Book of abstracts

Where science and policy meet: FMD RISK MANAGEMENT in a world of changing disease landscapes

Open session of the Standing Technical and Research Committees of the EuFMD
Cavtat (Croatia), 29-31 october 2014

Food and Agriculture Organization of the United Nations

European Commission

eufmd
european commission for the control of foot-and-mouth disease

The Open Session 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 held on a regular basis, with over 200 participants at the most recent Session 2012 in Jerez de la Frontera, following on from 2010 in Vienna, and 2008 in Erice, Sicily.

The 2014 Open Session theme is **"Where science and policy meet: FMD risk management in a world of changing disease landscapes"** and is held from 29 to 31st October 2014 in Cavtat, Croatia.

The 2014 Open Session has two parts, an Open Session of the Standing Technical Committee – to consider invited papers on topics of high importance for FMD control policy in free countries – and an Open Session of the Special Committee on Research which welcomes submitted papers relevant to progressive FMD control in endemic and at risk regions. Observers working in any area of FMD science or FMD management are welcome to attend.

We are offering online participation for those who cannot attend in person. The registration for this will take place closer to the date. Some data from our last session: Over 220 participants, 60 offered papers on FMD epidemiology, vaccines and control, and 17 Keynote talks, poster sessions and debates: all on the science underpinning progressive FMD control!

EuFMD Executive Secretary

Acknowledgements

The EuFMD Commission gratefully acknowledges the support of the European Commission and the EuFMD Member States, for funding the Committee meetings, Working Groups, and enabling the creation of the Fund for Applied Research (EuFMD-FAR) to address the need for funds to address specific technical issues of practical nature common to European risk managers.

Professor David Paton is thanked for his work as Chairperson of the Standing Technical Committee to oversee the development of the Fund and for guidance to the Executive Committee on technical issues. His Chairmanship has seen substantial change in the work and impact of the Committee since 2011 and has been highly appreciated. The work of Dr Eoin Ryan is also acknowledged as being instrumental in the establishment of the Training Programme, the EuFMD-FAR and to ensuring greater dialogue between policy and scientists in the EuFMD program.

The Open Session 2014 is made possible through the support of Dr Ulrich Herzog, President of the EuFMD Commission, Dr Nigel Gibbens, Focal Point for Research in the Executive, Dr Alf Füssel (DG-SANCO), and our host, Dr Mirjana Matausic, Ljupka Maltar, Tomislav Kis and their team. We would like to acknowledge on your behalf the EuFMD team, and in particular Enrique Anton, Cecile Carraz, Silvia Clementelli, Ida D'Alessandro, Erica Tomat and Ingrid van Woudenberg, who managed and undertook most of the many, major tasks involved with the Cavtat Session, and Melissa McLaws, Chris Bartels, Kees van Maanen and Emiliana Brocchi for assisting with the scientific programme. The city of Dubrovnik is thanked for its hospitality.

ORGANIZATION OF THE 2014 OPEN SESSION

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Prof David Paton

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Aldo Dekker (NL) *FMD research, vaccine evaluation*
Tsviatko Alexandrov (BG) *Contingency planning, wildlife surveillance*
Kate Sharp (UK) *Surveillance, risk management*
Sten Mortensen (DK) *Crisis management, contingency planning; epidemiology*

Pillar II

Labib Bakkali (FR) *FMD surveillance in REMESA, RESOLAB, European neighbourhood risk*
Giancarlo Ferrari (IT) *FMD surveillance and epidemiology, Progressive Control Pathway expert*
Michel Bellaiche (IS) *FMD surveillance and management, Israel/Mid-East*
Naci Bulut (TUR) *FMD surveillance in West Eurasia, vaccine quality and production*
Gregorio Torres (SP) *Epidemiology, surveillance systems, REMESA Mid-East*

Pillar III

Jean Francois Valarcher (SWE) *FMD virology, vaccine QA, surveillance, epidemiology, global*
Ron Bergevoet (NL) *Veterinary economist/FMD*
Katharina Stark (CH) *Veterinary epidemiology, surveillance, management; FMD field research*
Stephan Zientara (FR) *Epidemiology, surveillance systems, Europe/Africa/REMESA/West Eurasia*
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Day 1 :: Wednesday 29 October PLENARY ROOM

PLENARY SESSION

The changing disease landscape and its implications

SESSION I. OPENING

09:00 – 09:25 Opening - EuFMD

09:25 – 09:30 EuFMD at 60 - Overview video

09:30 – 09:45 Frenkel lecture (Keynote) *(W. Vosloo)*

09:45 – 10:00 State of FMD research review (Keynote) *(T. Knight Jones)*

10:00 – 10:30 The pressures affecting the current and emerging disease landscape (Keynote) *(H.J. Ormel)*

10:30 – 11:00 **Coffee/Tea break**

SESSION II. FMD TRENDS CHANGING DISEASE LANDSCAPE: GLOBAL CHALLENGE

11:00 – 11:30 Changing pathways: Lessons from recent pathogen migrations for FMD risk assessment (Keynote) *(S. Alexandersen)*

11:30 – 11:50 Update on current global situation for FMD: New outbreaks and threats (Keynote) *(D. King)*

11:50 – 12:10 Changing landscape for livestock production in Europe; directions and expected change in the next 20-30 years (includes wildlife issues) (Keynote: Ad-connect) *(A. Mottet)*

12:10 – 12:30 Changing landscape for livestock production and health in China and neighbouring countries; directions and implications for FMD management (Keynote) *(J. Edwards)*

12:40 – 13:30 **Lunch break**

SESSION III. THE CHANGING LANDSCAPE FOR FMD MANAGEMENT

13:30 – 13:50 Contribution of the Food and Veterinary Office of the European Commission to reinforce FMD import risk management measures, and animal disease emergency preparedness and early warnings systems in the EU *(F.J. Pérez Pérez)*

13:50 – 14:10 Prospects for FMD control *(K. Sumption)*

14:10 – 14:30 Risk management: the changing landscape for personal engagement in surveillance and risk management (Health, engaging livestock keepers as actors in animal health) (Keynote) *(A. Cameron)*

14:30 – 14:50 Control of FMD and other major infectious transboundary diseases towards better integration of control programmes (Keynote) *(J. Domenech)*

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PARALLEL SESSION

The changing disease landscape and its implications

12:40 – 13:30 **Lunch break**

SESSION P1. VIROLOGY AND VACCINOLOGY

13:30 – 13:45 Quantifying and predicting antigenic relationships: A comparison of two alternative approaches investigated using data from FMDV and influenza A *(W. Harvey)*

13:45 – 14:00 The introduction of positively-charged residues at the five-fold axis of the FMD virus SAT-type capsid enhances infection of cultured cells *(M. Chitray)*

14:00 – 14:15 A Thiazepino [4,5-a] Benzimidazole derivative inhibits the in vitro replication of Eurasian serotypes of FMD virus *(D.J. Lefebvre)*

14:15 – 14:30 Identification of FMDV strain in multivalent vaccines by using Loop Mediated Isothermal amplification *(M. Fiorucci)*

14:30 – 14:34 Identification of a novel antibody binding determinants of serotype O FMD virus *(M. Mahapatra)*

14:35 – 14:39 Establishment of a persistent FMD Virus infection in MDBK cells *(L. Kopliku)*

14:40 – 14:45 Comparative utility of the fetal goat tongue cell line ZZ-R 127 and fetal bovine kidney cell line LFBK-αvβ6 for virus isolation from clinical samples collected from animals experimentally infected with a FMD virus *(K. Fukai)*

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Day 1 :: Wednesday 29 October PLENARY ROOM

PLENARY SESSION

The changing disease landscape and its implications

14:50 – 15:20 **Coffee/Tea Break**

SESSION IV. THE MANAGEMENT LANDSCAPE: LOOKING AHEAD, HUMAN CREATIVITY AND NEW BUSINESS SOLUTIONS

15:20 – 15:40 The expertise landscape: The FMD360 training programme, a panoramic view *(N. Short)*

15:40 – 16:00 Changing landscape for FMD management in Africa (includes issues of growing export trade, gap in services and the last mile challenge; multi-TADs approaches and wildlife) (Keynote) *(S. Nuala)*

16:00 – 16:20 Foot-and-Mouth Disease Continuity of Business Planning for the U.S. Dairy Industry *(P.J. Hullinger)*

16:20 – 16:30 Discussion

16:30 – 16:45 Kill the virus, spare the cow: disinfection as a tool everyone can use – better *(P.L. Eblé)*

16:50 Poster session
Social programme

PARALLEL SESSION

The changing disease landscape and its implications

14:50 – 15:20 **Coffee/Tea Break**

SESSION P2. DIAGNOSTIC METHODS (1) - ANTIGEN/ANTIBODY

15:20 – 15:35 Results of the 2013 Proficiency Testing Scheme *(Anna B. Ludi)*

15:35 – 15:50 Development and validation of multiple non-structural protein antibody tests to confirm FMD infection in vaccinated animals *(K.Parekh)*

15:50 – 16:05 Ready-to-use ELISA kits for antibody to FMDV SAT1 and SAT *(E. Brocchi)*

16:05 – 16:09 Design of a PAN-FMD diagnostic bioreagent *(A.K. Van Dreumel)*

16:10 – 16:14 Validation of virus neutralisation test for all FMD to determine sero-prevalence in cattle in Eritrea *(A.Dekker)*

16:15 – 16:19 Development of lateral flow assay for antigen detection and serotyping FMDV *(K. Morioka)*

16:20 – 16:24 Development and comparison of various Elisa for the diagnosis of FMD *(M. Ali)*

16:25 – 16:29 Diagnostic observations of IZSLER Antigen ELISA kits for detection and serotyping of FMDV serotypes O, A, SAT1 and SAT2 in several North, West and East African countries *(K. Van Maanen)*

16:30 – 16:34 New competitive Elisas for FMD diagnosis by detection of non-structural or structural antibodies against FMDV *(L. Comtet)*

16:35 – 16:50 Meet with the 4 minute - presenters at their posters for Q&A
Posters in "Session P2" wall

16:50 Poster session
Social programme

Day 2 :: Thursday 30 October PLENARY ROOM

PLENARY SESSION

The FMD science and policy development landscape

SESSION V. FMD RISK MANAGEMENT IN FREE COUNTRIES (PILLAR I) – RISK ASSESSMENT AND EPIDEMIOLOGY

09:00 – 09:15 Framework to compare the importance of the different source regions for entry of FMDV into Europe *(M. McLaws)*

09:15 – 09:30 The enhanced passive surveillance system: A solution supporting data collection, integration and analysis for disease surveillance *(L. Holmstrom, K. Biggers)*

09:30 – 09:45 Quantitative risk assesment evaluating the transmission of FMD via fresh deboned beef produced from an endemic region *(G.T. Fosgate)*

09:45 – 10:00 Modelling FMD transmission in a feral pig-domestic cattle ecosystem *(M. Ward)*

10:00 – 10:15 Geographical facilitators and non-facilitators of FMD dissemination *(A.L. Rivas)*

10:15 – 10:19 Evaluating the transmission and survival of FMDV on environmental fomites *(E. Brown)*

10:20 – 10:24 Techniques to asses the risk of airborne spread of FMD and examine underlying uncertainties *(L. Burgin)*

10:25 – 10:29 Development of a standard model for FMD in the United States *(T. Boyer)*

from 10:30 Meet with the 4 minute - presenters at their posters for Q&A
Posters in "Session V" wall

12:30 – 13:30 **Lunch break**

SESSION VI. FMD RISK MANAGEMENT IN FREE COUNTRIES (PILLAR I)

11:00 – 11:15 Resource estimations in contingency planning for FMD *(S. Mortensen)*

11:15 – 11:30 Maximising efficiency with a surveillance strategy for FMD during an outbreak in a previously FMD-free country *(K. Walker)*

11:30 – 11:45 Evaluating vaccination strategies to control FMD: A model comparison study *(C. Cook)*

11:45 – 12:00 Impact of stakeholders influence, geographic level and risk perception on strategic decisions in simulated FMD epizootics in France *(M. Marsot)*

12:00 – 12:15 An adaptive management approach to Foot-and-Mouth Disease control *(M.J. Tildesley)*

12:15 – 12:19 Ensemble forecasting for epidemiology: How to combine multiple projections of FMD *(T. Lindström)*

12:20 – 12:24 Epidemiologic and economic impacts of applying alternative control strategies for FMD in feedlot operations *(T. Boyer, A. Delgado)*

12:25 – 12:29 Epidemiological and economic modelling to determine the cost to New Zealand of an FMD oubreak *(A. van Halderen)*

from 12:30 Meet with the 4 minute - presenters at their posters for Q&A
Posters in "Session VI" wall

12:30 – 13:30 **Lunch break**

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PARALLEL SESSION

The FMD science and policy development landscape

SESSION P3. PROGRESSIVE CONTROL OF FMD: TECHNICAL DEVELOPMENTS AND ISSUES FOR NON FREE COUNTRIES (PILLAR II)

09:00 – 09:15 Outbreaks of FMDV in Libya and Saudi Arabia during 2013 due to an exotic O/ME-SA/IND-2001 lineage *(K. Bachanek-Bankowska)*

09:15 – 09:30 Mass vaccination, immunity and coverage: Modelling population protection against FMD in Turkish cattle *(T.J.D. Knight-Jones)*

09:30 – 09:45 Modelling endemic FMD in Turkey *(P.M. Dawson)*

09:45 – 10:00 FMD health situation in Tunisia *(H.A. Heni)*

10:00 – 10:15 Serological survey in Libya to asses FMD viruses circulation and vaccine immune response *(G. Ferrari)*

10:15 – 10:19 Seroepidemiological study of FMD in livestock in Tripoli, Libya *(A. Dayhum)*

10:20 – 10:24 FMD in Libya and the control strategy *(I. Eldaghayes)*

from 10:25 Meet with the 4 minute - presenters at their posters for Q&A Posters in "Session P3" wall

12:30 – 13:30 Lunch break

SESSION P4. PROGRESSIVE CONTROL OF FMD: TECHNICAL DEVELOPMENTS AND ISSUES FOR NON FREE COUNTRIES (PILLAR II)

11:00 – 11:15 Promoting a risk-based strategy plan for FMD control in endemically infected countries: Development and examples from 4 countries *(C.J.M. Bartels)*

11:15 – 11:30 Serosurveillance and the PCP-FMD: Why are serosurveys useful, how are they being used and what are the gaps? *(M. McLaws)*

11:30 – 11:45 Vaccination of cattle only seems to be sufficient to stop transmission in most mixed populations of cattle and sheep *(P. Eble)*

11:45 – 12:00 ELITE: An electronic laboratory information tracking environment for supporting the Progressive Control of FMD in Pakistan *(K. Biggers)*

12:00 – 12:04 Molecular variability within the FMD virus O-Panasia-2 lineage *(B-Valdazo-González)*

12:05 – 12:09 Knowledge and perceptions of communal farmers concerning FMD at the wildlife/livestock interface of the Kruger National Park *(D.D. Lazarus)*

from 12:10 Meet with the 4 minute - presenters at their posters for Q&A Posters in "Session P4" wall

12:30 – 13:30 Lunch break

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Day 2 :: Thursday 30 October PLENARY ROOM

PLENARY SESSION

The FMD science and policy development landscape

SESSION VII. GFRA LED SESSION (1): VACCINE STABILITY

13:30 – 14:00 FMD virus stability: implications for vaccine efficacy (Keynote) *(T. Doel)*

14:00 – 14:15 Thermofluor analysis of the FMDV capsid and the effects of different solutions on stability *(J. Seago)*

14:15 – 14:30 A method based on the use of specific llama antibodies for quality control testing of FMD vaccines *(E. Pérez Martín)*

14:30 – 14:45 Evaluation of the immune responses of Nguni cattle vaccinated with Foot-and-Mouth Disease virus stabilised SAT2 antigens *(K. Scott)*

14:45 – 15:00 Demonstration of a high potency SAT2 vaccine in cattle and confirmation of efficacy in pigs against virulent challenge *(L. Mouton)*

15:00 – 15:30 **Coffee/Tea break**

SESSION VIII. GFRA LED SESSION (2): FMD ECOLOGY

15:30 – 16:00 Looking Forward from FMD Epidemiology to FMD Ecology (Keynote) *(R. Garabed)*

16:00 – 16:15 Mechanisms of persistence of FMDV in African buffalo populations: A briefing on current work at Kruger National Park *(A. Jolles)*

16:15 – 16:30 Foot-and-Mouth Disease Ecological Studies In Endemic Settings: Ongoing Studies in Vietnam and Pakistan *(J. Arzt)*

