samples during the first visit, one from 43 samples during the second visit and two from 112 samples during the fourth visit. All samples were also screened for peste des petits ruminants virus (PPRV), with four samples positive for PPRV RNA from both the first and fourth visit.

Discussion

Movement of goats through markets has been implicated in the spread of FMDV in Nepal. Detection of FMDV RNA in environmental swabs demonstrates FMDV infected goats have been present at the Khasibazar goat market, although the source of virus cannot be linked to specific animals.

Environmental sampling provides a simple, non-invasive method of detecting FMDV at markets, where clinical signs in animals may not be apparent and where individual sampling is not practical. Environmental sampling data could be used to supplement epidemiological data to identify and predict patterns of spread and highlight potential transmission risks in endemic and FMD-free countries.

APPENDIX 28: EFFECTIVE IN SILICO SEQUENCE-BASED PREDICTION OF FMDV VACCINE MATCHING

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Introduction

FMDV has a rich population structure, with a number of strains possibly co-circulating in the same geographic area and new variants rapidly emerging. Available vaccines only offer protection for a limited time, and are prone to the problem of antigenic drift. Hence the importance of serology-based vaccine matching techniques in tackling the virus and informing decision makers. However, those techniques are imprecise and costly; replacing them with in-silico predictions would represent a significant progress in the field.

Materials and methods

A large dataset of titres obtained from neutralisation assays on different FMDV types was used to train machine-learning algorithms. Quantities derived from whole-capsid sequencing information (including biochemical and structural indicators) were used to inform the prediction.

The predictors are able to reproduce observed titres with great accuracy (much greater than methods previously published in the literature). Accuracy appears to be largely independent of the FMDV type and vaccine used.

Discussion

Our novel strategy seems to represent a promising first step towards achieving reliable in silico vaccine matching prediction. Hopefully in the future datasets similar to the one we used will become available for more FMDV types and vaccines, allowing us to extend and explore the scope of our approach.

APPENDIX 29: IN-VITRO CORRELATES OF HETEROLOGOUS PROTECTION USING AVIDITY AND IGG-SUBTYPING ELISAS

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Introduction

Virus neutralisation tests (VNT) and the liquid phase blocking ELISA (LPBE) are the commonly-used vaccine matching methods, measuring how much an antiserum made against a vaccine strain will cross-react with a field virus. However, the results are often interpreted as how well a vaccine will protect against a given field strain, which is also dependent upon vaccine potency. ELISAs that measure antibody avidity and isotypes appear to provide a better correlation with protection than VNT alone and show good reproducibility. To extend the validation of these methods we have transferred the technology from INTA to WRLFMD, where panels of post-vaccination sera exist from previously conducted challenge studies.

Materials and Methods

Twenty-one day post vaccination serum samples (n=34) from studies which assessed the vaccine viruses A/MAY/97 and A22 IRQ against the field virus A/ASIA/G-VII were tested by VNT, LPBE, avidity and IgG-subtyping ELISAs at The Pirbright Institute with guidance from INTA.

Results

Antisera to the vaccine viruses A22 IRQ and A/MAY/97 have low VNT titres against the A/ASIA/G-VII field strain, giving rise to low r1 vaccine match results; however, the A/MAY/97 appears to protect in-vivo (PD50 > 6). Results will be provided from comparative tests on sera from two heterologous potency studies, analysed as indirect correlates of protection.

Discussion

Future plans include improving the purification method for the required test antigens so that they can be done routinely in diagnostic laboratories. To further validate these assays larger numbers of sera samples from potency tests should be tested. This could be achieved by transferring the methods used and/or sharing the sera with other laboratories that have established the techniques.

APPENDIX 30: ASSESSMENT OF EXISTING AND FUTURE VACCINE SELECTION TECHNIQUES – MOVING FORWARD

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Introduction

Since the 1980s the OIE Terrestrial Manual's has promoted r1-values – the ratio of heterologous to homologous antibody titre – as the main in vitro method for vaccine matching. The recommendation is to use virus neutralisation (VN), or failing that liquid phase blocking ELISA (LPBE) or complement fixation (CF) as the test to measure titre. As well as issues with the strength of the correlation between r1 value and vaccine match, there is a secondary problem of a lack of understanding that vaccine efficacy also depends on potency. New assays, especially avidity and IgG isotype ELISAs, have been developed since then that may improve our ability to calculate vaccine match and, more importantly, take account of potency, potentially giving a better insight into vaccine efficacy.

Materials and Methods

Collaborators were identified with data and/or antisera from heterologous and homologous in vivo challenge trials. The resulting data came from ~1000 animals, half of which underwent homologous challenge and half heterologous challenge. VN and LPBE data was available for nearly all of these animals, and avidity and IgG isotype data for ~10%. A Bayesian analysis was carried out to investigate the relationship between all of these assays and protection.

Virus neutralisation was found to be better correlated with protection than LPBE, in accordance with earlier studies. The limited data currently available for the new assays is also very promising, but further data is necessary to confirm these conclusions. A variety of antisera have already been identified for further testing, which will take place over the next year, but we would be pleased to broaden our collaboration through data and antisera from other challenge studies (homologous as well as heterologous).

APPENDIX 31: REINFORCEMENT LEARNING FOR CONTEXT-DEPENDENT CONTROL OF EMERGENCY OUTBREAKS OF FMD

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Introduction

Emergency disease control interventions can, do, and should adapt to reflect the progression of an outbreak. Reinforcement learning (RL) is a methodology that can be used in combination with epidemiological simulation models to search for, and test, control policies that are context-dependent, thereby recommending different actions as an outbreak progresses. Rather than testing the performance of a pre-determined set of control actions RL allows for the discovery of control policies. The resultant RL policies provide an upper bound on management performance against which simpler policies can be evaluated.

We apply RL to the control of a hypothetical FMD outbreak. The decision problem is to determine the extent of culling or vaccination around currently confirmed infected premises so as to stop the spread as quickly as possible. Management performance of the RL policy is compared against policies that do not adapt through time.

Results

In all simulated outbreaks, the policies generated by the RL algorithm were adaptive. That is, it was optimal to change control interventions through time. Most striking was how the optimal action changed as the area of the outbreak changed – for small outbreaks it was optimal to vaccinate in a large radius around infected premises but for larger outbreaks it was optimal to cull at a medium radius. All policies were heavily dependent upon carcass disposal capacity.

Discussion

We illustrate methodology for generating control policies that are tailored to each future outbreak scenario. Such methods can capitalise upon the wealth of stochastic epidemiological models currently available.

APPENDIX 32: INVESTIGATING THE BENEFITS OF AN ADAPTIVE MANAGEMENT APPROACH INVOLVING EMERGENCY VACCINATION USING SIMULATED FMD OUTBREAKS IN NEW ZEALAND

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Introduction

This study investigated personnel resource requirements and the performance of an early decision indicator (EDI) to trigger emergency vaccination.

Materials and methods

InterSpread Plus was used to simulate 5000 FMD outbreaks in New Zealand. Four response strategies were evaluated: Stamping-out only (SO) and SO plus vaccination initiated by three mechanisms: randomly started between days 11-35 of the response (VAC); started on day 21 (VACf); or deployed if the number of infected premises (IPs) or the estimated dissemination rates exceeded certain threshold values between days 11-35 (TRV).

