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Day

1

SUPPORT

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Dear colleagues,

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

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

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

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

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

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

DAY 1 Plenary session

YOUR AGENDA

1	OPENING
09.00	**OPENING**
09.30	**Keynote:** GLOBAL OVERVIEW. *K. Sumption*
10.00	**Keynote:** GLOBAL STATUS REPORT FOR FMD: TRACKING THE EMERGENCE AND SPREAD OF NEW VIRAL LINEAGES. *D.P. King*
2	THE SCALE OF THE PROBLEM
11.00	MODELLING FMD VACCINE REQUIREMENTS FOR MULTI-COUNTRY FMD OUTBREAKS IN EUROPE. *TBC*
11.15	EVALUATING VACCINATION STRATEGIES TO CONTROL FMD: A COUNTRY COMPARISON STUDY. *R. Sanson*
11.30	UNDERSTANDING VACCINE DEMAND IN THE ENDEMIC SETTING. *C. Miller*
11.45	HOUSEHOLD PERCEPTIONS OF RISK AS DRIVERS FOR ADOPTION OF FMD VACCINATION. *A. Railey*
12.00	MASS FMD VACCINATION IN CENTRAL MYANMAR, 2015-2016. *Y. Qiu*
12.15	ASSESSMENT OF THE RISK OF INCURSION OF EXOTIC FMD VIRUSES INTO SOUTHEAST ASIA. *C. Bartels*
3A	VACCINE SUPPLY
14.00	H.S. FRENKEL AND J. VAN BEKKUM, HOW THEY IMPROVED VACCINE AVAILABILITY AND QUALITY. *A. Dekker* *(Available upon request)*
14.15	VACCINE BANKS: POLICY OPTIONS EVALUATED USING THE EU EVALUATION FRAMEWORK. *R. Bergevoet*
14.30	THE CHALLENGES OF FMD VACCINE PRODUCTION. *P. Hudelet*
14.45	MUTUAL REGISTRATION OF VACCINES IN EAST AFRICA: PROGRESS AND ISSUES FOR BETTER ACCESS TO EFFECTIVE FMD VACCINES? *N.M. Aineplan*
14.45	*Discussion*

YOUR AGENDA

DAY 1 Plenary session

4A	BREAKING BARRIERS (pag.46)
16.00	ACCESS OF FARMERS AND CO-OPERATIVES TO VACCINES. *K. Mintiens*
16.15	EXPLORING PRIVATE AND PUBLIC SECTOR RIGHTS AND RESPONSIBILITIES IN PREVENTION AND CONTROL OF FMD: THE CASE OF RIGHT TO ACCESS VACCINES BY LIVESTOCK KEEPERS. *B. Ahmadi*
16.30	FMD RESEARCH GAP ANALYSIS WORKSHOP 2018. *M. Pérez-Filgueira*
16.45	BIOSAFETY BARRIERS. *K. Tjornhoj*
17.00	*Discussion*
from 17:30	POSTER SESSIONS

DAY 1 Parallel session

YOUR AGENDA

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COMPLETE GENOME SEQUENCE ANALYSIS OF OVER 140 FMD VIRUSES ISOLATED FROM FREE-LIVING AFRICAN BUFFALO (SYNCERUS CAFFER) IN ZIMBABWE. *N. Knowles*	14.15
EVOLUTION AND COMPETITION OF SAT STRAINS DURING BUFFALO TRANSMISSION IN A CONTROLLED CHALLENGE EXPERIMENT. *K. Scott*	14.30
FMDV EVOLUTIONARY DYNAMICS WITHIN INFECTED BUFFALOES AND ITS LARGE-SCALE CONSEQUENCES. *L. Ferretti*	14.45
ANTIBODY RESPONSES TO THE MAJOR ANTIGENIC SITES OF FMD VIRUS SEROTYPE O AFTER PRIMO-VACCINATION, RE-VACCINATION AND AFTER NATURAL EXPOSURE. *J. Biswal*	15.00
Discussion	15.15

IMMUNOPATHOLOGY (pag. 68)	4B
GENE SIGNATURES ASSOCIATED WITH FMD VIRUS INFECTION AND PERSISTENCE PART I: PERSISTENT FMDV INFECTION IN AN AIR-LIQUID INTERFACE MODEL OF BOVINE SOFT PALATE. *J. Valarcher*	16:00
GENE SIGNATURES ASSOCIATED WITH FMD VIRUS INFECTION AND PERSISTENCE PART II: TRANSCRIPTOMIC ANALYSIS OF ACUTE AND PERSISTENT FMDV INFECTION IN BOVINE SOFT PALATE. *F. Pfaff*	16:15
TRANSMISION OF FMD FROM PERSISTENTLY INFECTED CARRIER CATTLE TO NAÏVE CATTLE VIA TRANSFER OF OROPHARYNGEAL FLUID. *J. Artz*	16.30
Discussion	16.45
POSTER SESSIONS	from 17:30