16:30 – 16:45 An Emergent Strain of Foot-and-Mouth Disease Virus, Serotype Sat 3, Isolated From A Long-Horned Ankole Calf In The Queen Elizabeth National Park In Uganda *(G.J. Belsham)*

16:45 – 17:00 Recovery of Viral RNA And Infectious Foot-and-Mouth Disease Virus From Positive Lateral-Flow Devices *(V.L. Fowler)*

from 17:00 Poster session
Social programme

PARALLEL SESSION

The FMD science and policy development landscape

SESSION P5. PCP-FMD ASIA

13:30 – 14:45	SEAC FMD Roadmap: A risk-based approach to FMD control in SE Asia and China *(R.C. Abila)*
13:45 – 14:45	Epidemiological investigation of Foot-and-Mouth Disease incidences in southern peninsular India during 2013 *(G. K. Sharma)*
14:00 – 14:00	Using risk assesment to inform FMD policy in Mongolia *(M.E. Schuppers)*
14:15 – 14:30	Pig, cattle and buffalo value and social network analysis in Xayabury province of LAO PDR (Ad-connect) *(J. Hinrichs)*
14:30 – 14:45	Isolation and identification of FMDV types and its sequence analysis on the basis of P1 (Capsid protein gene) in Pakistan *(U. Waheed)*
14:45 – 14:49	Promoting a risk based strategic plan for FMD control: Improvement of risk assessment through real time training (RTT) *(C.J.M. Bartels)*
14:50 – 14:54	Epidemiological analysis of FMD outbreaks in South-East Asia and China (2010-2014) *(R.C. Abila)*
from 14:55	Meet with the 4 minute - presenters at their posters for Q&A Posters at the "Session P5" wall
15:00 – 15:30	**Coffee/Tea break**
15:30 – 17:00	Modelling network meeting (by invitation)
	GFRA meeting (by invitation)
from 17:00	Poster session Social programme

Day 3 :: Friday 31 October PLENARY ROOM

PLENARY SESSION

FMD future: responding to change in the global landscape

SESSION IX. IMPACT AND AFRICA

08:30 – 09:00 Global landscape of FMD control *(S. Metwally)*

09:00 – 09:15 What do we know about the economic impact of FMD in smallholder production? - Summary of the evidence *(J. Rushton)*

09:15 – 09:30 Impact of FMD on milk yield, mastitis and culling on a large-scale dairy farm in Kenya *(N.A. Lyons)*

09:30 – 09:45 Household level impacts of FMD on traditional livestock-keeping systems of Northern Tanzania *(M. Casey)*

09:45 – 10:00 Emerging massive FMDV outbreaks in Uganda and possible impact on PCP *(A. Chrisostom)*

10:00 – 10:05 The impact of FMD outbreaks in Mbala and Kazungula districts in Zambia *(F. Banda)*

10:05 – 10:10 The epizootiology of FMD in high risk zones in Kenya *(B. Kibore)*

from 10:10 Meet with the 4 minute - presenters at their posters for Q&A
Posters in "Session IX" wall

10:30 – 11:00 **Coffee/Tea break**

SESSION X. IMMUNISATION AND VACCINES

11:00 – 11:15 Vaccine evaluation on large-scale dairy farms using routine prophylactic schedules for FMD *(N.A. Lyons)*

11:15 – 11:30 Longitudinal studies on the immunogenicity of different vaccination methods of highly potent FMD oil vaccine for weaner pigs *(K-N. Lee)*

11:30 – 11:45 Maternal immune cells transferred through colostrum do not interfere with the immune responses to FMD vaccine *(A.V. Capozzo)*

11:45 – 12:00 Serotype O vaccine efficacy and challenge with different viruses from South East Asia in various species *(V. Vosloo)*

12:00 – 12:15 The field effectiveness of an O Manisa + O 3039 vaccine for the control of an O/ME-SA/IND-2001 FMD outbreak in a private dairy farm in Saudi Arabia *(A.M. Shamia)*

12:15 – 12:19 Overview on the performance of FMD Vaccines in Potency Tests using lp-ELISA: a perspective from a Manufacturer in South America *(Otto D. Mozzer)*

12:20 – 12:24 Detection of antibodies against NSP residues in the commercial FMD trivalent vaccines after multiple vaccinations in cattle and goats *(D. Tark)*

12:25 – 12:29 Early protection in sheep against heterologous challenge with serotype O FMDV using high potency vaccine *(J. Horsington)*

12:30 – 12:34 Testing the efficacy of O1 Manisa high potency vaccine against challenge with O/SKR/2010 (O MYA98) strain in sheep *(N.B. Singanallur)*

from 12:35 Meet with the 4 minute - presenters at their posters for Q&A
Posters in "Session X" wall

12:40 – 13:40 **Lunch break**

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PARALLEL SESSION

FMD future: responding to change in the global landscape

SESSION P6. DIAGNOSTICS (2): MOLECULAR DIAGNOSTIC DEVELOPMENTS

09:00 – 09:15 Development of probe-based real time RT-PCR assays for detection and serotyping of FMDVs circulating in West EurAsia *(S.M. Jamal)*

09:15 – 09:30 Development and evaluation of a multiplex conventional RT-PCR for simultaneous detection and typing of FMDV in West Africa *(K. Górna, Blaise-Boiseau)*

09:30 – 09:45 Develelopment of tailored specific real-time RT-PCR assays for detection of FMDV serotypes A, O, SAT 1 and SAT 2 circulating in East Africa *(K. Bachanek-Bankowska)*

09:45 – 10:00 Real-time RT-PCR for the rapid detection of FMDV in milk *(B. Armson)*

10:00 – 10:15 Realising the potential of simple isothermal molecular tools for field diagnosis of FMD *(E. Howson)*

10:15 – 10:30 Development and evaluation of multiplex reverse transcription loop mediated isothermal amplification assays combined with lateral-flow visualisation for the discrimination of FMD from other vesicular diseases *(V. L. Fowler)*

10:30 – 11:00 Coffee/Tea break

SESSION P7. TRANSMISSION AND FMD DIAGNOSIS IN NON-AFRICAN WILDLIFE

11:00 – 11:15 From sequence to prevalence: Phylodynamics of FMDV *(A. Di Nardo)*

11:15 – 11:30 Beyond the consensus: Investigating intra-herd variability of FMDV using the Illumina Miseq *(D.J. King)*

11:30 – 11:45 Probability of infection of cattle, sheep and pigs exposed to FMD virus aerosols *(J.L. Gonzales)*

11:45 – 12:00 A novel protocol to generate consensus level genome sequences for FMD virus and its application to sequencing a large outbreak *(G. Freimanis)*

12:00 – 12:15 Non-invasive sampling systems for the detection of FMDV in wild boar *(S. Mouchantat)*

12:15 – 12:19 Performance of diagnostic tests for FMD in red deer *(R. Kittelberger)*

12:20 – 12:24 Evaluation of two air samplers for detection and quatification of airborne FMDV *(C. Colenutt)*

from 12:25 Meet with the 4 minute - presenters at their posters for Q&A
Posters in "Session P7" wall

12:40 – 13:40 Lunch break

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Day 3 :: Friday 31 October PLENARY ROOM

PLENARY SESSION

FMD future: responding to change in the global landscape

SESSION XI. THE FUTURE OF VACCINATION

13:40 – 13:45 Developing technologies to understand duration of immnutity following FMDV vaccination of livestock species *(T. Golde)*

13:55 – 14:10 Novel vaccine strategies for the control of FMD in Africa *(F.F. Maree)*

14:10 – 14:25 Recombinant adenovirus expressing empty capsid of serotype A22 FMDV provides sterile immunity in cattle following homologous prime-boost vaccination *(S. Parida)*

14:25 – 14:40 Defining requirements and execution of international field trials for next generation FMD vaccines and diagnostics *(M. Colby)*

14:40 – 14:44 Development of a novel, recombinant, potent, and safe diva vaccine for FMD *(M. Ali)*

14:45 – 14:49 Follicular dentritic cells (FDCS): A key player in the pathogenesis of FMD virus? *(M. Habiela)*

15:00 – 15:04 Vaccine development, challenges and strategies for FMD control in Indian subcontinent *(S.N. Singh)*

15:05 – 15:19 Meet with the 4 minute - presenters at their posters for Q&A
Posters in "Session XI" wall

15:20 – 16:10 **Coffee/Tea break**

SESSION XII. FINAL KEYNOTE, CONCLUSIONS AND CLOSING

16:10-16:30 Lessons from delivery and impact assessment of human vaccination programmes: the GAVI experience (Keynote) (Ad-connect) *(S. Malvolti)*

16:10 – 17:10 Final Keynote + conclusions + report + appreciations *(Tbd)*

PARALLEL SESSION

FMD future: responding to change in the global landscape

SESSION P8. UNDERSTANDING FMDV DIVERSITY IN AFRICA

13:40 – 13:55 Evolution of FMDV during persistence in African buffalo *(M. Cortey)*

13:55 – 14:10 Genetic characterization of circulating FMD viruses from African buffalo (Syncerus caffer) and cattle in Kenya: Evidence for independent virus populations *(S.N. Wekesa)*

14:10 – 14:25 Multiple FMDV serotypes identified in Uganda during 2010-2013 *(K. Tjørnehøj)*

14:25 – 14:40 Identification of novel genotypes of FMDV recovered from African buffalo in Marromeu, Mozambique *(C.J. Kasanga)*

14:40 – 14:55 Emergence of antigenic variants of SAT2 FMD viruses at the wildlife/livestock interface in South Africa *(B. Blignaut)*

14:55 – 14:59 Challenges for FMDV diagnosis - Case study of outbreak confirmation in Uganda (2011-2014) *(C. Ayebazibwe)*

15:00 – 15:04 A vaccine matching study for SAT2 viruses in Southern Africa *(F.F. Maree)*

15:05 – 15:19 Antigenic and genetic characterization of FMDV serotype O circulating in East Africa *(K. Lloyd-Jones)*

from 15:20 Meet with the 4 minute - presenters at their posters for Q&A
Posters in "Session P8" wall

15:20 – 16:10 Coffee/Tea break

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Day 3:: FMD FUTURE: RESPONDING TO CHANGE IN THE GLOBAL LANDSCAPE

POSTER SESSION

FRENKEL LECTURE

W. Vosloo

AVAILABLE UPON REQUEST

notes

STATE OF FMD RESEARCH REVIEW

Theo Knight-Jones

In this report we use literature review and expert consultation to evaluate global FMD research progress since 2011 and highlight remaining knowledge gaps, incorporating ongoing work from 33 research institutes from around the world.

Global needs

Disease-free countries experience periodic FMD outbreaks and must maintain the capacity for rapid detection and control. Some countries, particularly in South America, are successfully controlling and eradicating FMD through mass vaccination. However challenges remain in proving FMDV freedom in vaccinated populations and the design, production and distribution of vaccines able to induce effective immunity to emergent field strains. In addition, many endemic countries have limited control programmes and within this group there is a need to understand the level of FMD economic impact and to identify control measures that are effective and feasible in smallholder systems, particularly where wildlife play an important role in FMD epidemiology. Underpinning all this is the requirement for comprehensive knowledge of the virus itself, its interaction with host species, and how we can most effectively prevent its replication and spread on both the individual and population levels.

Areas of progress

Since the last GFRA report, the USA has licensed a recombinant vector vaccine for use in cattle during an FMD outbreak. Elsewhere, alternative, improved recombinant vaccines are likely to prove valuable in years to come. While current vaccines often induce sub-optimal, strain-specific immunity, studies of the interaction of the virus with the immune system are allowing better vaccine design and testing. Coupled with advances in manufacturing and quality control, it is reasonable to expect improved vaccine based control programmes in the medium term future.

Advances in genetic analysis have allowed greater understanding of FMD transmission with increasing relevance to field control. Novel molecular techniques promise to further reduce diagnostic costs and improve accessibility.

FMD models are increasingly used to direct control policy although more ground testing is required. Commodity based trade is gaining wider acceptance as a possible way of opening up FMD free trade to poor farmers in areas where FMD is endemic in wildlife, without the economic drain and ecological impact of zonation and enclosure of wildlife. Previously neglected, the importance of field epidemiology in the evaluation of FMD outbreaks and control programmes has gained greater recognition.

Key knowledge gaps

Improved duration and breadth of vaccine immunity is required, as well as improved methods for estimating vaccine protection against rapidly-changing field strains. This must be underpinned by rigorous investigations of the immune systems of the various host species, which remain incompletely understood in many cases, and in neonatal animals in particular.

It is not known whether FMD can be controlled in endemic, smallholder systems by vaccination alone when biosecurity measures cannot be implemented effectively. Investigations are required to establish which approaches can control the disease within the constraints of this setting, including consideration of required vaccine potency, coverage and strategy.

Continued progress in molecular technologies and analyses are required, as they promise to provide great insights into virus evolution, ecology and epidemiology.

notes

A CHANGING WORLD AND CHANGING DISEASE LANDSCAPES

J. Lubroth and H.J. Ormel

In today's world humans have not only become increasingly linked to each other, but also to all other life on the planet. Human health has become ever more intertwined with the health of our environment and the animals that populate it.

Diseases emerge, spread and persist in humans, livestock, and wildlife, affecting all three with often devastating consequences. We are more in contact with animals than ever before, and livestock and wildlife are more in contact with each other. It is time for us to acknowledge the degree to which

human health is connected to the health of animals and the environment. It is time for us to focus on global health. For FAO this so-called 'One Health' includes not only zoonotic diseases, but also animal diseases that can disrupt livelihoods and the well-being of humans and animals. FAO is looking to animal health as an essential part of its strategic objectives to eradicate hunger, to make agriculture more sustainable, to reduce rural poverty and to make food systems more efficient. In the recent FAO flagship publication 'Changing Disease Landscapes', a Pressure-State-Response analysis framework is used to describe emerging animal diseases in their agro-ecological, socio-economic and political context.

Pressure

With regard to the pressures on global animal health we are all more or less aware of the rise of the world population, land pressure, marginalization of the poor, urbanization, deforestation, climate change and the inter-connectivity all over the world. Host environments and host availability to existing pathogens is changing rapidly. But do we adjust our husbandry-systems to these new trends? Is enough research done? Is the general public aware of these changes? Do decision makers know enough of these changing patterns?

State

Livestock health is the weakest link in our global health chain and disease drivers in livestock as well as in wildlife, are having increasing impacts on humans. Over 70 percent of human diseases originate in animals, and our expanding human population inhabits more wildernesses while becoming ever more reliant on animals for food. FAO statistics predict a rise of 65-70 percent of animal protein use until 2050. Health crises are happening already and getting enormous attention. Ebola, MERS and Avian Flu have an animal origin. Anti-microbial resistance and its link to intensive livestock production are gaining more and more political attention. But the impacts on livelihoods of non-zoonotic animal diseases, like Foot and Mouth disease, are highly under-estimated.

Respond

How do we respond? Firstly we must seek evidence to understand the problems and opportunities of the changing disease landscapes. Secondly, we must enable dialogue and

information exchange, not only among scientists and farming communities, but also to the urbanized populations and decision makers. Thirdly, we have to promote health-conscious innovation, improve the way we produce, buy, sell and consume animal products. Finally, we must recognize how globalization, population growth and technology push our markets and supply chains closer together to reveal growing threats with widespread impacts. In general FAO states that the best way to respond is to address disease at its source and to prevent animal diseases to disrupt societies. Prevention saves animals, saves livelihoods, and saves money.

FAO calls for the implementation of cohesive and concerted global efforts

Like the Global Foot and Mouth Control Strategy, towards health protection policies and strategies for sustainable development, with reference to the global One Health approach. In order to meet the food requirements of the growing global population, there is a need for the development of sustainable agricultural food systems that minimize the risk of emerging disease while protecting human health and conserving biodiversity and the environment.

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CHANGING PATHWAYS: LESSONS FROM RECENT PATHOGEN MIGRATIONS FOR FMD RISK ASSESSMENT

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Introduction

Traditionally we have looked mainly at potential international/transboundary transmission by indirect contact as happening by specific, often biological products and a few "things" such as vectors or boots or clothing. This concept needs to be changed due to the dynamics of the modern world where movements from A to B to…. Z of anything by any route are increasing in amount and complexity. In addition, farming is changing and intensifying and what was previously improbable are now changing to becoming possible.

Results and discussion

The presentation starts by giving a few examples and assumed routes of recent unexpected international spread and then focuses on a very recent example of international followed by national spread of a virus infection in swine by the feeding of spray dried porcine plasma to newly weaned piglets.