Parameters that were varied randomly included the number of personnel, whether airborne spread occurred, farm classes vaccinated and surveillance visits per person per day. Outputs, including time to first detection, total IPs, outbreak duration, total man-days, vaccine doses used and if and when the EDI trigger was fired, were collected for each iteration. Data were analysed using contingency table analyses, univariable tests and multivariable analyses.

Simulations were stopped if 90 days had elapsed without IPs or when the outbreak reached 365 days.

Results

IPs were highly right skewed (median = 10, interquartile range (IQR) = 3 – 34, range = 0 – 50,582); as was duration (median = 20 days, IQR = 7 – 39, range = 0 – 360). Logistic regression showed that the number of veterinarians available, the response strategy and the EDI trigger firing were associated with large or long epidemics.

Vaccination reduced the number of IPs and duration compared to SO, and the TRV strategy performed the best in terms of the fewest IPs and the shortest duration.

Discussion

This study showed the benefits of an adaptive management response to FMD outbreaks. Compared with previous studies it provided more information to support vaccination decision-making and insights into resourcing requirements when responding to FMD outbreaks in New Zealand.

APPENDIX 33: EVALUATING OPTIMAL CONTROL STRATEGIES FOR FMD WITH THE US DISEASE OUTBREAK SIMULATION

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Introduction

Spatially explicit simulation models can aid policy decisions and identify efficient control strategies for different scenarios. In two theoretical studies we analyzed (1) the effects of spatial clustering of premises on the course of Foot and Mouth Disease (FMD) outbreaks, and (2) the efficacy of different control strategies for controlling such outbreaks. In the U.S., the premises' locations are largely unknown below the county scale, necessitating assumptions about their spatial distribution. A better understanding of how such assumptions affect simulations, in combination with detailed analysis of control strategies would help develop more efficient preparedness plans.

Materials and Methods

The US Disease Outbreak Simulation (USDOS) is a data-driven, spatially explicit disease spread model simulating continental scale outbreaks. It was developed for the U.S. livestock demographic in a collaborative effort between Colorado State, Warwick, and Linköping Universities with support from the U.S. Department of Homeland Security Science and Technology Directorate.

Using USDOS, the effect of spatial clustering of premises on FMD outbreaks was analyzed analytically and through stochastic simulations in two sets of different spatial distributions of the U.S. cattle population—one random and one where the premises' locations were simulated to provide more realistic distributions.

Control strategies based on combinations of movement bans, culling and vaccination strategies were evaluated for simulated FMD outbreaks. The outbreaks were initiated in all parts of the U.S. and included realistic constraints on amount of time and resources that could be allocated regionally.

Results

Study one showed how realistic spatial distributions of premises can have a pronounced effect on epidemiological predictions, indicating unrealistic estimates of premises locations should be avoided. Study two revealed that control strategies are most efficient when applied rapidly with maximum resource allocation, but resource constraints may cause mitigation efforts to be outpaced for larger outbreaks. Substantial regional differences were identified, both in terms of the effect of clustering and choice of control strategy.

APPENDIX 34: BETWEEN-HERD TRANSMISSION DYNAMICS OF FMD IN KENYA RANGELANDS

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Introduction

Transmission of foot-and-mouth disease virus (FMDV) is driven by patterns of contact across multiple scales. Between-herd contact can be challenging to quantify, but can sometimes be directly measured through questionnaires or observation and/or indirectly inferred through phylogenetic data. Here, we present an overview of results from several studies related to transmission dynamics of FMD in Laikipia County and the Masai Mara Ecosystem (MME) in Kenya, both of which are characterized by pastoral cattle production and abundant wildlife.

Materials and Methods

We performed VP1 sequencing (Sanger and next-generation) on oropharyngeal fluid samples collected from cattle and wild buffalo in Laikipia. Maximum likelihood and Bayesian phylogenetic methods were applied to assess genetic clustering of FMDV within buffalo herds and wildlife-livestock transmission. In addition, data on between-herd contacts were collected from a combination of GPS-tracking of cattle and buffalo herds and questionnaires administered to herders in Laikipia and MME.

Results and Discussion

Molecular epidemiology: We recovered 75 SAT1 and SAT2 sequences from buffalo. For SAT1, separate phylogenetic clusters were found in different buffalo herds despite being sampled from a relatively small area, suggesting strong spatial and social mechanisms determining between-herd transmission. No SAT1 or SAT2 viruses were found in sympatric cattle that mix with buffalo, indicating that cross-species transmission is rare. However, at a broader spatial scale, SAT1 and SAT2 viruses found in buffalo were phylogenetically related to sequences associated with several cattle outbreaks elsewhere in central Kenya.

Dynamics of between-herd contact: Daily foraging contacts among cattle herds increased during the dry season driven by aggregations around water sources. This increases the vulnerability of contact networks to pathogen spread. In MME, GPS-tracking data on buffalo, impala, and cattle were analyzed to identify hotspots of between-herd and between-species interaction.

Results of these studies advance current knowledge of between-herd contact dynamics in buffalo and cattle populations in East Africa, thus contributing to the broader epidemiological understanding needed for more effective control measures in the region.

APPENDIX 35: USING NETWORKS OF LIVESTOCK MOBILITY TO IMPROVE CONTROL OF ENDEMIC FMD IN NORTHERN TANZANIA

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Introduction

Foot-and-mouth disease (FMD) remains endemic in East Africa, causing significant reductions in livestock production. Traditional livestock management systems relying on sharing of communal resource areas are predominant in this part of Africa. Unrestricted movements are widespread and are considered important drivers of FMD outbreaks in cattle. Yet they remain poorly understood. Unravelling livestock mobility patterns, particularly herd connectivity at resource areas and markets, is essential in order to determine disease dynamics and devise appropriate control strategies.

Materials and Methods

Focusing on areas of high-prevalence disease in northern Tanzania, our study mapped communal shared resource areas and investigated livestock herd movements around these areas across seasons. Information on livestock movements was collected in two districts representative of agro-pastoral and pastoral production systems – Serengeti and Ngorongoro – respectively. Data was generated through community-level participatory mapping, collection of livestock movement permits, and tracking of selected cattle herds using Global Positioning System (GPS) collars.

Results

For each village (n=140) in the two study districts, we identified the position of livestock resource areas (e.g. grazing and watering), markets, areas of livestock mixing, the volume of livestock traffic and frequency of interactions at specific points. Network analyses have revealed the influence of seasonality on livestock connectivity through shared resource areas and identified villages that act as key linkages across networks.

Discussion

Community structure matrices of connected villages have enabled us to uncover differences in the contact patterns of livestock movement networks that provide some understanding on how endemic FMD might spread in agro-pastoral and pastoral management systems. We are now using network vulnerability and resilience measures as proxies to demonstrate the effectiveness of interventions (e.g. targeted vaccination) at key transmission nodes (villages, communal grazing areas, etc.). Building on the network analyses, we will parameterise FMD virus transmission models that will allow us to assess the risk of transmission at shared resource areas in both settings.