YOUR AGENDA

DAY 1 morning / SESSION 2
HOUSEHOLD PREFERENCES FOR DIAGNOSTIC TESTING TO VACCINE MATCH IN AN ENDEMIC SETTING. *A. Railey*
DAY 1 afternoon / SESSION 3B
VP1 IS IMPORTANT IN HEPARIN SULPHATE BINDING OF FMDV STRAIN O MANISA. *A. Dekker*
REPLICATION DYNAMICS OF MIXED FMD VIRUSES IN VITRO. *E. Foglia*
USING HIGH THROUGHPUT SEQUENCING TO CHARACTERISE LOW-FREQUENCY DIVERSITY OF FMDV DURING VACCINE STRAIN ADAPTATION. *D. King*
IDENTIFICATION OF NOVEL ANTIBODY BINDING DETERMINANTS OF SEROTYPE O FMDV. *M. Mahapatra*

DAY 1

PARALLEL SESSION		PLENARY SESSION
	9:00h	1. Opening
	11:00h	2. The scale of the problem
3B. Virology	14:00h	3A. Vaccine supply
4A. Immunopathology	16:00h	4A. Breaking barriers

GLOBAL OVERVIEW

K. Sumption[1]

[1]*European Commission for the Control of Foot-and-Mouth Disease – FAO Rome*

AVAILABLE UPON REQUEST

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

D.P. King, V. Mioulet, A. B. Ludi, N. Knowles, B. Wood, A. Gray, B. Thapa, L. Henry, M. Azhar, H. Baker, G. Wilsden, C. Browning, M. Henstock, B. Statham, A. Bin-Tarif, K.Bachanek-Bankowska, J. Wadsworth, A. Di Nardo, DJ. Paton, N. Lyons, A. Morris, D. Wiseman, B. Johns, S. Belgrave and J. Maryan, on behalf of the OIE/FAO FMD Laboratory Network

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Summary of global situation

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.

Discussion

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.

MODELLING FMD VACCINE REQUIREMENTS FOR MULTI-COUNTRY FMD OUTBREAKS IN EUROPE

G. Garner[1], M. Hovari[1] and K. Sumption[1]

[1]European Commission for the Control of Foot-and-Mouth Disease – FAO Rome

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

Bradhurst RA, Roche SE, Kwan P and Garner MG (2015) A hybrid modelling approach to simulating foot-and-mouth disease outbreaks in Australian livestock. Front. Environ. Sci., 19 March 2015. http://dx.doi.org/10.3389/fenvs.2015.00017

EVALUATING VACCINATION STRATEGIES TO CONTROL FMD: A COUNTRY COMPARISON STUDY

T. Rawdon [1], G. Garner [2] , R. Sanson [3], M. Stevenson [4], C. Cook [5], C. Birch [5], S. Roche [2], K. Patyk [6] , K. Forde Folle [6], C. Dubé [7] , T. Smylie [8], Z. Yu [9]

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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.

UNDERSTANDING VACCINE DEMAND IN THE ENDEMIC SETTING

K. Sumption, C. Miller

European Commission for the Control of Foot-and-Mouth Disease – FAO Rome

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 at the herd level, 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

In many settings, individual livestock owners have the economic means to afford vaccination, but private access to FMD vaccines is very limited. 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. The current quantity of effective vaccines available on the global market is
unlikely to meet this demand.

Discussion

Lack of global FMD vaccine security affects everyone, and addressing this issue requires innovating thinking and a restructuring of public sector and private industry roles. 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.

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 neighbor 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.

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 (≥6PD50) 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.

ASSESSMENT OF THE RISK OF INCURSION OF EXOTIC FMD VIRUSES INTO SOUTHEAST ASIA

C.J.M. Bartels[1], J. Afonso[1], S. Sieng[1], and M. McLaws[1]

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.

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.

H.S. FRENKE AND J. VAN BEKKUM, HOW THEY IMPROVED VACCINE AVAILABILITY AND QUALITY

A. Dekker

AVAILABLE UPON REQUEST

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

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.

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

Noel M. Aineplan

Uganda National Drug Authority

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 immunologicals and veterinary pharmaceuticals. However, as only veterinary immunologicals 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.

ACCESS OF FARMERS AND CO-OPERATIVES TO VACCINES

K. Mintiens

AVAILABLE UPON REQUEST

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.

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.

BIOSAFETY BARRIERS

K. Tjornhoj

AVAILABLE UPON REQUEST

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.

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-hooved 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.

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 naïve animals were introduced into each group and transmission from carrier to naïve 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 snap-shots 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 competi-

tion 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.

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 oro-pharingeal 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 oro-pharyngeal 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.

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.

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 worldwide. The etiological agent (Aphtovirus, 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$_{\alpha v\beta 6}$, 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/MESA/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$_{\alpha v\beta 6}$ 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$_{\alpha v\beta 6}$ cells supports the growth of both serotypes with similar efficiency.

Discussion

Our results corroborate previous observations that LFBK$_{\alpha v\beta 6}$ are the preferable cell line for VI from field suspect samples, thanks to the speed of viral replication and to a wider suscepti-

bility 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 2.82x107 and 2.94x104 RNA copies/µl. 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 finding 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. Serologi-

cal 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.

4B
SESSION

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.

Materials and methods

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. Transcriptomic data will be presented in a joint communication.

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.

TRANSMISION OF FMD FROM PERSISTENTLY INFECTED CARRIER CATTLE TO NAÏVE CATTLE VIA TRANSFER OF OROPHARYNGEAL FLUID

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Introduction

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 $LFBK_{\alpha v\beta 6}$ 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.

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