In January 2014, approximately nine months following the initial detection of porcine epidemic diarrhea (PED) in the USA, the first case of PED was confirmed in a swine herd in southwestern Ontario. A follow up epidemiological investigation carried out on the initial and 10 subsequent Ontario PED cases pointed to feed as a common risk factor. As a result, several lots of feed and spray dried porcine plasma (SDPP) used as a feed supplement were tested for the presence of PEDV genome by real-time RT-PCR assay. Several of these tested positive supporting the notion that contaminated feed may have been responsible for the introduction of PEDV into Canada. These findings led us to conduct a bioassay experiment in which three PEDV positive SDPP samples (from a single lot) and two PEDV positive feed samples supplemented with this SDPP were used to orally inoculate 3-week-old piglets. Although the feed inoculated piglets did not show any significant excretion of PEDV, the SDPP inoculated piglets shed PEDV at a relatively high level for ≥ 9 days. Despite the fact that the tested PEDV genome positive feed did not result in obvious piglet infection in our bioassay experiment, contaminated feed cannot be ruled out as a likely source of this introduction in the field where many other variables may play a contributing role.

The study has been published online on 7 August 2014 and in the print version of the Journal Transboundary and Emerging Diseases, Volume 61, Issue 5, pages 397–410, October 2014 and is freely available as Open Access on: http://onlinelibrary.wiley.com/doi/10.1111/tbed.12269/pdf

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UPDATE ON CURRENT GLOBAL SITUATION FOR FMD: NEW OUTBREAKS AND THREATS

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This presentation will review the current situation regarding field outbreaks of foot-and-mouth disease (FMD) using laboratory data generated for clinical samples and sequences received to the WRLFMD (The Pirbright Institute) and partner laboratories within the OIE/FAO FMD Laboratory Network. These data are used to monitor the continued trans-boundary movements of FMD virus in Asia and Africa due to established FMD virus lineages, and to also provide recommendations about the suitability of vaccine strains that can be used to control these outbreaks. In addition to mapping epidemiological patterns in FMD endemic settings, the sequence data also reveal exotic and unexpected incursions of FMD virus into new regions and countries that can pose an increased risk for onward spread of the disease, including to FMD-free countries. During the past 12-24 months, particular concern has been raised about the expanding circulation of FMD virus lineages (such as O/ME-SA/PanAsia, O/SEA/Mya-98 and A/ASIA/Sea-97) in a number of East Asian countries, the movement of the A/ASIA/Iran-05 strain to cause FMD outbreaks in the Black Sea region of the Russian Federation, as well as new FMD outbreaks due to the O/ME-SA/Ind2001 lineage that have been detected in the Middle East (Saudi Arabia and UAE) and in North Africa (Libya, Tunisia and most recently possibly in Algeria). New tailored tools (lineage-specific real-time RT-PCR assays) are being developed to recognise a number of these important FMD lineages to complement other laboratory assays used for diagnosis. These data reinforce the role played by the OIE/FAO FMD laboratory Network to coordinate global surveillance to monitor the patterns of FMD virus movements and to recognise the emergence of new FMD virus lineages that may require new vaccines for control.

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CHANGING LANDSCAPE FOR LIVESTOCK PRODUCTION IN EUROPE; DIRECTIONS AND EXPECTED CHANGE IN THE NEXT 20-30 YEARS

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Introduction

The European livestock sector is dynamic and production is expected to grow and recover in the next decade, though at a slower pace than in the past. But it is facing growing challenges, including market developments, reformed policies, climate change and animal health threats.

Materials and methods

The analysis relies on different national and international databases, projections for EU production from the European Commission, OECD and FAO, and various contributions of experts of the European livestock sector available in the literature, including WUR and Idele.

Results & Discussion

European milk production should continue to grow over the next decade and exports are expected to increase, especially for cheese. The meat sector trend is more contrasted, with an expected growth in pig meat and poultry production but further decrease in beef and small ruminants' meat production and consumption.

Whereas greening is not expected to affect the main trend in the sector, the CAP reform should result in geographical shifts, especially in the dairy sector, through the convergence of direct support (flat rate/ha).

Adaptation to climate change will be a constraint, but not to everyone since length of growing season and yields are expecting to increase in Northern and Central Europe. Nevertheless, livestock vulnerability and vectors of animal diseases will be affected and could result in higher risk for the European livestock sector.

Gains in efficiency must and will continue. But the European livestock sector will have to strive for more protein self-sufficiency, which may lead to system change and relocation in the long term future.

CHANGING LANDSCAPE FOR LIVESTOCK PRODUCTION IN CHINA AND NEIGHBOURING COUNTRIES; DIRECTIONS AND IMPLICATIONS FOR FMD MANAGEMENT

J. Edwards

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CONTRIBUTION OF THE FOOD AND VETERINARY OFFICE OF THE EUROPEAN COMMISSION TO REINFORCE FMD IMPORT RISK MANAGEMENT MEASURES, AND ANIMAL DISEASE EMERGENCY PREPAREDNESS AND EARLY WARNING SYSTEMS IN THE EU

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Introduction

The Animal Health Audit Team (AHAT) of the Food and Veterinary Office of the European Commission (FVO) contribute to the implementation of effective animal health control systems and verify compliance with animal health standards within the EU, and in third countries in relation to their exports to the EU.

Summary of recent and current activities

In recent years, the main activities performed by the AHAT in relation to FMD are:

- Audits in non-EU countries to evaluate FMD risks associated with import of animal commodities. Since 2011, the AHAT evaluated the FMD situation in countries in South America (five), Southern Africa (three) and the EU-neighbouring region (e.g. Turkey, Russia, Belarus). The outcome of those audits has informed the decisions of the EU FMD import risk management system coordinated by the European Commission.

- Verification of the effective implementation of FMD eradication measures in Bulgaria in response to the outbreak of FMD in 2011. As a result of that audit, the FMD early warning system was further reinforced in that region of the EU.

- A multi-annual series of audits in MS that since 2012 aims to reinforce the existing exotic animal disease emergency preparedness and early warning systems in the EU. In addition, the AHAT organises an annual workshop with the MS to discuss the outcome of the audits, to highlight the best practices identified, and to let the MS share their experiences with the operation of animal health surveillance and disease outbreak simulation and management systems.

- A multi-annual series of audits in MS on verification of compliance with bio-security standards applied in laboratories and establishments handling live FMD virus. In light of some weaknesses and deficiencies found, a number of recommendations were made to ensure that adequate and effective FMD virus bio-containment measures are always in place.

Conclusion

The FVO contributes to the development of a more effective FMD control policy in the EU. The outcome of the FVO audits provides invaluable information on the operation of the FMD control

systems in MS and non-EU countries. This information is used to shape up and modernise FMD risk management measures that are fit-for-purpose and that successfully maintain the high FMD health status of the EU.

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PROSPECTS FOR FOOT-AND-MOUTH DISEASE CONTROL

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Introduction

This paper considers how the landscape for FMD control has changed over the past decades, and presents a SWOT analysis of prospects for extending the benefits of FMD control to populations where it has been so far out of reach. It posits that each previous age, from the 1500s, has brought a depth of valuable experience that has enabled, through a combination of quarantine and isolation, slaughter policies and use of vaccination, to achieve national scale FMD control and elimination from Europe and the America's, but that FMD has become less controllable in some settings by traditional isolation and slaughter policies as a result of increasingly complex animal production systems and livestock trading movements. Maintaining regions free of FMD is feasible only by continuous effort to maintain defences to limit entry of FMD virus and rapidly control incursions by publically acceptable and effective responses, and reducing the risk of such events. The GF-TADS Global Strategy for FMD Control has since 2012 been in place and it can be questioned why major international investments to support countries to develop national FMD strategic plans have not materialised. Despite this, we could be entering a Fifth Age of FMD Control, in which the tools, and benefits of FMD control become accessible at livestock keeper level even the least developed countries, if more emphasis is placed on using market based solutions (MBS) where Government scarce resources should be used to attract major private sector investments in animal health service delivery. Given the high proportion of livestock owning families in most FMD affected countries, where studies indicate 5-20% of these are "livestock entrepreneurs" , then in many if not all of the least developed countries a market potential exists for local information on risk and purchase of vaccine. In addition, even subsistence oriented livestock keepers are often willing to purchase affordably-priced animal vaccines and other livestock-related inputs, given their high returns in terms of food, insurance and other benefits. The difficulties to estimate market potential and develop business model that reach the farm doorstep is one supply side barrier; others include demand side barriers such as limited information and evidence of the returns to investment a lack of trust in veterinary medical products or lack of public information system that warn producers of risk and of the options for prevention.

Discussion

Historically most publically funded FMD control programmes have been driven by entrepreneurial class of livestock keepers, in Europe and elsewhere, with the progressive keepers eventually driving public policy to reduce risk to their business. Public policies, to enable quality vaccines onto the market, and public information to give confidence on these products and on the local risk, could catalyse FMD control in a country, as the mosaic of private efforts eventually lead to co-ordinated livestock sector demand for area or sector based containment of infection. Rather than focus only on nationally managed public campaigns, international efforts should consider

the market based solutions as part of the national risk based strategic plans. The MBS approach could also have much wider benefits than for FMD alone, as the generic issue is of access of livestock keepers to information and services in real time that are relevant to their daily business, and risk management, issues. Threats to progress on FMD control include national preference systems that limit access to international quality vaccines, reduced funding of international support to countries to develop appropriate national strategies, and failure to maintain R&D funding to deliver the vaccines needed by the market place.

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ENGAGING LIVESTOCK KEEPERS AS ACTORS IN ANIMAL HEALTH

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Livestock keepers, in general, want their animals to be healthy, as good health provides various benefits, such as increased productivity, income and status. The veterinary services, in general, have the same motivation. Why is it then, that it is so often so difficult to establish and maintain good relations between keepers and the veterinary services, for clinical services but especially for disease surveillance?

Disease surveillance information collection systems are frequently designed by national-level epidemiologists or decision-makers based on national-level priorities such as OIE reporting. Irrelevant data, such as local-level details and routine diseases are frequently excluded. The result is a set of data that meets national-level needs, but is of little interest to those responsible for providing it (livestock keepers) and those responsible for collecting it (field veterinary staff). An alternative approach was adopted, in which the animal health information collection and management system was designed solely to meet the needs of keepers and field staff. The result is a database containing much more detailed case data on *all* disease cases, not just those deemed important at the national level. Another aspect of this philosophy is the idea that surveillance data is not something to be extracted but a by-product of effective service provision.

In order to capture, manage, filter and summarise the data, powerful information management tools are required to support this approach, but these are readily available thanks to cloud computing services.

This paper examines a philosophical and sociological approach to keeper engagement and describes practical experience with such a system for disease surveillance in Indonesia.

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CONTROL OF FMD AND OTHER MAJOR INFECTIOUS TRANSBOUNDARY DISEASES TOWARDS BETTER INTEGRATION OF CONTROL PROGRAMMES

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Transboundary diseases are among the major limiting factors for livestock production. Their impact can vary from huge mortalities to reduced productivity and restricted market access. These diseases can also have an impact on food security, human livelihoods, nutrition and economic developments for small holders or more organized producers. Several diseases can be mentioned among them are Foot and Mouth Disease (FMD) in cattle or Peste de Petits Ruminants (PPR) in small ruminants, Other diseases can be either specific to certain species such as Pox and Goat Virus diseases in sheep and goats or Contagious Bovine Pleuropneumonia in cattle or they can affect several domestic animal species such as Brucellosis. Others are getting more importance since they can affect humans (zoonotic diseases) and/or they are emerging due to environmental changes such as vector borne diseases. Last but not least the impact of diseases due to intensification of animal production systems is expected to increase and in this regard some particular problems are to be carefully addressed such as the risks of the development of resistance to antimicrobials. Holistic approaches to control all these new or more ancient threats are needed. There is an evident interest to implement combined control programmes against several diseases at the same time with a specific focus on a flagship disease such as FMD in cattle or PPR in small ruminant. On the other hand, a holistic approach means that controlling diseases must consider the global environment context taking into account socioeconomic aspects as well as ecosystem, climatic and human population evolutions. It is therefore indispensable to define multisectoral and multidisciplinary approaches and to rely on effective veterinary services working at the country level in strong collaboration with other ministries or institutions in charge of human health, agriculture, wildlife and environment and, at international level, building on the FAO-OIE as well as the Tripartite FAO-OIE-WHO “One Health” agreements. Examples of such global integrated approaches will be presented and discussed.

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THE EXPERTISE LANDSCAPE: FMD 360 TRAINING PROGRAMME, A PANORAMIC VIEW

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Introduction

The rapid development of the Internet has provided the potential to introduce radical changes in the delivery of veterinary training. At the same time, the knowledge and skills required of professionals working worldwide in animal disease control are becoming increasingly diverse and sophisticated. This presentation draws on the recent experience of the RVC, EuFMD and other organisations to identify the most appropriate training opportunities for the future.

Materials and Methods

The EuFMD has taken a progressive approach to training in developing an e-learning platform in partnership with the RVC to extend field and classroom based training courses. Over 600 users are now registered on the platform, which hosts online induction and refresher courses to supplement existing workshops. Standalone multilingual e-learning courses have also been developed including the FMD Emergency Preparation Course as well as live webinars. User feedback research has highlighted the perceived value of this interactive and flexible learning system for participants.

Discussion

The opportunity now exists to further develop effective and affordable training resources that address a range of training needs for FMD control, and that can be flexible and repurposed when required. The challenge will be to match the requirements of key audiences (veterinarians, scientists, farmers and even students) with the most appropriate technical solutions. This will need to take into account a wide range of new issues around access, intellectual property, language, device usage, rapid response and digital literacy. Whilst this horizon scanning will be challenging, it is also worthwhile, given the exciting possibilities that lie ahead.

CHANGING LANDSCAPE FOR FMD MANAGEMENT IN AFRICA

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FMD CONTINUITY OF BUSINESS PLANNING FOR THE U.S. DAIRY INDUSTRY

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Introduction

If foot-and-mouth disease (FMD) was detected in the United States (U.S.), a national animal health emergency would be declared and livestock and allied industries would feel the immediate impacts of animal and product movement restrictions, animal quarantines, disease surveillance activities and other necessary measures implemented to control the disease. These controls, while necessary to contain the outbreak, have significant impacts on the normal business operations of uninfected livestock operations in affected regions, potentially disrupting interstate commerce. Such impacts are most disruptive to industries producing perishable products and utilizing 'just-in-time' supply models. One significantly impacted sector would be the U.S. dairy industry whose operations rely upon daily animal, product and other supportive movements, and do not have the capacity to store milk for more than 24-48 hours. Disruption of normal milk movement in the U.S. could affect the provision of milk and milk products, as well as create significant milk disposal, environmental and animal welfare issues.

Materials and methods

The United States Department of Agriculture (USDA) is collaborating in preparedness initiatives and pre-event, academia-facilitated emergency management planning efforts with states and livestock industries. Collectively these projects are called "Secure Food Supply" (SFS) plans. A key element, critical to a successful outcome from this initiative is the engagement and involvement of industry throughout the process.

Discussion

One specific SFS effort is the 'Secure Milk Supply' (SMS) Plan, and its initial goal is to develop agreed upon processes and procedures to pick up, transport, and pasteurize milk from uninfected farms in FMD control areas thus helping to maintain business continuity for dairy producers, haulers, and processors. The next phase of planning will address the management of milk during large outbreaks when depopulation is no longer pursued, as well as off-site calf rearing and other critical movements.

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KILL THE VIRUS, SPARE THE COW: DISINFECTION AS A TOOL EVERYONE CAN USE-BETTER

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Introduction

For the prevention and the control of FMDV outbreaks, disinfection of material contaminated with FMDV is important. Before use, the disinfectants should be tested for their virucidal effect against FMDV. We applied a method to test the efficacy of various disinfectants and to determine the effective dilution of those disinfectants

Materials and methods

We tested 108 samples for their disinfectant efficacy (contract research request). Most products were liquid

(n=63), some powder (n=46) and one product was supplied as tablets. Samples were administrated, stored etc., and per disinfectant 4 requested effective dilutions were made in hard water.

The laboratory method we used to test the disinfectants was a mixture of the UK methodology and the European Standard EN 14675:2006 "Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of virucidal activity of chemical disinfectants and antiseptics used in the veterinary field - Test method and requirements (phase 2, step 1). Shortly, it comprises of mixing FMDV type O BFS with a predetermined concentration of the disinfectant, sampling the mixture after 5'and 30' (where the effect of the disinfectant was stopped by dilution in medium), titration of the samples on secondary lamb kidney cells (in 3-fold), calculation of FMDV titres and calculation of the reduction of FMDV growth. As controls, hard water and 1% NaOH were included.