APPENDIX 36: LIVESTOCK MOBILITY IN WEST AFRICA: NETWORK ANALYSIS AND APPLICATIONS

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Introduction

In West Africa, livestock mobility is a complex phenomenon involving different types of movement (commercial and transhumance), different spatial scales (from local/village level to international) and different time scales (a day for commercial movements till several month for transhumant). In this work we present some analysis on livestock mobility network data in Senegal and Mauritania for 2014.

Materials and Methods

Data have been collected by the CNERV in Mauritania and by the DSV in Senegal. In both countries, a system of movement certification has been put in place: every time a herd is moved a certificate should be issued indicating Origin and Destination of the movements as well as the herd's size, the convoy type and other information.

Results

The analysis has shown the existence of two mobility patterns throughout the year: the first related to routine movements from January to August; the second strictly connected to the religious festivity of Tabaski. These mobility patterns are different in terms of animals involved, the means of transportation and destinations. Results from these analyses were used to estimate the rate of transmission of infection from one node to the other and to identify the nodes playing a pivotal node in the diffusion of diseases.

Discussion

These results can be used by Veterinarian offices and public health officers to inform control policies (prioritization of vaccination, market's closure etc) and surveillance network according to the period of the year, the species involved and the diseases considered.

APPENDIX 37: QUALITATIVE ASPECTS OF THE IMMUNE RESPONSES RELATED TO PROTECTION AGAINST FMDV CHALLENGE IN CATTLE

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Introduction

We have shown that multivalent vaccines, as well as revaccination protocols, could confer complete protection in a well-established vaccine-matching model for cattle using the A24/ Cruzeiro and A/Arg/2001 FMDV strains. Experimental groups did not show significant differences among them for a wide range of immunological parameters; however, differences were found between protected and unprotected animals, mainly in the isotype and avidity profiles of the immune sera. In this report, we described some qualitative aspects of the humoral responses found in protected bovines.

Materials and Methods

Serum samples from each vaccination protocol [n=5 each: single dose monovalent 10 µg (1) or 40 µg of A24/Cruzeiro (2); trivalent A24/Cruzeiro-O1/Campos-C3/Indaial (3), or two doses of the 10µg of A24/Cruzeiro vaccine (4)] were assayed using a set of newly developed serological tests. These new ELISA-based protocols were used to quantify avidity according to the isotype of the antibodies against A24/Cruzeiro and A/Arg/2001 FMDV strains as well as the amount of A/Arg/2001-specific antibodies generated by the other three heterologous strains.

Results

Protected animals showed significantly lower IgG1/IgG2 ratios than non-protected ones. Protected cattle also presented higher mean avidity indexes (AI) for antibodies against the A/ Arg/2001 but also against the A/24 and O1/Campos strains. Such significant differences were also observed when analyzing IgG1 and IgG2 isotypes separately. Strain-specific analyses also shown that both the C3/Indaial- and O1/Campos-specific antibodies showed cross-reactivity against the A/Arg/2001 strain in a similar manner.

Discussion

Our results would indicate the existence of a higher proportion of cross-reactive IgG2 antibodies, compared to the IgG1 isotype, in the protected individuals. However, higher mean AI against A/Arg/2001 strain found in protected cattle, was not restricted to a specific IgG subclass and it was also detected for other strains (A24/Cruzeiro and O1/Campos). The O1/ Campos and C3/Indaial vaccine antigens present in the trivalent formulation effectively generated A/Arg/2001 cross-reactive antibodies observing a similar proportion.

APPENDIX 38: SECURE BEEF SUPPLY IN THE U.S. PLANNING FOR CONTROL AND CONTINUITY OF BUSINESS IN AN FMD OUTBREAK

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Introduction

Response to an FMD introduction in the U.S. would include movement controls, enhanced biosecurity and surveillance, depopulation and potentially vaccination. Control efforts would be disruptive to livestock industries and could cause long term economic damage. Contrasting short term goals of disease control and business continuity must be balanced to reach the long term goals of disease eradication and livestock industry survival.

Materials and Methods

USDA APHIS Cattle Health Programs funded the “Secure Beef Supply plan”. University personnel identified working groups of academic, industry and regulatory representatives to develop guidance for Managed Movement, Biosecurity, Surveillance, and Contingency Planning. Plans focus on: 1.) controlling disease transmission risk through managed movement and enhanced biosecurity; and 2.) early detection through active observational surveillance of cattle. The goal is to identify procedures and provide training materials to decrease transmission risk while supporting substantial industry business continuity.

Results

Guidance documents and training materials were produced for beef producers, packers, regulatory officials and veterinarians on Managed Movement, Biosecurity, Surveillance and Contingency Planning. Training materials focus on recognition of FMD signs and implementation of biosecurity and surveillance principles to decrease disease transmission risk. Documents and materials support criteria to allow movement permits for low risk movements in order to support continuity of business. Documents and materials are available at www.securebeef.org.

Discussion

The response to an FMD introduction in the U.S. would be disruptive and risk severely damaging a large portion of the livestock industries. A well planned response that balances the needs for disease control and business continuity in the livestock industries is necessary. This will require stakeholder training to minimize disease transmission risk during essential business practices while control measures are implemented. The Secure Beef Supply plan is an attempt to provide guidance to all stakeholders and optimize disease response and control.

APPENDIX 39: MODELLING THE IMPACT OF REGIONAL MOVEMENT CONTROL POLICIES FOR FMD OUTBREAKS IN DISEASE FREE COUNTRIES

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Introduction

The revenue of livestock farms in disease free countries is largely based on the movement of animals, either through selling animals to other farms or moving animals to slaughter. Therefore, adopting any form of movement restrictions has substantive economic consequences for the livestock industry. In this paper, we use state of the art mathematical models to investigate the cost-effectiveness of local and regional movement control upon outbreaks of FMD in the UK. Such policies, if implemented effectively, could balance the need of the containing and controlling the spread of infection with the economic incentive of maximising business continuity for a large number of unaffected farms.

Methods and Results

We use a sophisticated spatial stochastic model of foot-and-mouth disease (FMD) that has been extensively previously utilized previously to investigate optimal interventions for FMD outbreaks. In this paper, upon notification of an infected farm, we introduce livestock movement bans within a given radius of the notified farm. We then determine the radius that minimizes the overall cost of the outbreak. When determining cost, we take a national perspective, considering direct costs to the farms, as well as the agricultural sector, exports and the tourist industry. We show that for FMD, in contrast with past policy, the economically optimal strategy is to ban movements in a relatively short radius around infected farms; the precise balance between disease control and maintaining 'business as usual' varies between different regions of the country.

Discussion

Our model predictions demonstrate that movement restrictions have a dramatic impact on the cost of livestock diseases such as FMD, implying that large-scale movement bans are generally prohibitively expensive. This work suggests that movement controls need to be carefully matched to the epidemiological and economic consequences of the disease, and optimal bans can have substantial financial benefits.

APPENDIX 40: MODELLING MANAGEMENT STRATEGIES FOR VACCINATED ANIMALS AFTER AN OUTBEAK OF FMD AND THE IMPACT ON RETURN TO TRADE

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Introduction

An incursion of foot-and-mouth disease (FMD) in a previously FMD-free country can cause significant economic damage from the immediate and prolonged closure of FMD-sensitive markets. Whilst emergency vaccination may help with disease containment, the presence of vaccinated animals complicates post-outbreak surveillance and the recovery of FMD-free status.