To analyse the effect of different combinations of ingredients we calculated the percentage of tests (combination of disinfectant and test dilution) that produced at least 4 ^{10}log reduction. (Note that we matched disinfectants with similar active ingredients, but that does not mean that the concentration of these active ingredients were the same).

Results

In total 73 of the 108 products contained acid as an active ingredient. Most products contained citric acid, others malic acid or combinations of those two acids. The experiments were performed with different sets of disinfectants on 10 different days. The average titre obtained with the hard water control in the experiments was 7.45 ^{10}log pfu/ml with a standard deviation of 0.35 ^{10}log pfu/ml. In all experiments, the NaOH control reduced the virus titre more than 4

^{10}log in 5 minutes.

From the 73 disinfectants containing acid a total 71 (97%) were effective (4 ^{10}log reduction) in one of the dilutions tested within 5 minutes after mixing. Almost no increase in efficacy was observed with acid based disinfectants when incubated for 30 minutes. In the other 37 disinfectants not containing acid, only 10 (27%) were effective in one of the dilutions tested within 5 minutes. This difference is statistically significant ($p < 0.001$, fisher exact test).

Conclusion

Our method is simple for testing the efficacy of disinfectants against FMDV. Although the efficacy of disinfectants is often evaluated after 30 minutes of incubation, we believe that efficacy after 5 minutes is more relevant for use of disinfectants in field situations. Disinfectants containing acid are more effective against FMDV than products that not contain acid.

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FRAMEWORK TO COMPARE THE IMPORTANCE OF THE DIFFERENT SOURCE REGIONS FOR ENTRY OF FMDV INTO EUROPE

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Introduction

In its recent opinion on vaccine banks for major animal diseases, the European Commission highlighted the importance of selecting vaccine strains on the basis of a risk assessment informed by up-to-date knowledge of the FMDV serotypes and strains distribution and of the likelihood of their spreading to the EU (EC SANCO/7070/2010). To our knowledge, no such risk assessment is available today. Therefore, the study objective was to develop an updated framework to compare the FMD regional pools importance for entry of FMDV into Europe.

Materials and methods

We updated and refined the risk assessment framework developed by McLaws *et al*. (McLaws 2010). This additional work consisted for each country of i) updating the FMD occurrence level, ii) evaluating the importance of the FMDV transmission routes using proxy indicators and iii) computing a weighted linear combination of the transmission route scores that aims to describe the country contribution to FMD release into Europe. National results were further combined at FMD pool levels to provide recommendations on the most important pools to consider for the EU vaccine banks preparation.

Results

Study analysis is still on-going and preliminary results will be available for the EuFMD Open Session.

Discussion

Providing updated recommendations on the relative importance of the different FMD pools is one part of a process of prioritizing antigens for the EU vaccine banks, the other is the analysis of trends in the circulation of serotypes and strains in the priority pools (conducted by the WRL since 2010). Possible combinations of these two activities need to be explored. Identifying priority pools could also inform the international efforts to improve FMD surveillance.

Finally, possibilities to improve access to data that are critical for this risk assessment and the best frequency at which the risk assessment should be updated will be further discussed.

notes

THE ENHANCED PASSIVE SURVEILLANCE SYSTEM: A SOLUTION SUPPORTING DATA COLLECTION, INTEGRATION AND ANALYSIS FOR DISEASE SURVEILLANCE

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Introduction

The future of disease surveillance and risk management requires systems capable of providing real-time situational awareness of disease prevalence and location and taking advantage of existing data streams (e.g., veterinarians, livestock owners, diagnostic laboratories) in order to detect disease events and trends. The Enhanced Passive Surveillance (EPS) system provides a platform to rapidly collect and integrate animal health data in real-time to provide early detection of potential disease outbreaks or changes in endemic disease.

Materials and methods

Working closely with U.S. agricultural industries, veterinarians, diagnostic laboratories, and state/federal animal health officials, mobile applications were developed that allow participants to enter data on healthy and sick animals into the EPS system directly from the field using mobile devices and web-based reporting tools. Submitted data are assimilated into a common integrated display, called the EPS Analyst Workstation (AWS), and combined with veterinary diagnostic laboratory and climate/environmental data sources for monitoring and analysis.

Results

The EPS system is being piloted in the U.S. by 61 veterinary practitioners and two diagnostic laboratories reporting on cattle, cervids, equine, poultry, small ruminants, and swine species. Since July 2012, over 21,077 healthy and syndromic reports have been submitted, representing the animal health status of over 1.3 million animals within four States. Eleven state/federal animal health officials have been trained on the EPS AWS and its use for determining baselines and trends.

Discussion

Piloting of the EPS system has had many successes, including obtaining industry buy-in, demonstrating security of information, integrating data streams, and customizing the mobile applications to meet daily industry requirements and encourage participation. The system is being expanded to all U.S. livestock/poultry industries and wildlife. The EPS system is adaptable with broad applications in the international community for early disease detection and animal health monitoring. The system provides a low-cost, low-maintenance solution supporting real-time situational awareness, surveillance, and risk management.

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QUANTITATIVE RISK ASSESSMENT EVALUATING THE TRANSMISSION OF FOOT-AND-MOUTH DISEASE VIA FRESH DEBONED BEEF PRODUCED FROM AN ENDEMIC REGION

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Introduction

Article 8.6.26 of the OIE's Terrestrial Animal Health Code provides an international standard for the safe importation of chilled or frozen deboned beef from FMD infected countries or zones. The objective of this investigation was to determine whether a formal value-chain risk management approach would provide 'equivalence' in terms of risk management to with the existing international standard (Article 8.6.25).

Materials and Methods

A value-chain approach for the reduction of FMD risk was developed for the Zambezi Region (ZR) of Namibia using integrated HACCP and commodity-based trade (CBT) principles. A quantitative, stochastic risk assessment was conducted on three independent scenarios, *viz.* 1) present operating procedures (Current), 2) theoretical application of Article 8.6.25 and 3) developed value chain system (VCA). Analyses were conducted independently for different cuts of beef and exposure assessment was based on swine consuming waste beef.

Results

The mean (range) number of FMD virus contaminated carcasses that could pass through all safeguards on an annual basis was estimated as 0.48 (0, 8), 0.18 (0, 4), and 0.02 (0, 2), for the Current, Article 8.6.25, and VCA scenarios. Estimated probabilities of infection of swine were consistently highest for 'Current' and lowest for the VCA. The probability (range) that a box of fillets would cause FMD virus infection in exposed swine in the importing country was 4.3x10-8 (0, 1.4x10-4), 1.6x10-8 (0, 8.7x10-5), and 2.1x10-9 (0, 5.3x10-5) for the Current, Article 8.6.25, and VCA scenarios, respectively.

Discussion

A risk management system based on integration of HACCP and CBT approaches along a defined beef value chain documented equivalence with existing international trade standards. This system has the potential to benefit many thousands of resource poor cattle farmers and offers a system whereby both wildlife conservation and commercial beef production can be accommodated in FMD endemic areas.

notes

MODELLING FOOT-AND-MOUTH DISEASE TRANSMISSION IN A FERAL PIG–DOMESTIC CATTLE ECOSYSTEM

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Introduction

Feral pigs are an invasive species in Australia that cause agricultural, economic and environmental damage and are a reservoir for important zoonotic diseases. Since elsewhere they have been implicated in the spread of emergency trans-boundary diseases such as classical swine fever and FMD, feral pigs are perceived as a major biosecurity threat in Australia.

Materials and Methods

To estimate potential FMD spread and control in a mixed feral pig-domestic cattle ecosystem in northern Australia, feral pig and grazing cattle distributions were created within a GIS. Aerial survey methodology, expert opinion and local knowledge were used to determine species abundance and density.

A susceptible-infected-resistant disease spread model was coded and parameterised based on published literature and expert opinion. Pig–to–pig transmission can occur when home ranges of infectious and susceptible groups overlap. Cattle–to–cattle transmission is based on infection pathways (shared watering points, proximity, indirect contact or cattle movements). Transmission between pigs and cattle is based on daily herd home range overlap.

Results

Outbreaks were predicted to be ongoing after 6 months in most simulations, with more cattle herds infected than feral pig herds (median 907 vs. 22, respectively. Simulations were sensitive to assumed transmission parameters. Assuming only pig–to–pig transmission, the infection routinely died out. In contrast cattle–to–cattle, cattle–to–pig or pig–to–cattle transmission produced outbreaks in >75% of simulations. A control strategy targeting feral pigs was not predicted to be successful, with outbreaks still developing in 87% of the simulations. Control targeting both pigs and cattle resulted in smaller outbreaks (less herds depopulation, shorter outbreak period and less land area affected) in cattle and in pigs.

Discussion

If FMD is controlled in cattle in the modelled ecosystem, it is likely to be self-limiting in feral pigs. This has important implications in terms of disease response and resource management.

notes

GEGRAPHICAL FACILITATORS AND NON-FACILITATORS OF FMD DSSEMINATION

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Introduction

One major property of complex systems is emergence: a new and informative pattern is revealed when the complex system is assembled. The properties of complex systems have not yet been demonstrated in epidemics.

Materials and methods

To elucidate whether epidemics display complex system-related properties, a pattern recognition-oriented method was developed and evaluated with geo-referenced data collected in the first 11 epidemic weeks of the 2001 Foot-and-Mouth Disease (FMD) epidemic that took place in Uruguay. Three-dimensional (3D) data analysis, together with map-based assessments, explored relationships among the number and location of FMD-infected farms and five county-related variables: farm density, road density, river density, and the ratio of road (or river) length/ county perimeter.

Results

A distinct pattern identified 11 counties as possible 'facilitators' (F) of epidemic spread. The remaining 264 counties were suspected to be 'non-facilitators' (NF). Most F counties were geographically clustered. In the first 9 epidemic weeks, F counties displayed a statistically significantly higher road density and a lower proportion of river segments in the county perimeter (natural barriers) than NF counties. F counties showed higher (early) and lower (late) case density than NF counties. Emergence was demonstrated: when case data were analyzed in 'facilitator' counties, together with area and road density data, three non-overlapping (very early, plateau, and resolution phase-related) subsets were observed; however, the same data structure only revealed two subsets in NF counties. Because F and NF counties displayed overlapping area and road density data distributions, such variables, alone, did not predict emergence. Several 3D analyses that measured geographical structures but did not consider FMD case data characterized the early epidemic stage.

Discussion

Geographical structures likely to facilitate the initiation of epidemics are not randomly distributed. Complexity analysis provides an alternative to identify such structures before epidemics occur.

notes

EVALUATING THE TRANSMISSION AND SURVIVAL OF FOOT AND MOUTH DISEASE VIRUS ON ENVIRONMENTAL FOMITES

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Introduction

Foot and mouth disease virus (FMDV) can survive outside of the animal host, which provides a potential transmission route. Survival of virus in the environment is a very important determinant of this risk of transmission. The objective of this study was to assess the risk a contaminated environment represents for transmission of FMDV.

Materials and methods

Firstly, four direct contact pair-transmission experiments were carried out whereby successful transmission of FMD between calf donors and receivers occurred. Following this, pilot indirect transmission experiments were conducted to investigate the transfer of FMDV through fomites. The same four unclean rooms were used to challenge sentinel calves (one per room) for an eight hour period. During the exposure time, air and fomite samples were taken. After exposure, the calves were monitored for two weeks for clinical signs and shedding of virus.

Results

None of the sentinel calves became infected following the eight hours of exposure. However, positive PCR results were obtained from nasal swabs and probang samples taken at the end of the exposure period. Presence of FMDV viral genome in the environment was confirmed by PCR. The virus concentration in the air was similar in the four rooms ranging from $10^{-1.3}$ to $10^{-2.9}$ equivalent $TCID_{50}$/litre of air. The virus concentration on the walls ranged from $10^{2.06}$ to $10^{2.38}$ equivalent $TCID_{50}/m^2$. No live virus was recovered from these samples. Following these results the impact of the contaminated environment (faeces, surfaces, urine, water) on virus survival is being evaluated.

Discussion

The results of this study suggest that an eight hour exposure period may not be long enough for a successful transmission to occur. Quantitative information on the survival rate of the virus in excretions and on fomites will help to better quantify the level of virus to which sentinels were exposed.

notes

TECHNIQUES TO ASSESS THE RISK OF AIRBORNE SPREAD OF FOOT AND MOUTH DISEASE AND EXAMINE UNDERLYING UNCERTAINTIES

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Introduction

Understanding the transmission mechanisms of foot and mouth disease (FMD) can aid the development of control strategies. One potential route for transmission between farms involves transportation of the virus as an aerosol in the atmosphere. This transmission route is important as it offers a potential pathway for spread even after a ban on animal movement has been implemented during the early stages of an outbreak. We examine how an atmospheric dispersion model may be used to support decisions related to planning or emergency response. Additionally we examine the uncertainties in the characterisation of virus emissions and meteorological variables driving downwind transport.

Materials and Methods

The Met Office atmospheric dispersion model NAME (Numerical Atmospheric-dispersion Modelling Environment) is a Lagrangian particle model used for response to a variety of hazardous events. Using hypothetical FMD outbreaks we use NAME to examine the influence of uncertainties in aerosol emissions. The impact of wider environmental conditions and atmospheric circulation patterns are also examined to provide information for strategic planning.

Results

The analysis provides an ensemble output of emission profiles indicating the likelihood of atmospheric spread due to aerosol size and other release characteristics. The size of the aerosol, wind speed and atmospheric stability have the greatest impact on atmospheric residency of the virus at distances beyond a kilometre.

Discussion

This analysis provides valuable additions to the tools required for the management of an FMD outbreak. Understanding how meteorological variables can influence FMD spread offers useful information for emergency response and, with the development of risk incursion maps, strategic planning. Our assessment of the uncertainties underlying this route of transmission provides guidance on the robustness of modelling outputs and where future research on aerosol characterisation should be focussed.

notes

DEVELOPMENT OF A STANDARD MODEL FOR FOOT-AND-MOUTH DISEASE IN THE UNITED STATES

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Introduction

Emergency planning focuses on identifying control activities that offer the greatest potential for minimizing severity and duration of outbreaks. In order to establish realistic scenarios for evaluating control strategies, modelers must specify the susceptible livestock population, determine herd-level disease state transitions, and characterize animal movements, direct contacts, and indirect contacts between livestock production types. Our objectives were to define the susceptible livestock population and to establish parameters estimating pathogen transmission and control to facilitate the development of a standard model for FMD in the United States.

Materials and Methods

A microsimulation model, the Farm Location and Animal Population Simulator, was used to distribute livestock farms throughout the United States based on data sourced from the 2007 U.S. Census of Agriculture. An extensive literature review, solicitation of expert opinion from agricultural extension specialists and industry representatives, and comprehensive applications of survey responses from CEAH national studies were integrated to identify production types, to establish regions, and to estimate parameters for animal movement, contact, and control. Interspread Plus® (v.5.1.3.12) was used to model the spread and control of FMD in the United States.

Results

The standard model for FMD comprises 1.92 million farms distributed across twenty-three production types among bovine, caprine, ovine, and porcine classes in five geographic regions. Currently, the model incorporates twenty-six movement types and includes control measures associated with movement restrictions, surveillance, epidemiologic investigation and tracing, depopulation, and vaccination. Model outputs provide estimates of the numbers of herds and animals infected, depopulated, and vaccinated.

Discussion

This model provides a readily available and well-defined tool that can be used to address animal health questions of regional or national scope while incorporating local-area aspects related to animal populations, density, and production characteristics. Future objectives include data collection, refinement of model parameters, and application of the model to evaluate control strategies.

notes

RESOURCE ESTIMATIONS IN CONTINGENCY PLANNING FOR FOOT-AND-MOUTH DISEASE

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Introduction

Preparedness planning for a veterinary crisis is important to be fast and effective in the eradication of disease. For countries with a large export of animals and animal products, each day in an epidemic will cost millions of euros due to the closure of export markets. This is important for the Danish swine industry, which had an export of €4.4 billion in 2012.

Materials and methods

The purposes of this project were to: 1) estimate the resources needed during an outbreak of FMD in Denmark, 2) identify areas, which can delay the control of the disease, and 3) develop an iterative tool, which can easily be updated, when knowledge is gained from other veterinary crises or during an outbreak of FMD.

A stochastic simulation model was developed in InterSpread Plus to simulate spread of FMD in Denmark. The personnel and resource needs were estimated using results from this model.

Results

We estimated that the need for personnel would peak on day 7 with a requirement of approximately 170 veterinarians, 70 technicians and 45 administrative staff. However, the need for personnel in the Danish Emergency Management Agency (responsible for the hygiene barrier and initial cleaning and disinfection of the farm) would peak already on day 4 with a requirement for almost 500 persons, mostly recruits.

On average, 53,000 animals were culled during the simulated epidemics, leading to a daily need for rendering capacity of up to 210 tons for swine and 379 tons for ruminants.

Discussion

Based on results from the stochastic simulation model, it was possible to create a simple model in excel to estimate the requirements for personnel and materiel during an FMD outbreak in Denmark. The model can easily be adjusted, when new information on resources appears from management of other crisis or from new model runs.

notes

MAXIMISING EFFICIENCY WITH A SURVEILLANCE STRATEGY FOR FOOT-AND-MOUTH DISEASE DURING AN OUTBREAK IN A PREVIOUSLY FMD-FREE COUNTRY.

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Introduction

New Zealand has never had an outbreak of foot-and-mouth disease, but preparedness planning for responding to an incursion is a high priority for New Zealand's Ministry for Primary Industries. As part of FMD preparedness in New Zealand, a sampling and diagnostic surveillance strategy was developed to guide rapid and efficient detection of infected farms, through to proof-of-disease-freedom surveillance.

The strategy establishes the appropriate diagnostic testing algorithm, specimen types and sample size numbers to use for different farm situations, allowing rapid deployment of an effective, pre-validated surveillance programme to diagnose infected properties, including pre-clinical ones, with the greatest efficiency, accuracy and speed.

Methods

Factors assessed in development of the strategy include:

- the available diagnostic tests for FMD in the country, and the sensitivity and specificity of these and parallel and series combinations thereof;
- the practicalities of obtaining certain sample types and numbers, and of laboratory resource requirements;
- the design prevalences and desired confidence in results for the surveillance programme; and
- a generalised time-line of virus and antibody presence in an individual animal.

Farm-level sampling and testing is determined by:

- the surveillance category of a property, the probable time since exposure to virus, and the presence or absence of clinical signs; and
- the species, and number of animals and management groups present on a farm.

Results

The sampling guidelines are presented as a flowchart on the pre-specified laboratory submission form, enabling the field veterinarian to easily determine the appropriate samples to take for

a particular farm. The same submission form identifies for the laboratory which of the pre-established testing algorithms the samples should follow, and their priority.

Discussion

The diagnostic surveillance strategy is designed to improve efficiency in an outbreak by streamlining decision-making at the farm and the laboratory. It will reduce reliance on clinical surveillance and enhance pre-clinical diagnosis, hopefully aiding in faster delimitation of an outbreak and ongoing management.

notes

EVALUATING VACCINATION STRATEGIES TO CONTROL FOOT-AND-MOUTH DISEASE: A MODEL COMPARISON STUDY

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Introduction

Vaccination is increasingly being recognized as a potentially important tool in controlling Foot-and-Mouth Disease (FMD), although considerable uncertainty exists as to how and when it should be used. Simulation models can offer insights into the effectiveness of control strategies and act as decision-support tools when comparing scenarios and control strategies. This study involved five countries (Australia, New Zealand, United States of America, the United Kingdom, and the Netherlands) comparing a range of vaccination strategies.

Materials and Methods

The scenario used was a hypothetical, multifocal outbreak in 38 counties of the UK, with detailed history and spread of disease. The population at risk, first infected premises (IP) and premises infected during the silent spread phase were given to each modelling group. Modellers then simulated disease spread with a series of pre-specified control strategies.

Model capabilities, parameter estimates and control strategies were defined so each group could configure their model to represent equivalents.

Vaccination scenarios were selected taking into account contingency plans and policy priorities of all countries involved, including: timing, size of vaccination zones, species vaccinated, and deployment of vaccination.

Results

Results recorded were number of IPs, spatial distribution of IPs, outbreak duration and number of vaccinated premises. All models demonstrated that vaccination with 'stamping-out' of IPs reduced the predicted size and duration compared to a 'stamping-out' alone. All models demonstrated the benefits of vaccination were greater when deployed earlier, that suppressive

vaccination zones were more beneficial than protective vaccination zones, and out of the sizes of suppressive zones evaluated, 3 km radius gave the best results.

Discussion

This study has shown that certain vaccination strategies are effective and robust to substantial differences in model designs. These results should increase end-user confidence in conclusions drawn from model outputs and can be used to support and develop effective policies for FMD control.

notes

IMPACT OF STAKEHOLDERS INFLUENCE, GEOGRAPHIC LEVEL AND RISK PERCEPTION ON STRATEGIC DECISIONS IN SIMULATED FOOT AND MOUTH DISEASE EPIZOOTICS IN FRANCE

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Comparison of control strategies against animal infectious diseases allows determining optimal strategies according to their epidemiological and/or economic impacts. However, in real life, the choice of a control strategy does not always obey a pure economic or epidemiological rationality. The objective of this study was to analyze the choice of a foot and mouth disease control strategy as a decision-making process in which the decision-maker is influenced by several stakeholders (government, agro-food industries, public opinion). For each of these, an indicator of epizootic impact was quantified to compare seven control strategies. We then determined how, in France, the optimal control strategy did vary according to the relative weights of stakeholders and to the perception of risk by the decision-maker (risk-neutral/risk-averse). When the scope of decision was national, whatever their perception of risk and the stakeholders' weights, decision-makers chose a strategy based on vaccination. This consensus concealed marked differences between regions, which were connected with the regional breeding characteristics. Vaccination-based strategies were predominant in regions with dense cattle and swine populations, and with a dense population of small ruminants, cattle and swine densities being medium. These differences between regions suggested that control strategies could be usefully adapted to local breeding conditions. We then analyzed the feasibility of adaptive decision-making processes depending on the date and place where the epizootic starts, or on the evolution of the epizootic over time. The initial conditions always explained at least half of the variance of impacts, the remaining variance being attributed to the variability of epizootics evolution. Thus adaptive strategies changing dynamically according to the evolution of the epizootic appear feasible. A further study will permit to elaborate and evaluate a steering tool for the control of FMD epizootics in France, based on the combination of a control panel and indicators.

notes

AN ADAPTATIVE MANAGEMENT APPROACH TO FMD CONTROL

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Introduction

In the early stages of a disease outbreak, there will be significant uncertainty regarding the epidemiological characteristics of the outbreak and hence the accuracy of predictive models. Whilst this uncertainty will take time to resolve, it is vital that intervention strategies are introduced at an early stage in order to have maximum effect. Adaptive Management (AM), is a structured decision-making approach to solving dynamic problems that accounts for the value of resolving uncertainty via real-time evaluation of alternative models. Here we present an investigation into the use of AM for outbreaks of foot-and-mouth disease (FMD).

Methods and Results

We use predictions of the impact of competing intervention strategies for outbreaks of FMD in the UK as predicted by multiple models to quantify the effect of model uncertainty on decision-making. During the 2001 UK FMD outbreak, had AM been used to determine optimal control policies, the approach predicts a saving of up to £20.1M in terms of livestock saved. AM would have recommended a more conservative initial approach than would a fixed strategy. We also investigate the optimal initial control strategy that should be introduced for future outbreaks using multiple FMD models in an ensemble (AusSpread, DADs, Interspread, NAADSM and the Warwick model). The optimal control policy is dependent upon the weight of belief attached to individual models and the objective of the control strategy (e.g. minimizing farms affected, livestock lost, cost or duration of outbreak).

Discussion

Formal incorporation of a policy to update future management actions in response to information gained in the course of an outbreak can change the optimal initial response and result in significant cost savings. Adaptive management provides a framework for using multiple models to facilitate public-health decision-making and an objective basis for updating management actions in response to improved scientific understanding.

notes

ENSEMBLE FORECASTING FOR EPIDEMIOLOGY: HOW TO COMBINE MULTIPLE PROJECTIONS OF FMD

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Introduction

Ensemble modeling offers the possibility to combine several projections. In multi-model ensembles, different assumptions about the transmission process can be incorporated into the projections. In other fields of research this been demonstrated to make more robust predictions. We propose that this is a promising approach for epidemiology but there is a need for the development of methods to combine projections. This requires weighting of models, which can be done in four different ways: 1) equal weights to all models, 2) by ability to reproduce observed dynamics, 3) by consensus between model projections and 4) through expert opinions.

Materials and methods

We propose that methods used in Climate modelling, particularly the Bayesian Reliability Ensemble Average (BREA) could be adapted such that it can be used for epidemiology. Focusing on ensemble prediction of the efficiency of different control strategies, we apply the methodology to two case studies: (A) various parameterizations of the Warwick model for the 2001 UK FMD outbreak and (B) a multi-model ensemble, including NAADSM, InterSpread+, AusSpread, ExoDis, CVI and the Warwick model, simulated for the Silver Birch exercise.

Results

We conclude that the BREA method has promising potential for the field of epidemiology, yet requires modifications to account for the lack of available outbreak data. Through various adjustments, we show that all four weighting schemes mentioned above can be incorporated. By using a hierarchical Bayesian framework, we further reduce the effect of which projections are included in the ensemble.

Discussion

We propose that ensemble modelling is a beneficial approach because it obviates the need to choose a single model to inform policy decisions. Rather, the uncertainty about the underlying process can be incorporated by combining several models. Our work reveals that it is beneficial to preserve model differences, rather than aiming for a deliberate consensus between them.

notes

EPIDEMIOLOGIC AND ECONOMIC IMPACTS OF APPLYING ALTERNATIVE CONTROL STRATEGIES FOR FMD IN FEEDLOT OPERATIONS

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Introduction

Emergency response exercises have recognized issues associated with traditional control of FMD in large feedlots. The depopulation and disposal of large numbers of animals poses difficult challenges for environmental management and resource requirements. Alternative methods are needed for minimizing disease spread while allowing animals to reach their intended purpose.

Materials and methods

The spread and control of FMD within a 7 state region of the US was modelled for 4 strategies: stamping out (S-O) with ring vaccination (baseline strategy), modified S-O with no depopulation of feedlots and ring vaccination surrounding infected herds; modified S-O with no depopulation or vaccination of feedlots; modified S-O with no depopulation of feedlots, ring vaccination surrounding infected herds, and vaccination of infected feedlots. The effect of strategy and start location on outbreak characteristics was explored using Kruskall-Wallis comparisons and logistic regression. Epidemiologic results were fed into an economic modelling framework to estimate national economic impacts for each strategy and a minimum marketed beef price for feedlot operations under which that strategy becomes viable.

Results

None of the alternative strategies resulted in an increased number of infected herds or animals, while all alternative strategies resulted in a significant decrease in the number of depopulated herds and animal numbers ($p<0.004$). Baseline strategy resulted in shorter outbreaks (32 vs 46 median days for other strategies; $p<0.004$). Changes in national fed cattle prices were largely driven by trade assumptions. Analysis of impacts on individual feedlot operations is ongoing.

Discussion

Allowing feedlot cattle to recover and enter normal market channels post-outbreak does not appear to have serious epidemiologic consequences. Economic viability of alternative strategies is expected to be driven in large part by trade implications.

notes

EPIDEMIOLOGICAL AND ECONOMIC MODELLING TO DETERMINE THE COST TO NEW ZEALAND OF A FOOT-AND-MOUTH-DISEASE OUTBREAK

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Introduction

There have many trade flow and farming pattern changes since the impact of a foot-and-mouth disease (FMD) for New Zealand was last quantified in 2003. An updated assessment was undertaken to allow for informed decision making in the current review of the New Zealand FMD strategy.

Materials and methods

Epidemiological modelling was undertaken using Interspread Plus (ISP), a stochastic simulation model, using the pre-agreed New Zealand Standard Model input parameters. Three baseline scenarios were chosen from the iterations- small, medium (the mean iteration) and large. A vaccination and a disease free zoning scenario were also selected.

Three input shocks we used to model the economic impact for each epidemiological scenario:

- animal product export impacts
- Tourism impacts.
- Eradication costs

The scenarios were run through a dynamic Computable General Equilibrium (CGE) model of the New Zealand economy. Actual 2011/2012 data were used as counterfactual.

Results

The net present value GDP losses for the period 2012- 2020 varied from NZ$ 6.1 billion for the small outbreak to NZ$16.2 billion for the large outbreak. By far the largest proportion of costs was due to export losses, with disease control costs accounting for a relatively small proportion of the total costs.

Discussion

The significant cost of an FMD incursion to New Zealand is not unexpected. However, this review has highlighted the wide impacts of the disease across a range of sectors, as well as its potential social impacts. It provides information on the scale of disruption to the primary processing industries, and quantifies the scale of stock numbers that will need to be managed as animals

can no longer be processed for export. This modelling study reaffirms the value of investment in FMD prevention and preparedness, and provides very useful guidance on areas of mitigation where efforts should be focused.

notes

FMD VIRUS STABILITY: IMPLICATIONS FOR VACCINE EFFICACY

T. Doel

AVAILABLE UPON REQUEST

THERMOFLUOR ANALYSIS OF THE FMDV CAPSID AND THE EFFECTS OF DIFFERENT SOLUTIONS ON STABILITY

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Introduction

The structural integrity of the FMDV capsid is a major determinant of efficacy for FMD vaccines. The FMDV capsid is both pH and temperature labile. Current FMD vaccines are frequently produced from inactivated virus, a process which further reduces capsid stability. Likewise, storage conditions can greatly affect the integrity of the FMDV capsid. We have used new technology to characterise the effects different solutions have on the thermostability of the FMDV capsid.

Materials and methods

We have used thermofluor-based technology to investigate the thermostability of the FMDV capsid of O, SAT 1, SAT 2 and SAT 3 serotypes following incubation in different solutions. As an indicator of capsid disassembly, viral genome release was monitored using a dye sensitive to the presence of nucleic acid during a slow increase in temperature. Assays were performed using an RT-PCR machine and purified virus samples.

Results

We show that thermostability of the FMDV capsid can be increased, by up to 10^{o}C, in the presence of sugars and monovalent and divalent cations.

Discussion

Here we describe the use of thermofluor-based technology to analyse the effects different solutions have on thermostability of the FMDV capsid. This methodology allowed accurate determination of the temperature at which the FMDV capsid dissociated and offers a simple tool to optimise storage conditions and analyse the stability of FMDV before and after inactivation.

notes

A NEW METHOD BASED ON THE USE OF SPECIFIC LLAMA ANTIBODIES FOR QUALITY CONTROL TESTING OF FMD VACCINES

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Introduction

One of the main handicaps of inactivated FMD vaccines is the instability of viral particles. A facile method to measure antigen integrity is needed. Here we describe an ELISA using specific recombinant llama antibody fragments (VHHs) able to discriminate between intact viral capsids (146S) and dissociated capsid subunits (12S) to measure antigen stability.

Materials and methods

Phage display libraries were generated from llamas immunised with multiple FMDV serotypes (O, SAT2, Asia, A) and VHHs specific for FMDV 146S particles were selected by biopanning. Selected VHHs were tested for their ability to bind 146S and 12S FMDV antigen by double antibody sandwich (DAS) ELISA. The effect of buffers, temperature and pH on the stability of the three FMDV serotypes was also investigated.

Results

Two VHHs (M170F and M3F) were earlier described to be specific for O1Manisa 146S and 12S subunits, respectively (Harmsen et al., 2011). These antibodies cross-reacted with serotype counterparts OUKG and O Tur/05/09. In addition, M3 cross-reacted with A and Asia serotypes but was still specific for 12S. We isolated two novel 146S specific VHHs. One VHH (M377F) was specific for 146S SAT2 and was able to bind both ZIM2 and Eritrea SAT2 subtypes. Finally, one VHH (M332F) was highly specific for 146S Asia 1 Shamir and was shown to be strain specific since it didn't bind Asia Bahrain. VHHs generated for A serotype were not specific for intact or disassembled particles.