We present enhancements to the Australian Animal Disease Spread Model (AADIS) that allow comparisons of post-outbreak management strategies for securing proof-of-freedom from FMD and return to trade. These include: surveillance of previously infected areas (according to configurable sampling regimes), in order to obtain statistical confidence of freedom from FMD, and economic assessment of retaining vaccinated animals in the population compared to removing them to waste or for salvage.

Materials and methods

A case study is presented that compares vaccinate-and-retain, vaccinate-and-remove-to-waste and vaccinate-and-remove-for-salvage strategies, and their impact on the recovery of FMD-sensitive markets (per OIE guidelines).

Results

Removing vaccinated animals resulted in higher post-outbreak management costs but lower overall costs (due to reduced trade losses), than retaining them. Under the assumptions of the study there were no cost benefits of salvaging vaccinated animals. The potential salvage revenue was offset by trade losses associated with the increased time required for removal, and the delay in regaining markets.

Discussion

The new modelling capability will support the development and refinement of post-outbreak management policies that facilitate the earliest possible recovery of FMD-free status and return to trade.

APPENDIX 41: VACCINE EFFICACY OF FMD VIRUS-LIKE PARTICLES PRODUCED BY THE BACULOVIRUS EXPRESSION SYSTEM

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Introduction

Vaccine production is currently carried out in high containment manufacturing facilities, resulting in very high production costs and lack of production capacity. There is also room for improving the stability of conventional killed foot-and-mouth disease virus (FMDV) vaccine, and the time to market should be improved in case of new outbreaks. To address these issues, a system to express FMDV virus like particles (VLPs) was developed. The possibility of producing VLP-based FMD vaccines in conventional facilities greatly increases the flexibility and significantly lowers costs of commercial manufacturing. The utility of VLPs can be further enhanced by improving their thermostability and stability at low pH by introducing structure-guided amino acid changes in the VLPs.

Materials and Methods

VLPs were expressed in insect cells using the baculovirus expression system and used to formulate vaccines with an appropriate adjuvant. Cattle were vaccinated with these vaccines and the virus neutralising antibody titres (VNT) were measured. Vaccine efficacy was also determined in vaccination-challenge experiments.

Results

Mutations that have been predicted to enhance VLP stability were cloned into baculovirus expression constructs and stabilized VLPs could be expressed for A, O, Asia1, and SAT2 strains at satisfying yields. VLP-based vaccines induced neutralising antibodies in cattle consistent with protection. This was confirmed by high containment cattle challenge experiments in several cases.

Discussion

Virus-like particles have now the potential to be a commercially viable alternative to conventional killed vaccines.

APPENDIX 42: A CURRENT PERSPECTIVE ON ADENOVIRUS 5-VECTORED FMD VACCINES

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Introduction

Over the years, viral vectors have been used to deliver FMDV structural proteins, with the aim of synthesizing virus-like particles (VLPs) in infected cells, potentially inducing both, humoral and cell-mediated immunity. Numerous groups have focused on using different viral vectors, including vaccinia virus, fowlpox virus, attenuated pseudorabies virus (PRV) or “single cycle” Semliki forest virus (SFV). Most of these FMD vaccines were tested in a limited number of livestock species, usually requiring vaccination boosts and offering partial protection in swine and/or cattle.

To date, the most successful strategy to induce protection against FMD in livestock, as an alternative to the inactivated whole FMDV vaccine, is the use of a recombinant-replication- defective human adenovirus type 5 coding for FMDV capsid proteins and also the 3Cpro that allows for the in vivo assembly of VLPs (Ad5-FMD). These vaccines protect swine and cattle from clinical disease as early as 5 days post vaccination. In fact, an Ad5-FMD serotype A24 vaccine has been recently granted licensure for emergency use in cattle in USA. The main advantages of this vaccine platform are: i) it does not require a high containment facility for production; ii) it has intrinsic DIVA capabilities; iii) it does not require the adaptation of field strains to vaccine production in cell culture; and iv) it is genetically stable. However, the Ad5-FMD technology requires further industrial development for large scale production and broad use internationally, especially in developing countries. In this section we will discuss recent developments aimed at improving vaccine potency, vector stability, route of delivery, all strategies focused on achieving fast protection with one vaccine dose, at the lowest cost possible and with not significant side effects. Furthermore, we will discuss current challenges and possible solutions aimed at improving Ad5-FMD vaccine performance.

APPENDIX 43: FMD-LL3B3D VACCINE PLATFORM: SAFE, HIGHLY POTENT, FULLY DIVA COMPATIBLE, INACTIVATED FMDV VACCINES

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Introduction

Traditional chemically-inactivated foot-and-mouth disease virus (FMDV) vaccines are used to control FMD around the world in spite of drawbacks - (1) large quantities of virulent FMDV are used, with the risk of virus escaping from manufacturing facilities or incomplete inactivation during the vaccine formulation process; (2) traditional vaccines produced from wild type FMDV are not fully compatible with a DIVA approach, since small amounts of nonstructural proteins (NSPs) may still be present; and (3) they do not fully protect animals from persistent infection.

Materials and methods

A novel, marked FMD-LL3B3D vaccine platform under development by Zoetis Inc. and The United States Department of Agriculture - Agricultural Research Service, consists of an attenuated virus platform containing negative markers in the NSPs 3B and 3Dpol. This vaccine platform allows for the easy exchange of capsid coding sequences. In contrast to wild-type FMD vaccine viruses, the FMD-LL3B3D vaccine platform viruses induce no clinical signs of FMD and no shedding of virus in cattle or pigs when inoculated as a live virus. This vaccine platform uses existing FMD vaccine manufacturing technology without the biosafety risk associated with FMD vaccine production.

Results

Cattle immunized with a variety of inactivated FMD-LL3B3D vaccine constructs formulated with a proprietary adjuvant system were protected from challenge with parental virus. Two negative markers allow the FMD-LL3B3D vaccines to be fully DIVA compatible.

Discussion

This vaccine platform, currently undergoing development, provides opportunities for safer and higher potency FMD vaccines in support of global disease control and eradication programs.

APPENDIX 44: AN OVERVIEW OF REVERSE GENETIC APPROACHES TO ENHANCED FMD VACCINES IN AFRICA

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Introduction

The control of FMD in Africa is complicated by several factors, including the unique epidemiological situation where five of the seven serotypes are present on the African continent; the antigenic diversity of each serotype; maintenance of the virus by persistently infected African buffalo, instability of SAT type vaccines; and the socio-economic conditions in Africa. The most successful way to manage the disease in Africa is via regular livestock vaccination programmes and physical separation of wildlife and livestock. Here we discuss the progress that has been achieved in the development of improved vaccine seed viruses, tailored for the conditions in Africa, using structural design and reverse genetics.

Materials and methods

We compared vaccines based on genetically modified or structurally stabilised vaccine and wild type antigens in eliciting protective immunity against live virus challenge. Epitope-replaced mutant viruses were also assessed in terms of antigenic distance using virus neutralisation assays and reactivities to SAT2-specific monoclonal antibodies (mAbs).