Discussion

Here we describe a simple, quick and economic methodology that allows us: 1) to quantify the content and integrity of the antigen contained in a FMD vaccine; and 2) to monitor the stability of the antigen under physical (temperature) and chemical (excipients) conditions, which are important for vaccine storage and formulation. Moreover, we believe that such methodology would be extremely useful for the study and development of mutant viruses with greater stability, as well as for the study of the integrity of FMDV virus-like particles in subunit vaccines.

notes

EVALUATION OF THE IMMUNE RESPONSES OF NGUNI CATTLE VACCINATED WITH FOOT-AND-MOUTH DISEASE (FMD) STABILISED SAT2 ANTIGENS

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Introduction

FMD viruses, especially SAT2 serotypes, are unstable during heating leading to irreversible dissociation into pentamers causing loss in immunogenicity, and requiring frequent booster vaccinations. The hot climate in African regions necessitates improved stability with less reliance on a cold chain. The adjuvant used in the vaccine formulation has also an enormous effect on the efficacy and potency of the vaccine. We investigated whether a structurally stabilised SAT2 antigen vaccine could improve immunogenicity in cattle.

Materials and methods

Using reverse-genetics we produced recombinant FMD viruses with enhanced thermal stability. To evaluate the potency of current wildtype SAT2 antigen versus two thermo-stable SAT2 antigens, we immunized groups of 7 calves with two doses of 6.0 µg/dose (0 and 42 dpv) formulated with ISA206 or aqueous-saponin adjuvants. Two animals were left as controls. Animals were challenged at 162 dpv. Immune responses were evaluated with different assays as markers of protection and compared with clinical scores.

Results

While control animals developed lesions in the feet, all vaccinated animals, except one from the saponin-adjuvant group were protected from challenge. The kinetics of neutralizing antibodies <56 dpv are similar between all vaccinated groups, although titers were higher for wt followed by stabilized antigens (ISA206). After second dose there is no statistical difference in VNT titers between vaccinated groups. By 14dpv all groups increased their total antibody titers except the saponin group. The second vaccination elicited similar total antobody titers in all groups except one month after boost the saponin group showed decreasing titers.

Discussion

Stabilised antigens are important for improved African FMD vaccines. Novel immune assays in combination can be used as markers of protection in assessing immune responses. The oil adjuvant maintained antibody levels longer than the aqueous adjuvant. Other assays including isotyping, avidity and interferon gamma will help to identify differences in vaccines.

notes

DEMONSTRATION OF A HIGH POTENCY SAT2 VACCINE IN CATTLE

AND CONFIRMATION OF EFFICACY IN PIGS AGAINST VIRULENT CHALLENGE

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Introduction

FMD infection with SAT strains have been well studied in cattle, but not in pigs. Merial investigated the efficacy of a SAT2 vaccine in both species.

Materials and methods

PD50 study in cattle

Four groups of 5 cattle were vaccinated with a decreasing dose (volume) of a commercial double oil emulsion vaccine containing SAT2 antigen. Another group of 2 cattle was left unvaccinated. All animals were challenged in the tongue 28 dpv, by 10 000 cattle ID_{50} of a virulent SAT2 Saudi Arabia virus. After humane euthanasia (D36), all animals were inspected for FMD lesions.

Efficacy study in pigs

On D0, a commercial SAT2 vaccine was administered by IM route to 1 group of 5 pigs. Another group of 5 pigs was left unvaccinated. All pigs were challenged on D28 with 100 000 $TCID_{50}$ per 0.1 ml of a 2nd-pig-passaged SAT2 Saudi Arabia virus, in the bulb of the heel. Clinical and virological (oropharyngeal swabs) monitoring were humanely performed until D36.

Results

PD50 study in cattle

Both controls developed FMD lesions on all feet. A strong dose-response relationship was demonstrated in the vaccinates. The potency of the vaccine was estimated ≥ 59 PD50/dose.

Efficacy study in pigs

One control pig, with feet and tongue FMD lesions, showed lameness and was euthanized 4 dpi. On D36, in 2 out of 4 remaining controls, generalisation to the tongue was observed. Only 1 of the vaccinates showed a doubtful lesion of another foot than the inoculated one. All vaccinates remained virologically negative after challenge, although all controls were positive.

Discussion

The vaccine tested provides a very high level of clinical protection against SAT2 challenge in cattle. Its efficacy against virulent challenge was confirmed in pigs, although the role of pigs in the spread of SAT2 serotypes has not been reported so far.

notes

LOOKING FORWARD FROM FMD EPIDEMIOLOGY TO FMD ECOLOGY

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Introduction

At their cores, epidemiology is a description of risk, and ecology is a description of interactions. While both disciplines increase our understanding of disease at a population level, prevailing questions in foot-and-mouth disease virus (FMDV) research relate to ecology more than epidemiology. For example, what are the effects of movement, host and environment factors, clinical versus sub-clinical disease, co-circulation of viruses, and human behaviour on FMDV maintenance?

Materials and methods

We conducted a literature review of population-level FMDV risk and interactions relating to FMDV transmission. These publications are summarized. Also, we provide examples of studies that contrast data collection and analysis under epidemiologic and ecologic paradigms (Cameroon and UK).

Results

The vast majority of recent FMDV epidemiology studies are descriptions of serotypes circulating in various locations and general risk factors for disease. The methods employed in these analyses include calculations of means and percentages, contingency table analyses, and regression models. Ecological studies are comparatively rare for FMDV and use dynamical mathematical models to interpret the complex interplay between hosts and the pathogen and the mechanisms that drive the interaction, in a relatively small area.

Discussion

In general, published ecological studies have used less globally comprehensive disease data than epidemiologic studies. This is likely because most surveillance and regulatory data on FMDV is collected without the resolution necessary for ecological investigations. While some fundamental questions regarding FMDV epidemiology and biology remain to be answered, the ecological questions also need to be answered to understand the forces driving disease persistence. For these studies, detailed data from a few locations are more important than superficial global coverage. Thus, in addition to global collaborative efforts to improve surveillance, there is a need for harmonized smaller-scale research projects to answer the next generation of questions about FMDV and to move towards global disease control.

notes

MECHANISMS OF PERSISTENCE OF FMDV IN AFRICAN BUFFALO POPULATIONS: A BRIEFING ON CURRENT WORK AT KRUGER NATIONAL PARK

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The persistence of rapidly transmitting, acute pathogens in their natural host populations represents one of the fundamental puzzles in disease ecology: highly contagious, rapidly immunizing pathogens tend to reduce the pool of susceptible hosts to very low numbers, increasing their own risk of extinction during epidemic troughs. Our current work at Kruger National Park, South Africa, investigates how FMDV overcomes these challenges and persists in isolated populations of its reservoir host, the African buffalo. Here we present an overview of our theoretical, observational and experimental approaches, and preliminary results of this work-in-progress. Initial mathematical models of FMDV dynamics in African buffalo populations suggest that transmission between successive calf cohorts alone is not a plausible mechanism for long-term FMDV persistence in realistically sized buffalo populations. We hypothesize that carrier animals play a key role in disease persistence, and are investigating triggers for recrudescence of such carrier hosts, focusing on (i) protein-calorie restriction during the dry season and (ii) co-infections with respiratory pathogens. In addition, behavioral data from our buffalo study herd show pronounced heterogeneity in contact frequency among individuals, which may accentuate variation among hosts in their contribution to FMDV transmission.

notes

FOOT-AND MOUTH DISEASE ECOLOGICAL STUDIES IN ENDEMIC SETTINGS: ONGOING STUDIES IN VIETNAM AND PAKISTAN

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Introduction

Little information is available about the natural cycle of foot-and-mouth disease (FMD) in natural settings. We have engaged various GFRA partners and carried out prospective studies including serological, antigenic and genetic aspects of FMD virus infections among different livestock species and production systems in Pakistan, and Vietnam in order to gain insight into the natural ecology.

Materials and methods

Integrative studies were performed in the field, using a multidisciplinary approach combining clinical and subclinical surveillance, longitudinal studies and transmission studies and field "vaccine matching" using pooled sera from in-country vaccinated cattle or buffalo.

Results

In Vietnam, dairy farms were at lower risk of FMDV infection and farms with larger capacity and/or history of infection in 2010 were at higher risk. Furthermore, while buffalo showed the highest risk of being FMDV-infected, beef cattle had the highest carrier risk. In Pakistan phylogeographic analysis showed widespread distribution of multiple genotypes indicating fluid intra-country transmission pattern. Longitudinal studies in buffalo demonstrated carrier animals maintaining either one or multiple serotypes over multiple months of subclinical infection. Sequence analysis determined a close regional relationship with neighbouring countries of viruses (serotypes A, O, Asia1) circulating in either Pakistan or Vietnam. In country vaccine monitoring in Pakistan demonstrated circulating serotype Asia 1 and A strains that were not well covered by the vaccine formulation being used at the time.

Discussion

Descriptive epidemiology and modelling of animal-specific and herd-level variables described herein provide valuable information regarding risk factors for FMD in endemic settings. Surveillance including sampling of both clinical sand subclinical sampling provides a more in-depth view of FMD ecology.

notes

AN EMERGENT STRAIN OF FOOT-AND-MOUTH DISEASE VIRUS, SEROTYPE SAT 3, ISOLATED FROM A LONG-HORNED ANKOLE CALF IN THE QUEEN ELIZABETH NATIONAL PARK IN UGANDA

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Introduction

As part of a study of FMDV transmission between wildlife, especially the African buffalo (*Syncerus caffer*), and domestic animals, groups of Ankole cattle (ca. 6 months old) were introduced into Nyakatonzi (Kasese district), in close proximity to the QENP and sampled on a regular basis.

Materials and methods

Blood and probang samples were obtained before and after entry of the animals to the farm from which they moved regularly into the QENP for pasture and water. Sera were assayed for antibodies to the FMDV NSPs, positive sera were then tested for serotype specific antibodies by SPBE. RNA was extracted from the probang samples and analyzed for the presence of FMDV genomes using real time RT-PCR assays (RT-qPCR). FMDV was isolated on BTY cells from probang samples and analysed by serotype specific antigen ELISA and full genome sequencing.

Results

FMDV was isolated from a probang sample which had been shown to contain FMDV RNA. The virus was identified as SAT 3 by antigen ELISA and this was confirmed by sequencing of the complete virus genome. Comparison of the VP1 coding sequence indicated its closest relatives (ca. 80% identity) were viruses (topotypes V and VI) isolated previously from buffalo in Uganda. The complete virus genome was about 80% identical to SAT 3 viruses from southern Africa that were isolated about 50 years ago.

Discussion

Some 16 years after the last isolation of a SAT 3 FMDV from Uganda, a new isolate has been obtained from an apparently healthy long-horned Ankole calf which had been newly introduced

into the QENP. This is the first isolation of a SAT 3 FMDV from cattle in East Africa. The VP1 coding sequence was about 20% different from its closest relatives within Uganda and up to 36% divergent from SAT 3 viruses from southern Africa.

notes

RECOVERY OF VIRAL RNA AND INFECTIOUS VIRUS FOOT-AND-MOUTH DISEASE VIRUS FROM POSITIVE LATERAL-FLOW DEVICES

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Introduction

Foot-and-mouth disease Virus (FMDV) is an economically important, highly contagious *picornavirus* that affects both wild and domesticated cloven hooved animals. In developing countries, the effective laboratory diagnosis of foot and mouth disease (FMD) is often hindered by difficulty in the transportation and storage of clinical material resulting in inadequate preservation. This can result in a compromised ability to detect and characterise FMD virus in samples. Furthermore, the high cost of sending infectious virus material and the biosecurity risk it presents emphasises the need for a thermo-stable, non-infectious mode of transporting diagnostic material.

Materials and methods

This paper investigates the potential of using FMDV Lateral Flow Devices (LFD's) for dry transportation of clinical material for subsequent nucleic acid amplification, sequencing and recovery of infectious virus by electroporation.

Results

Cell culture isolates and epithelial suspensions from four FMDV serotypes were added to the LFD's after which it was possible to recover FMDV viral RNA that could be detected using real-time RT-PCR. Using this nucleic acid, it was also possible to recover VP1 sequences and also successfully utilise protocols for amplification of complete FMD virus genomes. When eluted RNA was directly inoculated onto susceptible cell cultures there was no infectious virus recovered, however following electroporation into BHK-21 cells and subsequent passage, infectious virus could be recovered. The LFDs could be stored for periods of one month at temperatures as high as at 37°C.

Discussion

Therefore, these results support the use of the LFD to be used for the dry, non-hazardous transportation of samples from FMD endemic countries to international reference laboratories. Official biosecurity guidelines for the transport of these inactivated sample types should now be considered and agreed.

notes

GLOBAL LANDSCAPE OF FMD CONTROL

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Since the global FMD control strategy brought to light and adopted by FAO and OIE member countries at the 2nd global conference on FMD in June 2012 , several initiatives were made to establish an enabling environment to make FMD control a feasible option particularly for countries that are affected the most by this disease. Progressive control pathway for FMD (PCP-FMD) was introduced as the guiding tool for national control approach in which standard control measures are applied in a step-wise and monitored manner. Out of 87 FMD-affected countries, about 60 nations are currently engaged at various levels in the implementation of PCP-FMD worldwide in the quest to reduce or eliminate FMD virus circulation by 2020-2025. Some regions are making progress in FMD Control such as South America and South East Asia. However still a number of countries in Asia, middle East and Africa are endemic for FMD.

For an effective implementation of the global FMD strategy and to resolve some of the anticipated challenges, regional roadmap platforms have been successfully used to assess the progress of FMD control achieved in accordance to PCP-FMD guidelines by member countries of a given FMD virus pool. These regional platforms also permit the formulation of harmonized national and regional programs and project proposals to be presented for investment and support by the governments and development agencies. This is best achieved by bringing together the chief veterinary officers (CVOs), national FMD disease experts, regional organizations and development partners. The PCP-FMD stage acceptance process is done during these regional roadmap meetings by a regional advisory group which is made of elected three CVOs and leads of epidemiology and laboratory networks in each region. Technical guiding documents and protocols to facilitate the take-up by countries have been developed and more in the pipeline to support the implementation of PCP-FMD such as guiding documents for national strategic control plans, sero-surveillance, FMD outbreak investigation and reporting, vaccination strategies, post vaccination monitoring, socioeconomic impact studies and others.

For countries to engage in investing in FMD control, cost benefit analysis needs to be taken into account to demonstrate the benefit of FMD control. It has been predicted in an economic model that for countries in low PCP stages, the benefit may take some time before it outweighs the startup cost and that in some cases may diminish the incentive to embark on a control program. Therefore regional and international support for countries in lower stages of the PCP pathway is critical. This may represent a challenge in adopting a control strategy for low income countries, that are mostly in PCP-FMD stage zero or one. Therefore public -private partnership and resource mobilization from development partners must be encouraged to stimulate the initial engagement in the control activities. For countries in higher PCP-FMD stages (i.e.) 3 and 4, the investment in the FMD control program that is eligible for OIE official endorsement is linked to the overall revenue from livestock export and other benefits related to FMD-free status. Nevertheless countries might choose not to progress beyond stage 2 if benefits do not outweigh the costs. Even at stage 2 or 3, sustainable management of FMD can be attained for reduced

disease burden and risk of spill over to unaffected zones, between production systems or to neighbouring countries.

Vaccination is an important component of FMD control globally and an essential measure for reducing the incidence of the disease which represents a major setback in the effective implementation of the Global control Strategy. Some issues with vaccination are the costs of FMD vaccine, shortage in supply, relatively short term immunity, availability of infrastructures to guarantee the cold chain and quality of vaccines produced in some regions. All these challenges must be urgently addressed by the veterinary authorities, FMD research community and the industry.

Conclusions

Global FMD control is feasible and the PCP-FMD approach, along with the reinforcement of veterinary services, used in regional roadmap platforms has been gradually gaining acceptance, better understood by the affected countries and implemented in FMD national control strategies. Political will and engagement of international community are crucial for the startup and sustainability of the FMD control programs. At the same time, FMD can be an important driver to improve animal health system and subsequently permitting control of other high impact transboundary and emerging animal diseases. The principles of PCP-FMD can be adapted and employed in control and eradication of other transboundary animal diseases.