Results

Inter- and intra-serotype chimeric vaccines conferred protective immunity. However, capsid swapping, during the production of chimeric vaccine seed viruses, may transfer other undesirable traits such as capsid instability and poor cell culture adaptation, which are limitations that were overcome by site-directed mutagenesis of the amino acid(s) associated with improved performance. Recently, we compared SAT2 viruses, containing mutations adjacent to the inter-pentameric interfaces to improve the conformational stability of the capsid, as vaccine antigen and found it to elicit superior protective immune responses in cattle. Using charge-dampened mutants the reactivity of anti-FMDV antibodies in the sera from structurally stabilized SAT2 FMD vaccinated animals against dominant antigenic sites on the structurally-exposed protein targets were assessed.

Discussion

Chimeric viruses, enhanced epitopes, cell-culture adapted viruses and stabilised are important improvements to African FMD vaccines. Any applied research into the development of novel FMD vaccines and disease control strategies for Africa need to enable a fit-for-purpose approach to FMD control in Africa.

APPENDIX 45: RATIONAL DESIGN OF ATTENUATED FMDV VACCINES BY ELEVATION OF –CPG- AND –UPA- DINUCLEOTIDE FREQUENCIES

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Introduction

Live, attenuated, vaccines have classically been developed by serial passage of virus in tissue-cultured cells: progeny viruses are analysed for attenuation throughout this process. Often, attenuation is based upon a small number of key mutations which may back-mutate during vaccine production, or, following administration: reversion to virulence. Modern molecular biology, in combination with reverse genetics, has facilitated FMDV attenuation by deletion/mutation of virus proteins, or, by transposition of RNA secondary structures. An alternative method - Synthetic Attenuated Virus Engineering (SAVE) - produces attenuated viruses by the rational design of genomes to include high numbers of synonymous mutations. Initially attributed to alteration of codon-pair bias, it has been shown that attenuation is due to elevation of –CpG-/–UpA- dinucleotide frequencies.

Materials and methods

Using synthetic biology we have produced FMDV ‘replicon’ systems which allow us to quantify RNA replication in real-time, in live cells. Our replicons allow the rapid determination of replicative fitness and, constructed as ‘cassette’ systems, allows genomic regions to easily be replaced. In this manner the various strategies of FMDV attenuation have been compared.

Results

Data will be presented comparing the replicative fitness of a wide range of published modified FMDV genomes, together with our SAVE genomes, in a range of cell-types. Replicons have been converted into infectious copies and virus rescued.

Discussion

Attempts in the 1960s to produce live, attenuated, FMDV vaccines via the ‘classical’ method were unsuccessful – most probably through a lack of genetic stability. Attenuation by SAVE, however, involves high numbers (many 100s) of such mutations providing a very much higher degree of stability. Our attenuation strategy is the introduction of synonymous mutations into the replication proteins (alone) producing a ‘pre-attenuated’ genomic ‘backbone’ into which capsid proteins from any sub-type can be inserted to produce a vaccine: a rapid response to new outbreak strains.

APPENDIX 46: FIELD TRIAL TO ESTIMATE THE EFFECTIVENESS OF THE VACCINATION PROGRAM IMPLEMENTED IN THE MAGHREB REGION

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Introduction

Routine or emergency vaccination are strategic tools to control FMD. Preliminary estimates of vaccine effectiveness can be obtained by confined field studies, contributing to optimizing control programs.

Field trials to evaluate effectiveness of FMD vaccines currently used in the Maghreb region have been designed; here we report the results obtained from the trial conducted in Morocco.

Materials and Methods

A bivalent vaccine (A/Eritrea-98 6PD50 and O-Manisa/O-3039 3PD50) was administered to 20 naïve calves and 20 previously vaccinated cattle. Sera were checked before and 30 days post vaccination (DPV), with an intermediate sampling 5 or 10 DPV for vaccinated and naïve cattle respectively. The level of virus neutralizing (VN) antibodies against the vaccine strains and the field viruses was determined; in addition, sera were titrated using IZSLER ELISA kits.

Results

In previously vaccinated cattle, vaccination elicited a strong and fast increase (up to 10X, 5 DPV) of neutralizing antibodies, suggestive of protective immunity, with overlapping titres against the two type O vaccine strains and the field virus O-ALG/1/2014 (lineage O-Ind2001); antibodies had further increased at 30 DPV, reaching average titres of $\geq 3 \text{ Log}_{10}$. Analogous trend was observed for type A, though titres to the vaccine strain were 3-fold higher than those against the field virus A/Algeria/1/2017 (despite both belong to the same lineage A/Africa/G-IV).

All naïve calves seroconverted from negative to positive after vaccination, but the level of VN antibodies remained lower than in the boost-vaccinated group. The best immune response was observed against the vaccine strain A/Eritrea-98, with 95% animals overcoming the presumed protective threshold, whilst only about 50% achieved sufficient immunity against the other FMDV strains tested.

ELISA provided results consistent with VNT for boost-vaccinated cattle, whilst it was less sensitive to detect antibodies in prime-vaccinated calves.

Discussion

A booster vaccination is necessary to elicit a strong and fast increase of antibodies, cross-neutralizing field circulating viruses. Simple and feasible field trials enable producing relevant information for improving FMD control and preparedness against reoccurrence of outbreaks.

APPENDIX 47: MODELLING THE IMPACT OF FARMING PRACTICES UPON VACCINE EFFECTIVENESS IN ENDEMIC SETTINGS – A CASE STUDY IN KENYA

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Introduction

In many lower and middle-income countries in Africa, the Middle East and Southern Asia, livestock owners bear a significant impact as a result of regular FMD epidemics. However, lack of information on localized risks and appropriate prevention measures affects their ability to effect control. Additionally, the inherent uncertainty in disease reporting, inconsistent implementation of interventions and the use of imperfect vaccines means that there are significant challenges for policy makers and local farmers in managing the disease. It is therefore crucial to develop bespoke modelling tools that can appropriately capture farming practices in endemic regions and to establish appropriate controls accordingly.

Methods

In this work, we use epidemiological, demographic and behavioural transect study data gathered through a series of EuFMD training workshops to develop a cross scale mathematical model to predict the spread of FMD in Nakuru County, Kenya. The model is then utilized to investigate the potential for reactive vaccination to reduce the incidence of FMD in the future.

Results

Analysis of the transect study data indicates that farmers that use common grazing and water sources are at increased risk of infection with FMD. The mathematical model results show that the use of high efficacy vaccines is crucial to decrease the incidence of FMD in Nakuru, even when vaccine coverage is relatively low. In addition, in the presence of limited resources, targeting control towards those farms that are at increased risk through shared resources can have beneficial effects when controlling future outbreaks.

Discussion

the fact that traditional proximity-based models are inappropriate to capture FMD transmission in countries such as Kenya, where local farming practices can have a significant effect upon infection risk. We have also highlighted the urgent need for procurement and deployment of high efficacy vaccines to ultimately assist in progression towards disease freedom.