In this paper, the achieved milestones, success stories and challenges in the implementation of the global FMD control by FAO and OIE will be discussed.

notes

WHAT DO WE DO KNOW ABPUT THE ECONOMIC IMPACT OF FMD IN SMALLHOLDER PRODUCTION? SUMMARY OF EVIDENCE

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Abstract

Foot-and-mouth disease (FMD) is most prevalent in regions where households are most dependent upon livestock, which raises the question of what is known about the burden of the disease on smallholder producer. The paper employs a method of looking at the burden in terms of direct losses that include production changes through disruptions in meat and milk production and more subtle changes in fertility leading to different herd structures. It also includes the human reaction to disease such as the treatment of sick animals, the preventative measures such as vaccination and measures that lead to a loss of revenue such as local, national and international markets. An important aspect leading to estimating burden is information on the prevalence of disease, and in particular the prevalence in the smallholder systems. What the authors have found is that there is a general lack of empirical data on this key aspect of estimating disease impact. They have also looked for specific studies that have collected primary data to estimate impacts on meat and milk production and changes in fertility. Overall this literature is sparse with studies focused on small regions of countries and with no systematic method of data collection and analysis. The conclusions are that FMD causes impacts, but the capture of the data needs to be improved underpinned by impact assessment methodology. The authors will present how this could be achieved through linking more technically orientated projects to a global system of data capture.

notes

IMPACT OF FOOT-AND-MOUTH DISEASE ON MILK YIELD, MASTITIS AND CULLING ON A LARGE-SCALE DAIRY FARM IN KENYA

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Introduction

Foot-and-mouth disease (FMD) can be devastating in free countries yet its economic impact is less well characterised in endemic settings, despite such knowledge being essential when allocating scarce resources in disease control. This study uses field data from an outbreak on a large-scale dairy farm in Kenya to estimate effects on milk yield, mastitis, and culling.

Materials and methods

An outbreak of FMD (SAT2) occurred on a large-scale dairy farm in Nakuru Country during September 2012. Milk production by cases and non-cases were compared using a generalised linear model and generalised estimating equations with an autoregressive correlation matrix. Lactation yield predictions were made using historic lactation data from the same herd and compared to the actual production using a multiple linear regression model. Mastitis and culling rates were analysed using survival analysis and multivariable Cox proportional hazards regression.

Results

Overall, no statistically significant difference in milk yield was seen between cases and non-cases. Cows in parity 3 produced significantly less milk than predicted if <200 days in milk (DIM) during the outbreak. Cows in parity ≥4 produced significantly less milk irrespective of lactation stage during the outbreak with the greatest impact on cows 101-200 DIM producing on average 547.4 kg (95% confidence interval [CI] 295.6-799.1) less than predicted. No impact was detected among parity <3 cows.

Cows with FMD had a higher rate of culling in the 12-month period following the outbreak (HR=1.7, 95%CI 0.90-3.4, P=0.10). There was some evidence of an increased mastitis rate for cows with FMD during the first month after the onset of the outbreak (HR=2.9, 95%CI 0.97-8.9, P=0.057).

Discussion

The results of these studies provide new data on the impact of FMD on large-scale dairy farms

in Kenya. Similarities in genetics and feeding make these results generalizable to other farming systems in East Africa.

notes

HOUSEHOLD LEVEL IMPACTS OF FOOT-AND-MOUTH DISEASE ON TRADITIONAL LIVESTOCK-KEEPING SYSTEMS OF NORTHERN TANZANIA

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Introduction

The potential for livestock to contribute to the livelihoods of the poor is recognised. Foot-and-mouth disease (FMD) ranks high amongst diseases constraining pro-poor growth in developing countries. Impacts, hence demands and incentives for control, are likely to differ across settings, production systems and segments of the society. Such heterogeneities are poorly characterised, hence well-informed control policies benefiting those mostly affected by the disease are lacking.

Materials and methods

Household questionnaire (n = 123) data were generated across three production systems of northern Tanzania (pastoralist / agro-pastoralist / rural smallholder), including: (1) income types; (2) perceived importance of FMD compared to other livestock diseases; (3) frequency of outbreaks; (4) morbidity and mortality due to outbreaks; and (5) outbreak impacts on herd production and performance.

Results

Livestock sales were the most important income source across production systems, followed by crop- and milk-related income. FMD was the disease of greatest concern to agro-pastoralists and was ranked second by pastoralists, but was of less concern to smallholder farmers. In 81.8% [95% CI: 64.5-93.0%] of pastoral and 80.0% [56.3-94.2%] of agro-pastoral households, respectively, at least one FMD outbreak was reported in the past year, whereas 39.5% [25.0-56.5%] of herd-owners reported two or more, and 25.6% [13.5-41.2%] three or more outbreaks. Cattle suffered the highest morbidity (52.0% [51.0-53.0%]). Overall mortality was low (1.0% [0.9-1.2%]). Adult female cattle were especially affected (70.9% [68.9-70.9%]) and impacts on milk production were considerable: 84.1% [73.3-91.8%] and 67.4% [51.5-80.9%] of respondents reported decreased milk production in cattle and goats, respectively, while 62.3% [47.9-75.2%] stopped selling milk. A loss of traction capacity affected 66.1% [52.6-77.9%] of respondents.

Discussion

We provide evidence that FMD has important consequences for livestock-dependent communities in Tanzania. FMD control in these systems has the potential to reduce vulnerability through increased milk and crop production.

notes

EMERGING MASSIVE FMDV OUTBREAKS IN UGANDA AND POSSIBLE IMPACT ON PCP

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Introduction

Unusual massive occurrence of FMD outbreaks was encountered between May and July, 2014 hence the need to study the pattern of occurrence and the serotypes involved.

Materials and methods

FMD outbreaks were reported in the districts of Kotido and Napak. During field investigations, outbreaks had spread over the entire Karamoja region and many other surrounding districts: Moroto, Soroti, Ngora and Mbale. Epithelial samples were picked from symptomatic animals (31/150) and stored in PBS and liquid nitrogen. As part of preliminary serotyping, 23 samples were subjected to antigen ELISA. One or two farms were selected per district to maintain biosecurity. Information on outbreaks was collected through interaction with the DVO´s, farmers, reports and field observations.

Results

A total of 9/23 samples tested positive for FMDV antigen ELISA (Serotype O): Kaabong (5/12), Napak (2/3), Mbale (1/3), Moroto (1/4), and Ngora (0/4). All the FMDV outbreaks were blamed on suspected animal movements especially due to cattle restocking programmes and communal farming.

Discussion

Compared to annual FMD outbreaks in 2011 (22), 2012 (15) and 2013 (8), Uganda by 14th of July 2014 registered 23 outbreaks in two months (Kotido, Nakapiripirit, Abim, Kasese, Kween, Bugiri, Napak, Nwoya, Mbale, Alebtong, Ngora, Moyo, Amudat, Kaabong, Moroto, Sironko, Bukedea, Kumi, Soroti, Kapchorwa, Pallisa, Bukwa, Lamwo district). More investigations are required to serotype all outbreaks. The fact that Uganda is surrounded by 5 other countries and FMD is Transboundary, massive outbreaks can be a regional problem with potential to reverse the progress achieved by East African along PCP.

notes

INVESTIGATION OF FOOT-AND-MOUTH DISEASE OUTBREAKS IN MBALA AND KAZUNGULA DISTRICTS IN ZAMBIA

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Introduction

Foot-and-mouth disease (FMD) is an acute, highly contagious viral infection of domestic and wild cloven-hoofed animals. It is endemic in Zambia, with periodic outbreaks occurring in different geographical areas of the country. This study was conducted in 2012 to investigate the presence of FMD virus (FMDV) in FMD-suspect cases in cattle from the Kazungula and Mbala districts of Zambia.

Materials and methods

Sixty epithelial tissues or oesophageal-pharyngeal (OP) scrapings (probang samples) were collected from Mbala (n = 51) and Kazungula (n = 9) and examined for the presence of FMDV. The samples were investigated by reverse transcription polymerase chain reaction (RT-PCR), antigen enzyme linked immunosorbent assay (ELISA) and sequencing of the 1D region that encodes the major viral protein, VP1.

Results

Twenty-two samples (36.7 %) were positive by RT-PCR with cycle threshold (Ct) values ranging from 13 to 31. Four samples from Mbala (North Zambia on border with Tanzania) were serotyped as SAT 2 by antigen ELISA and two Kazungula samples serotyped as SAT 1. Phylogenetic analysis characterised the Mbala SAT 2 isolates as belonging to topotype IV and were similar to viruses from ecologically and socio-economically linked border districts of South West Tanzania. The Kazungula SAT 1 isolates were classified in topotype III.

Discussion

These findings indicated that at least two different epizootics have occurred in Zambia in 2012. Furthermore, regular interaction between buffalos from the Mosi-o Tunya Park and domestic animals from surrounding areas could contribute to the occurrence of regular FMD outbreaks in Kazungula, whilst the uncontrolled animal movements across borders between Mbala and Nsumbawanga could be responsible for disease outbreaks in Mbala. In-depth molecular biological studies should be conducted to elucidate the complex epidemiology of FMD in

Zambia, thereby providing valuable information needed for the rational control strategy of FMD in Zambia and neighbouring countries.

notes

THE EPIZOOTIOLOGY OF FOOT-AND-MOUTH DISEASE IN HIGH RISK ZONES IN KENYA

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Introduction

The nonstructural protein (NSP) ELISA test was used because it is able to discriminate animals that have been infected by wild virus from those that have been vaccinated using either purified/ semi-purified vaccines (Diego *et al.,*1997).

Materials & methods

Serum from several collections at foot-and-mouth disease laboratory were subjected to the nonstructural protein Elisa for screening with positive titrated for serotypes using liquid phase blocking Elisa (LPBE).

Results

The Kenya/Uganda borderland and the north rift disease free zone had the highest FMDV seroprevalence of 95% and 97.5%. The seroprevalence was also highest in Mt Elgon national park and Turkana-Pokot-Trans Nzoia-Uasin Gishu-Nakuru-Nairobi stock route at 100% and 80.5% with serotype A being the most prevalent in all these zones. The southern pastoral regions had a mean seroprevalence of 58.2% when compared with the northern one which had mean FMDV seroprevalence of 49.4%. The seroprevalence of FMD was higher in non-pastoral regions at 58.6% compared to that of pastoral areas at 53% although the difference was not statistically significant (p=0.52, 95%CI)

Discussion

The Kenyan borderlands remain porous with uncontrolled movement in search of water and pasture. The livestock interact freely with roaming wild life in these areas therefore acting as virus maintenance zone. The sub-optimal use of FMD vaccination in relation to the population size of livestock increases the risk of susceptible animal populations. The study was an important step in understanding the disease epidemiology and in the implementation of the progressive control pathway.

notes

VACCINE EVALUATION ON LARGE-SCALE DAIRY FARMS USING ROUTINE PROPHYLACTIC SCHEDULES FOR FOOT-AND-MOUTH DISEASE

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Introduction

Vaccine evaluation for foot-and-mouth disease traditionally relies upon experimental methods like vaccine potency tests (or serological correlates) and vaccine matching (VM). There are many other determinants of vaccination effectiveness (VE) so field-based evaluation of vaccines should be regularly performed. This study presents data from outbreaks on three large-scale dairy cattle farms in Kenya and Iran using routine vaccination.

Materials and methods

Farm A (n=350, Kenya, serotype O) vaccinated all animals >6 months old every four months. The last recorded outbreaks were in 2004 and 2010. Farm B (n=650, Kenya, SAT2) vaccinated all animals every 4-6 months irrespective of age. The last outbreak was in 2004. Farm C (n=3,500, Iran, Asia-1) vaccinated animals >2 months old every four months, with a booster one month after the primary dose. No outbreak had occurred before. For all, the last dose was <4 months before the outbreak. Farms A and B used local quadrivalent (O/A/SAT1/SAT2) aqueous whilst Farm C used trivalent (Asia-1 Shamir/O/A) aqueous vaccine. Where possible, VM and VP1 sequencing was performed at the World Reference Laboratory, UK.

Results

In Farm A and C, the incidence was highest among youngstock with evidence of a protective effect of maternal antibodies. The incidence was relatively low among older animals. R-values <0.3 for Farm C indicated poor match between the vaccine and field strains. Conversely, on Farm B there was a low incidence among younger animals, but around 70-80% in older animals. No VM was available at the time of writing, but the VP1 sequence was 13% different to that published for the vaccine strain.

Discussion

Although it is not possible to estimate vaccine effectiveness from such data, these patterns

indicate problems with various aspects of vaccine performance, perhaps related to cold chain or vaccine schedules. The effectiveness of SAT2 vaccination is a particular concern.

notes

LONGITUDINAL STUDIES ON THE IMMUNOGENICITY OF DIFFERENT VACCINATION METHODS OF HIGHLY POTENT FMD OIL VACCINE FOR WEANER PIGS

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Introduction

From 2011, all the susceptible livestock in the Republic of Korea have been vaccinated with double oil emulsion FMD vaccine. For pig, there are still room for improvement of the current vaccines by modulating the route and times of vaccination and volume and antigen payload of single dose. In this study, we compared different schemes of vaccination in weaner pigs using commercial vaccine to the finishing stage.

Materials and methods

56 piglets aged seven weeks were randomly allocated into 7 groups (G1-G7) and immunized at the age of 8 weeks intramuscularly or intradermally in different volume with highly potent FMD oil vaccine. The booster vaccination was done in four weeks later for G3, G4, G6 and G7, and the blood was taken 0, 2, 4, 6, 8, 12, 16 weeks from the first vaccination for serological assays. Intradermal delivery was done using needless device (IDAL, MSD).

Results

About half of the weaner pigs had maternally derived antibodies above the cutoff level. Regardless of the route or volume of the vaccine administered, only the groups of pig boosted showed the increase of the antibody titer at 6 and 8 weeks after the first vaccination, whereas the groups of pig vaccinated once and for all showed the decrease of the antibody titer, more noticeably at the finishing stage except for the G1 in which the highest volume of vaccine, 2.0ml, was given per each head of pigs.

Discussion

After more extensive field study, the current scheme of national FMD vaccination for weaner pigs may be replaced with the new scheme delivering smaller volume of vaccine in intradermal route. We expect that this new scheme would greatly diminish the concerns of side effects of oil vaccines and increase the productivity of pork meat production with comparable protective immunity to the current scheme.

notes

MATERNAL IMMUNE CELLS TRANSFERRED THROUGH COLOSTRUM DO NOT INTERFERE WITH THE IMMUNE RESPONSES TO FOOT-AND-MOUTH DISEASE (FMD) VACCINE

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Introduction

Little is known on the influence of maternal antibodies and cells transferred through colostrum in the immune responses of calves to the currently used FMD vaccines. In this study, we evaluated the humoral and cellular immune responses induced by vaccination of colostrum-deprived calves and of calves that received equivalent amounts of colostrum preparations that differed in the presence or absence of maternal immune cells but were equivalent in terms of quantity and quality of anti-foot-and-mouth disease virus (FMDV) antibodies.

Materials and methods

The current oil-adjuvanted commercial tetravalent vaccine used in Argentina was applied to 30 days-old calves that were deprived of colostrum (n=3), fed with whole immune-colostrum (WC) from their mothers (n=4) or with a cell-free colostrum, containing only anti FMDV antibodies (n=3). The animals were bled periodically before vaccination, and weekly after vaccination. Immune responses were determined in terms of T-cell proliferation, IFNγ production against all vaccine strains, total and neutralizing antibodies, avidity and isotypes of anti O1/Campos antibodies.

Results

Anti-FMDV cell-mediated immune responses were detected in non-vaccinated calves that received WC. All vaccinated calves developed IFN-γ and lymphoproliferative responses, irrespectively of the colostrum received. Colostrum-deprived animals responded to vaccination with a primary IgM response (14 dpv) followed by an increase of IgG1 titers, a profile comparable to that obtained in adult animals. Conversely, all colostred calves showed a decrease in antibody titres after vaccination, following the decay curve for maternal antibodies. The higher the initial antibody titer, the greater the fall in titres observed after vaccination.

Conclusions

This study demonstrates for the first time that maternal immune cells transferred to the calves though colostrum do not modify the immune responses to FMD vaccine, and confirms the interference of maternal antibodies in the induction of humoral responses in colostred calves.

notes

SEROTYPE O VACCINE EFFICACY AND CHALLENGE WITH DIFFERENT VIRUSES FROM SOUTH EAST ASIA IN VARIOUS SPECIES

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Introduction

Foot and mouth disease virus serotype O consists of eight known topotypes and within each, significant genetic and antigenic variation exists. In addition, new variants frequently arise that may escape protection provided by current vaccines. O1 Manisa is one of the most widely used vaccines, but the outbreak in South Korea has led to the development of a new vaccine using the outbreak strain, O/SKR/2010. In our studies both vaccines were compared against the outbreak strain while O1 Manisa was also tested against a current outbreak strain from Vietnam (O/VIT/2010).

Materials and methods

O1 Manisa and O/SKR/2010 vaccines were tested in cattle and pigs against challenge with O/SKR/2010. The efficacy of O1 Manisa against challenge with O/SKR/2010 in sheep was also determined. In addition, pigs vaccinated with O1 Manisa were challenged with O/VIT/2010. All vaccines were high potency (>6PD_{50}) with double oil emulsion as adjuvant.