APPENDIX 48: GENETIC CHARACTERIZATION OF FMDV RESPONSIBLE FOR OUTBREAKS IN NIGERIA DURING 2016: RESURGENCE OF THE NOVEL FMD- SAT1 TOPOTYPE

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Introduction

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

Materials and methods

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

Results

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

Discussion

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

APPENDIX 49: SEROLOGICAL AND MOLECULAR EPIDEMIOLOGY OF FMD VIRUSES IN AGRO-PASTORALIST LIVESTOCK HERDS IN THE KACHIA GRAZING RESERVE, NIGERIA

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Introduction

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

Materials and methods

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

Results

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

Discussion

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

APPENDIX 50: COMPLEX CONCOMITANCE OF FMDV STRAINS IN NIGERIA

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Introduction

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

Materials and methods

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

Results

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

Discussion

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

APPENDIX 51: FOOT-AND-MOUTH DISEASE IN SMALL RUMINANTS AND IN WILDLIFE IN NORTHERN NIGERIA

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Introduction

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

Materials and methods

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

Results

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

In wildlife, 44.7% of the samples tested positive whereas 55.3% tested negative. Of the positive samples, 76.5% reacted with SP and NSP, 11.8% reacted with SP only and 11.8% with NSP only. Of the samples reacting with SP, 6.7% was mono-specific for serotype O, 40.0% mono-specific for A and 53.3% reacted with 2 or 3 serotypes (O, A and SAT2).

Discussion

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

APPENDIX 52: SEROTYPING SEROPREVALENCE OF FMD IN CATTLE FROM UGANDA SURVEILLANCE 2014-2018

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Introduction

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

Material and methods

The study was designed to conduct FMDV surveillance in cattle from widespread regions of Uganda from 2014-2018. Serum samples were subjected to the FMDV antibody detection against non-structural proteins (NSP) using the Priocheck FMDV NS ELISA kit and for serotyping the Priocheck FMDV type A and O kits following manufacturer's instructions. For SAT serotyping the in-house SPCE test was performed (ARC, South Africa). Briefly, Rabbit anti-serum raised to SAT serotype specific FMDV was used to capture the SAT type-specific antigen, thereafter the competition between guinea-pig antiserum and antibodies present in the test serum were measured. The percentage inhibition was calculated with >50 % classified as positive.

Results

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

Discussion

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

APPENDIX 53: FOOT-AND-MOUTH DISEASE IN BURUNDI

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Introduction

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

Materials and methods

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

Results

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

Discussion

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

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

**APPENDIX 54: DOES THE AFRICAN BUFFALO REALLY SPREAD FMD?
A SERO-SURVEY OF FMD IN CATTLE AROUND MANA POOLS CONSERVATION PARK OF NORTHERN ZIMBABWE**

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Introduction

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

Materials and Methods

The study investigated whether the serological picture in cattle explains buffalo/cattle contact patterns observed at the periphery of LZ-MP TFCA. 1238 cattle sera were collected from 2 districts in a two-stage random sampling protocol. Samples were tested for antibodies to the non-structural protein of FMD virus using the Enzyme Linked Immunosorbent Assay (NSP-ELISA). NSP positive sera were subjected to the Liquid Phase Blocking ELISA (LPBE). Risk factors perceived to influence FMD virus infection in cattle were interrogated using a questionnaire.

Results

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

Conclusion

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

APPENDIX 55: CONTROL METHODS OF FMD IN BENIN BY TRIAL VACCINATION AND MEDICINAL PLANTS

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Introduction

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

Material and methods

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

Result

The NSP rates from farm 1 were 54%, 62.5% and 48.97% on days 0, 30 and 120, respectively without a significant difference. However, in Farm 2, the NSP rate of day 30 (71.19%) was not differed significantly from that of day 120 (75%). 32 medicinal plants were listed by breeders. *Vitellaria paradoxa* is the most cited with three types of recipes. Thus medicinal plants such as *Citrus limon* L., *Pterocarpus erinaceus*, *Acacia nilotica* L, *Lannea acida*, *Khaya senegalensis* were each involved in two types of recipes. Barks are involved in 14 types of recipes and maceration is the method most used with 27% types of recipes followed by the powder with 24%.

Discussion

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

APPENDIX 56: MOLECULAR CHARACTERISATION OF FMDV DETECTED DURING 2015 - 2018 IN TANZANIA: INSIGHTS FOR VIRUS DIVERSITY AND EVOLUTION IN AFRICA

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Introduction

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

Methods

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

Results

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

Discussion

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

Acknowledgement

This work was funded by Wellcome Trust Intermediate Fellowship Grant WT104017MA and the Government of Tanzania.

APPENDIX 57: FMD SURVEILLANCE AND CONTROL IN MALI

Dr. Abdoulaye Diaouré VSF-international

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

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

Considering the realities of the livestock system in Mali (extensive system with transhumance but also an intensive system with crossbreeding of local and imported breeds) has pushed to determining 3 areas for this study. Western and Eastern area to cover the herds' transboundary movements and The peri-urban area of Bamako to cover crossbred dairy cows.

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

Key words

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

APPENDIX 58: A GVII-2015, A NEW HIGH POTENCY VACCINE WITH BROAD PROTECTION AGAINST A/ASIA/G-VII THREAT

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Introduction

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

Materials and methods

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

Results

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

Discussion

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

APPENDIX 59: INTRADERMAL APPLICATION OF FMD VACCINES FOR PIGS

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Introduction

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

Materials and methods

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

Results

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

Discussion

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

APPENDIX 60: EFFICACY OF A/MAY/97 FMDV VACCINE AGAINST HETEROLOGOUS CHALLENGE WITH A FIELD VIRUS FROM THE EMERGING A/ASIA/G-VII LINEAGE IN CATTLE

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Introduction

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

Materials and methods

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

Results

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

Discussion

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

APPENDIX 61: A SIMPLE UNIVERSAL TEST TO QUANTITATE 146S ANTIGEN DURING PRODUCTION OF FMD VACCINES

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Introduction

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

Materials and Methods

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

Results

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

Discussion

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

APPENDIX 62: IMPROVING THE DURATION OF IMMUNITY FOR FMD VACCINES

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Introduction

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

Materials and methods

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

Results and Discussion

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

APPENDIX 63: THE USE OF REVERSE GENETICS TO FACILITATE THE GROWTH OF FMDV FOR THE PRODUCTION OF VACCINES

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Introduction

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

Materials and Methods

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

Results

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

Discussion

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

APPENDIX 64: MULTIPLEX REAL-TIME RT-PCR FOR DETECTION OF FMDV, RIFT VALLEY FEVER VIRUS AND BOVINE VIRAL DIARRHEA VIRUS IN BULK TANK MILK

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Introduction

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

Materials and Methods

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

Results

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

Discussion

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

APPENDIX 65: EMERGENCY SUPPLY OF FMD DIAGNOSTIC KITS: REAGENT BANKS AND IDVET SOLUTIONS

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Introduction

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

Materials and Methods

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

Conclusion

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

APPENDIX 66: RESULTS FROM AN INTER-LABORATORY EXERCISE TO EVALUATE NON-STRUCTURAL PROTEIN ELISA KITS

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Introduction

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

Method and Results

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

Discussion

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

APPENDIX 67: RESULTS OF THE 2016 AND 2017 PROFICIENCY TESTING SCHEMES FOR FMD DIAGNOSTIC METHODS

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Introduction

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

Materials and Methods

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

Results

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

Discussion

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

APPENDIX 68: THE INTERDEPENDENCE OF FMDV PATHOGENESIS, CHALLENGE SYSTEM, AND OUTCOME OF VACCINE STUDIES

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Introduction

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

Materials and Methods

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

Results

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

Discussion

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

APPENDIX 69: GOOD CORRELATION BETWEEN VACCINE MATCH IN POTENCY TESTS AND R1-VALUE

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Introduction

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

Heterologous potency = match x homologous potency.

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

Materials and Methods

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

Results

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

Discussion

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

APPENDIX 70: POTENCY ASSESSMENT OF FMD VACCINES USING STANDARDISED SEROLOGICAL ASSAYS

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Introduction

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

Materials and methods

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

Results and discussions

Significant differences ($p < 0.05$) were found in the titre of the control serum tested in the three laboratories, in both the ELISA and VNT. Only a small difference was found in the mean titre in protected and non-protected cattle. In both the ELISA and VNT, a very significant ($p < 0.01$) influence of the laboratory was found on the correlation between antibodies titres, but also between standardised titres, and protection. In the ELISA, slope the correlation between antibodies and protection was lower when titres of the sera collected at day 21 post-challenge were analysed in comparison with the titres found on the day of challenge (for experiments performed in Brussels and Pirbright, these were the same). The slope was steeper when analysing the results obtained in VNT compared to ELISA.

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

APPENDIX 71: DETECTION OF FMDV O/ME-SA/IND-2001E IN JORDAN

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Introduction

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

Materials and methods

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

Results

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

Discussion

During this period, viruses of the FMDV/O/ME-SA/Ind-2001e sublineage have spread to the Middle East, North Africa and Southeast Asia. The nucleotide sequence of the VP1 gene of the O/JOR/1/2017 sublineage is very similar to that of viruses isolated from Saudi Arabia in 2016, indicating possible introduction of this strain to Jordan through animal movement or other transmission routes from Saudi Arabia.

APPENDIX 72: THE ASSOCIATION BETWEEN BORDER PROVINCES OF TURKEY AND FMD OUTBREAKS

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Introduction

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

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

Materials and methods

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

Results

The results of this study were presented in the OS18.

Discussion

The discussion were presented in the OS18.

APPENDIX 73: INTEGRATED RISK-BASED STRATEGIC PLANS FOR FIVE PRIORITY DISEASES IN THE PALESTINIAN AUTHORITY

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Introduction

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

Materials and methods

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

Results

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

Discussion

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

Key words

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

Acknowledgement: Khawla Alnjoum, Ludo Plee and Azzam Saleh of FAO-GZ.

APPENDIX 74: EMBEDDING PROGRESSIVE CONTROL FOR FMD IN THE POLICY AGENDA FOR LIVESTOCK PRODUCTION IN THREE COUNTRIES IN SOUTHEAST ASIA

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Introduction

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

Material and methods

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

Results

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

Discussion

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

Acknowledgements: Australia's Department of Foreign Affairs and Trade, Australia's Department of Agriculture and Water Resources, Ministry of Foreign Affairs and Trade, New Zealand.

APPENDIX 75: TRANS-POOL MOVEMENT OF TWO FMDV VIRUS SEROTYPE A LINEAGES: A/ASIA/G-VII AND A/AFRICA/G-IV

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Introduction

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

Materials and methods

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

Results

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

A/AFRICA/G-IV: Sequence data analyses show grouping of sequences based on geographical origin into East and West African clusters. These analyses also indicate the origin of the 2017 Algeria and Tunisia outbreaks in West Africa.

Discussion

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

APPENDIX 76: SOCIO-ECONOMIC IMPACT OF FMD OUTBREAKS AND CONTROL MEASURES AT DIFFERENT SCALES IN MONGOLIA: FROM NATIONAL LEVEL GROSS LOSSES TO HERDERS' FOOD SECURITY

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Introduction

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

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

Material and Methods

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

Results

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

Discussion

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

APPENDIX 77: RETROSPECTIVE FMD OUTBREAK REPORTS FROM UGANDA AND TANZANIA BORDER DISTRICTS (2011-2016): IMPLICATIONS FOR FMD CONTROL BY VACCINATION

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Introduction

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

Materials and methods

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

Results

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

Discussion

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

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

APPENDIX 78: UPDATE OF FMD IN THE MAGHREB REGION: VACCINATION ISSUES

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Introduction

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

Materials and methods

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

Results

The viral lineage of serotype O that exist in North Africa is O/ME-SA/Ind-2001, which is normally present in the Indian Subcontinent, which proves the long distance movements of genotype. The most used method to choose the vaccine strain is in vitro vaccine matching. This test, gives different results of 2 isolates (Morocco, Algeria) belonging to the same topotype, and even more, a difference in the results for 2 viruses of the same topotype isolated in Algeria has been shown.

The virus prevailing in Morocco and Algeria, have a poor in vitro vaccine matching of O1Manisa vaccine strain, while a study using the in vivo efficacy, support the use of the O1 Manisa vaccine to control the same topotype. The A/AFRICA G-IV was isolated in Algeria in 2017. The vaccine matching shows that the isolates correspond to two available vaccine strains, with r1 limit values.

Discussion

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

APPENDIX 79: ASSESSMENT OF FMD VACCINES IN MONGOLIA AND THE ROLE OF BACTRIAN CAMELS

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¹ State Central Veterinary Laboratory, Mongolia; ² Veterinary and Breeding Agency, Mongolia; ³ Provincial Veterinary Laboratory, Orkhon, Mongolia; ⁴ General Agency Specialized Inspection, Mongolia; ⁵ Veterinary Drug Quality Control Laboratory, Mongolia; ⁶ The Pirbright Institute, UK; ⁷ European Commission for the Control of Foot-and-Mouth Disease (EuFMD), Food and Agriculture Organisation of the United Nations, Rome, Italy.

Introduction

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

Material and methods

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

Results

Between January 2017 and April 2018, outbreaks were reported in cattle, sheep, goats and camels in 9 different provinces affecting 1,277 herders. During this time, the province level incidence in different species ranged from 0.02-2.3% in cattle, 0-0.17% in sheep, 0-0.07% in goats and 0-1.7% in Bactrian camels. From the latter, virus has been successfully isolated and is awaiting sequencing.

The PVM studies revealed higher titres in response to oil based vaccines for all strains. Although similar in cattle and sheep, in camels titres were significantly lower. Significantly higher titres were seen with a two dose primary course.

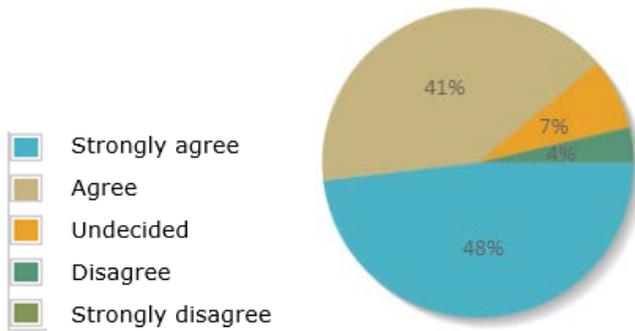
Discussion

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

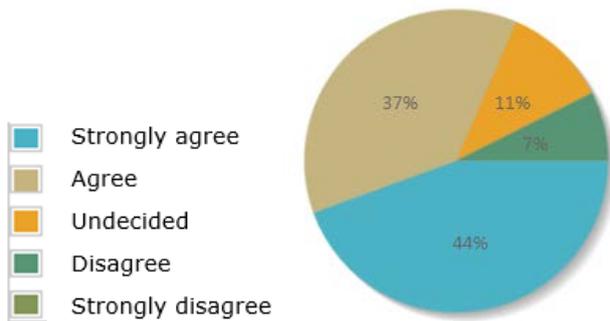
APPENDIX 80: MOBILE APPLICATION RESULTS

The Weigh In section of the mobile application invited participants to provide their feedback on a number of 'big ideas' using a five-point likert scale from strongly agree to strongly disagree. A summary of these 'big ideas' and participant feedback is presented below.

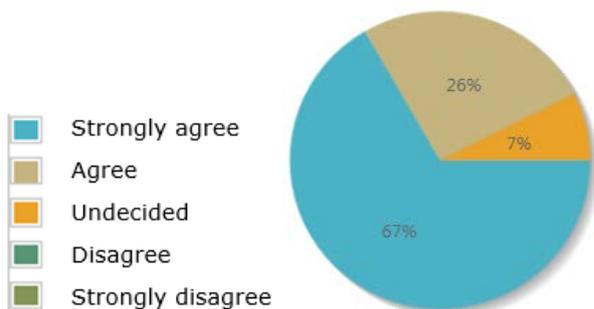
Idea 1: Improving vaccine availability needs urgent attention by both the public and private sectors and a new form of partnership is needed to achieve real change in vaccine supply.



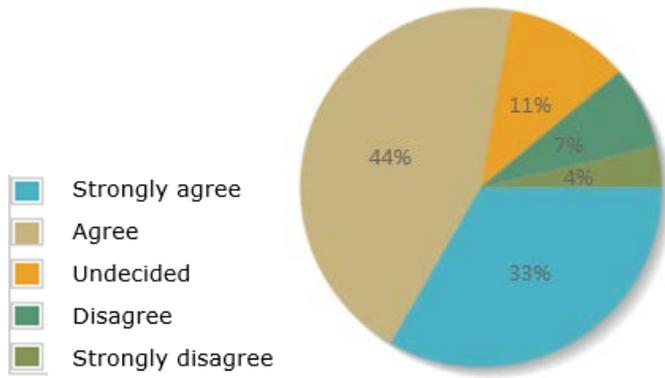
Idea 2: Development of a public-private professional network is needed to increase global security in the supply of effective vaccines.



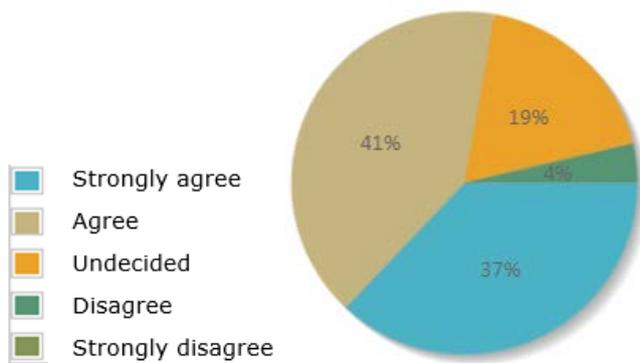
Idea 3: Quality vaccines are not enough, the barriers that prevent their availability must be addressed.



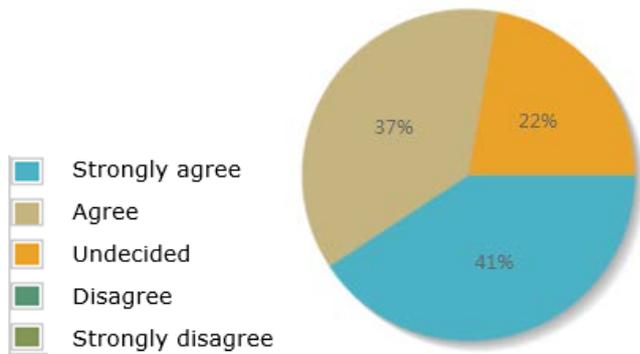
Idea 4: Inadequate access to effective vaccines in endemic regions is an animal welfare issue.



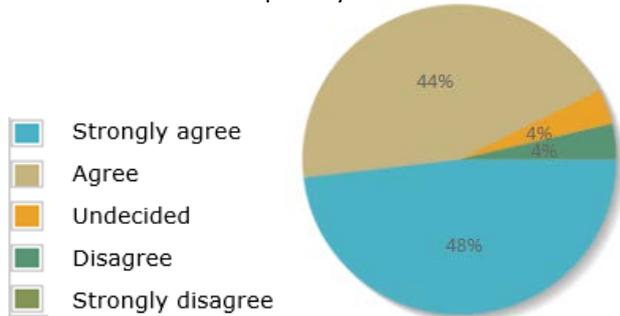
Idea 5: There exists a willingness to pay for vaccine for FMD, but further work is needed to understand the drivers for adoption and barriers to uptake.



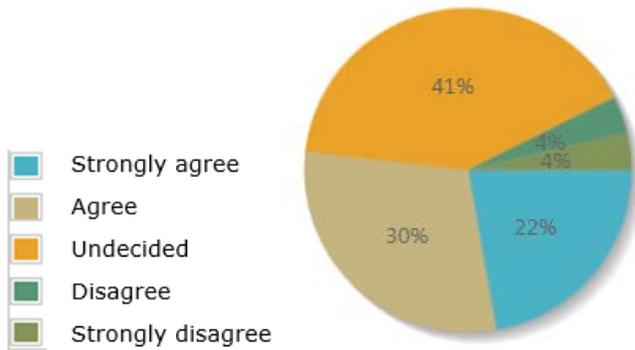
Idea 6: In many endemic settings, livestock keepers should have the right to access effective vaccines to protect their livestock and livelihoods.



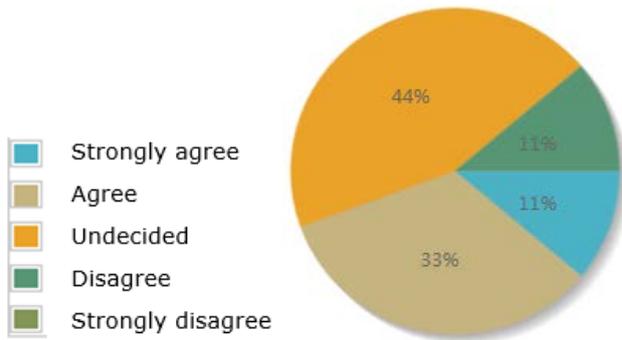
Idea 7: Further work to quantify the unmet demand for vaccines and predict future growth is needed.



Idea 8: The traditional vaccine bank model is poorly equipped to respond to trans-pool movements of viruses, and innovative approaches to vaccien resources are required.



Idea 9: Application of new modelling tools are needed to address the range of policy and operational issues related to vaccine resources.



Idea 10: EuFMD Biorisk Management Committee should examine the question of what level of FMD attenuation would be safe for use as a modified live vaccine in an endemic or free region.