Results

The O1 Manisa vaccine protected cattle 21 days post-vaccination (dpv) against heterologous challenge. However, at smaller doses (1/4 and 1/16 dose), the vaccine failed to protect all cattle. The newly developed O/SKR/2010 vaccine was efficacious against homologous challenge, even at lower doses. Both high potency vaccines failed in pigs at 21 dpv. In contrast, O1 Manisa partially protected pigs as early as 4 and 7 dpv when challenged with O/VIT/2010. Sheep vaccinated with O1 Manisa and challenged with O/SKR/2010 were protected 4 dpv when challenged by infected in-contact donor sheep. However, when sheep were challenged by needle injection into the coronary band, a double dose of vaccine was required to provide protection 7 dpv.

Discussion

High potency O1 Manisa vaccine is efficacious in cattle against at least one of the recently circulating O viruses isolated in South East Asia. However, the vaccine provided different levels of protection in pigs based on the challenge virus. Sheep showed different levels of protection based on the route of infection.

Acknowledgments

National Veterinary Company and Regional Animal Health Laboratory VI, Vietnam; Pirbright Institute, United Kingdom; Plum Island Animal Diseases Centre, USA, National Centre for Foreign Animal Diseases, Canada; Merial, United Kingdom.

notes

THE FIELD EFFECTIVENESS OF AN O MANISA + O 3039 VACCINE FOR THE CONTROL OF AN O/ME-SA/IND-2001 FMD OUTBREAK IN A PRIVATE DAIRY FARM IN SAUDI ARABIA

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Introduction

Saudi Arabia counts several large dairy farms holding between 5,000 and 70,000 high producing Holstein Friesian cows on a zero grazing basis. Its geographical situation at the crossroad between Asia and Africa poses a continuous threat of new FMD virus variants introduction. FMD outbreaks in Saudi Arabia, endemic like all Arabic Peninsula, are mostly caused by Serotype O viruses. Animals are vaccinated 3 times per year with broad spectrum high potency vaccine (>6PD50) . Same vaccines are used for emergency vaccination during outbreaks.

Materials and Methods

On August 5th, 2013 an FMD outbreak occurred in Al Safi Private dairy farm, 100 km South East of Riyadh. The farm has 3,500 ha and counts 45,000 cows split in 7 production units, and calves, growers and young stock split in 5 units according to the age (1,700 to 8,000 heads). All animals are vaccinated every 4 months with high potency Aftovaxpur® quadrivalent vaccine (Merial) containing O Manisa + O 3039, A Iran 05 + A Saudi 95, Asia1 Shamir, SAT2 Eritrea. The first clinical signs of FMD erupted in 4 animals in one unit, 3 months after the last routine vaccination. The farm implemented within 24 hours sanitary (sick animals isolation, immediate slaughter, movements stop from affected unit to the rest of the farm) and prophylactic measures (emergency vaccination of the unit with double dose Aftovaxpur® + other units with single dose).

Results

The outbreak was contained rapidly without transmission to the other units. After 7 days and 18 cases out of 2759 susceptible animals in the concerned unit, no more case was observed. Virus isolation at WRL Pirbright identified FMD virus strain O/ME-SA/ind-2001/KAR 13 as the source of the outbreak. 11 animals showed seroconversion to 3ABC NSP, proving virus circulation.

Two origins were suspected, first the numerous farm laborers coming from India where the strain is present; secondly the presence, beside the unit of a cull and sales unit with calves coming from the outside.

Discussion

This successfully controlled FMD incursion illustrates how appropriate sanitary and prophylactic measures can contain an FMD outbreak in short time (7 days) with minor cases (18), involving a recent lineage heterologous to the vaccine strains, within one of the world highest density of dairy population. Routine vaccination has created favorable conditions for a quick immune response.

The O/ME-SA/ind-2001/KAR 13 strain was controlled thanks to the synergistic combination of the 2 immunodominant strains O Manisa and O 3039.

notes

OVERVIEW ON THE PERFORMANCE OF FMD VACCINES IN POTENCY TESTS USING lp-ELISA. A PERSPECTIVE FROM A MANUFACTURER IN SOUTH AMERICA

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Introduction

Serological methods has been proved a very important tool to control FMD vaccines quality in substitution of animal testing as PD_{50} or PGP. Test parameters have an important role on balancing the criteria used on rejecting or approving serial batches. This work discloses, from the perspective of a vaccine manufacturer in South America, how serological tests parameters could influence availability of vaccine for eradication programmes.

Materials and methods

Data from internal and official vaccine controls in Brazil are used to demonstrate the impact of test parameters on test performance. Results from two different lp-ELISA methods are presented: PANAFTOSA in Brazil and CEVAN in Argentina.

Results

It is demonstrated that depending on test parameters adjustment and method used to calculate potency, lp-ELISA could result in a highly variable method. Moreover, results obtained internally at the manufacturer laboratories could be quite different that results obtained at the official laboratories, showing very low robustness of the methods when parameters are not well defined and controlled.

Discussion

The final objective of a quality control test is to accept or reject a serial batch of a determined quality based on acceptance criteria within a calculated risk range. The acceptance criteria must adequately consider the balance between the user´s risk and the manufacture´s risk. The risk of the user can be defined as the probability that a bad batch is approved. The risk of the manufacturer can be defined as the probability that a good batch is rejected. Unbalanced criteria and test parameters not well defined could affect availability of good batches to eradication programs.

notes

DETECTION OF ANTIBODIES AGAINST NSP RESIDUES IN THE COMMERCIAL FMD TRIVALENT VACCINES AFTER MULTIPLE VACCINATIONS IN CATTLE AND GOATS

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Foot and mouth disease (FMD) is the most contagious disease of cloven-hoofed mammals, and cause huge economic losses of the farmers and country. Detection of antibodies to non-structural protein (NSP) has been used to identify past or ongoing infections with any of the existing serotypes of FMDV. Since severe damages due to FMD outbreaks in South Korea from November 2010 to April 2011, South Korea adopted vaccination policy throughout the country for all susceptible animals. For the purpose of finding infected animals by detecting humoral immune responses to NSP of the FMD virus, antibodies induced by contaminated minute NSP contained in less pure FMD vaccine can be problematic for serological screening. This study was aimed to see whether antibodies against NSP could be induced in repeatedly vaccinated cattle, pigs and goats.

From March 2013, approximately 131 cattle, pigs and goats were kept on seven farms in Korea. At the beginning of this study, all of the animals were approximately 6 weeks of age. All animals were vaccinated once a month from at 8 weeks of age with oil adjuvanted, inactivated purified FMD trivalent vaccines against FMD types O, A and Asia 1 (Merial Animal Health Ltd or Intervet Co.). Blood samples were collected by jugular vein puncture at the time of vaccination and monthly thereafter. Those sera were tested by NSP ELISA (Median Diagnostics & Bionote, Korea; PrioCHECK® FMDV NSP, Netherlands) and SP ELISA (PrioCHECK® FMDV type O, Netherlands; Pirbright LPB A and Pirbright LPB Asia1, United Kingdom) according to the manufacturer's instructions.

All animals were 14 month old and vaccinated 12 times. NSP antibodies were not detected in all animals except for 6 cattle and 7 goats which were tested as positive after 5 times multiple vaccination. However, the NSP-seropositive cattle and goats showed negative results in antigen test by real-time RT-PCR. The SP-positive prevalence was increased in all three types depending on receiving vaccination times and the prevalence of type O and of Asia1 showed slightly higher levels than that of type A. We found that a multiple vaccinated animal can produce antibodies against minute NSP in commercially produced vaccines. It was unlikely that the NSP-positive reaction from tested samples in this study was caused by natural FMD virus infection. Currently, there is no confirmatory diagnostic standard method to support NSP ELISA for detection of true NSP antibodies induced by wild type FMD viruses. Thus, further study is required to develop the additional methods for confirmation of suspicious NSP-positive samples. Finally we strongly recommend that NSP screening for national serological surveillance needs to be carried out in young ruminant animals less than 2 year old.

notes

EARLY PROTECTION IN SHEEP AGAINST HETEROLOGOUS CHALLENGE WITH SEROTYPE O FOOT-AND-MOUTH DISEASE VIRUS USING HIGH POTENCY VACCINE

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Introduction

In 2009–2011, spread of a serotype O virus belonging to the SEA topotype (O SKR) led to the culling of over 3.5 million cattle and pigs in Japan and Korea. The O1 Manisa vaccine (belonging to the ME-SA topotype) was used at high potency in Korea to limit the expansion of the outbreak. However, no data are available on the spread of this virus, or the efficacy of the O1 Manisa vaccine in sheep. The ability of the O1 Manisa vaccine to protect sheep from direct in-contact challenge with O/SKR/2010 was investigated.

Materials and methods

Eight sheep were vaccinated with 1 ml FMDV O1 Manisa DOE vaccine (>6 PD_{50}) and challenged 4 days later by continuous direct contact with donor sheep. Donor sheep were inoculated with FMDV O/SKR/2010 24 hrs prior to contact with the vaccinated animals, or unvaccinated controls (n=4). The sheep were divided into six rooms, with two donors and 2 contact sheep per room. Samples (blood, saliva and nasal swabs) were collected daily for 10 days, then weekly to 35 days post-contact. Probang samples were collected weekly to 35 days post-contact.

Results

All O1 Manisa vaccinated sheep were protected from clinical disease. None had detectable antibodies to FMDV 3ABC, no virus was isolated from nasal swabs, saliva or oro-pharyngeal fluid and none became carriers. Three of the four control sheep became infected, two clinically.

Discussion

The O1 Manisa vaccine prevented clinical disease, local virus replication and the establishment of persistent infection in sheep following direct contact challenge with O/SKR/2010 at 4 days post-vaccination.

notes

TESTING THE EFFICACY OF O_1 MANISA HIGH POTENCY VACCINE AGAINST CHALLENGE WITH O/SKR/2010 (O MYA98) STRAIN IN SHEEP

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Introduction

The efficacy of different doses of a high potency O_1 DOE Manisa vaccine (>6 PD_{50}) was tested in sheep challenged with O/SKR/2010 at either 7 or 14 days post vaccination (dpv).

Materials and Methods

Three groups of sheep (n=7 each) were vaccinated intramuscularly with a full dose (1 ml), ½ dose (0.5 ml) or ¼ dose (0.25 ml) O_1 Manisa vaccine and challenged by intraepithelial heel bulb inoculation (10^5 CID) at 7 dpv with cattle derived O/SKR/2010. Samples were collected regularly until 6 days post-challenge (dpc). Following this, two more groups of 7 sheep each were vaccinated with 2 ml of vaccine and challenged 7 and 14 dpv, while a third group received 1 ml of vaccine and was challenged 14 dpv. Blood, nasal swabs and oropharyngeal fluid (probangs) were collected until 35 dpc. Naive controls (n=4 for each trial) were included.

Results

Sheep that received 1 ml or less of vaccine were not protected 7 dpv, except for one that had received 0.5 ml of vaccine. No FMDV antibodies were detected in any of the sheep at the time of challenge. Three of the sheep that had received 2 ml of vaccine 7 days prior to challenge were protected, although only one had detectable antibodies to FMDV structural proteins. At 14 dpv, four of the seven challenged sheep were protected, regardless of dose. Most of these animals had sero-converted. Intermittent virus shedding was detected 1–35 dpc in all vaccine groups and only one sheep vaccinated with 2 ml of vaccine and challenged at 7 dpv became a carrier.

Discussion

Clinical protection was not evident in sheep vaccinated with the recommended sheep dose and challenged 7 dpv using the coronary band route of infection. Improved protection was observed at higher doses or when sheep were challenged 14 dpv. It is not clear whether the stringency of the viral challenge may have determined the suboptimal vaccine efficacy.

notes

DEVELOPING TECHNOLOGIES TO UNDERSTAND DURATION OF IMMUNITY FOLLOWING FMDV VACCINATION OF LIVESTOCK SPECIES

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Introduction

The progressive control of FMDV will require vaccine formulations with significantly greater duration of immunity than the present products. Understanding the adaptive immune response to FMDV is critical in designing these new vaccines. We have developed new technologies, common in the analysis of the immune response of humans to vaccination, to characterize the immune response in cattle and swine. This will provide a critical capacity to reformulate present vaccines or design new vaccines with enhanced performance characteristics for the progressive control of FMDV.

Materials and methods

We have developed a number of assays using multicolour flow cytometry to analyse the immune response to FMDV at a single cell level. These include dendritic cells that are critical to stimulation of antigen specific T cells while concurrently activating T and B cells via cytokine production. New methods were also developed for tracking the antigen specific T and B cells harvested from blood or lymphoid tissues.

Results

Our results indicate that dendritic cells are clearly inhibited during the acute phase of FMDV infection and this inhibition is no longer significant when analysing vaccinated animals following challenge. B cells producing anti-FMDV antibody are surprisingly short lived in the circulation of infected animals, while presence of anti-FMDV antibody follows a much longer kinetic. The presence of anti-FMDV B cells in the draining lymphoid tissues is being assessed. Finally, we have adapted technologies developed for human to cattle and swine, to track antigen specific T cells in response to FMDV.

Discussion

The technologies described here will allow for a more detailed understanding of the process that triggers the development of cells that mediate long term immunity, specifically memory T cells and memory B cells. This knowledge will allow better vaccine design and assessment for the development of FMDV vaccines that have long duration of immunity.

notes

NOVEL VACCINE STRATEGIES FOR THE CONTROL OF FOOT-AND-MOUTH DISEASE IN AFRICA

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Introduction

The control of FMD in Africa is complicated by several factors, including the unique epidemiological situation where six of the seven serotypes are present on the African continent, the antigenic diversity in each serotype, maintenance of the virus by persistently infected African buffalo, instability of SAT type vaccines and the conditions in Africa. Not surprisingly 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 new vaccine technologies tailored for the conditions in Africa.

Materials and methods

We have compared vaccines based on genetically modified virus particles as vaccine antigen and multimeric protein-FMDV fusions as antigen display systems in eliciting protective immunity against live virus challenge. The genetically modified vaccine antigen includes structurally stabilised capsids and epitope-replaced mutants, while the multimeric vaccines are based on African horsesickness VP7 trimers and bacterial flagellin as epitope display systems.

Results

Previous work in cattle and pigs demonstrated that protection against FMD could be achieved following vaccination with inter-and intra-serotype chimeric vaccines. We have also explored multimeric protein display systems to display relevant epitope regions of FMD capsid protein VP1. When used as vaccine antigens these multimeric protein fusions with 36 – 120 FMD amino acid sequences did not protect cattle against live virus challenge, although good insert-specific immune responses were elicited in Guinea pigs. We have now designed various inter- and intra-serotype chimeric viruses with stabilised capsids as vaccine antigens.

Discussion

Chimeric, stabilised antigens less reliant on a cold-chain are an important improvement 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.

notes

RECOMBINANT ADENOVIRUS EXPRESSING EMPTY CAPSID OF SEROTYPE A22 FMD VIRUS PROVIDES STERILE IMMUNITY IN CATTLE FOLLOWING HOMOLOGOUS PRIME-BOOST VACCINATION

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Introduction

Conventional inactivated FMD vaccines do not induce sterile immunity and may allow viral replication at epithelial surface giving rise to the carrier state in some vaccinated animals following live virus challenge. Since the naso-pharynx is the major portal of entry in FMDV infection and is the site of FMDV persistence, induction of potent adaptive immune responses at this site by local delivery system may generate pre-existing mucosal antibody that could prevent the initial viral colonisation. Therefore, the main aim of this study is to develop and evaluate a viral vector based intranasal FMDV empty capsid vaccine in cattle.

Materials and methods

The A22 FMDV P1-2A and 3C genes were cloned into defective human adenovirus vector where FMDV P1-2A-3C genes were placed under the tetracycline operator 2 (TetO2) sites. This rAdVFMD empty capsid vaccine has been evaluated as intranasal as well as parenteral vaccine in cattle following the homologous prime-boost vaccination and virulent FMD virus challenge.

Results

The recombinant AdVFMD virus vaccine administered intramuscularly in cattle induced sterile immunity and provides full protection upon challenge with virulent FMD virus using a nebuliser and mask. Intranasal vaccinated cattle were clinically infected.

Discussion

This pilot cattle study envisages the efficacy of rAdVFMD parenteral vaccine in cattle with no replication of virus whereas intranasal vaccinated cattle showed clinical infection. The presence of adenovirus receptor (CAR) at the baso-lateral border of the respiratory epithelial cells perhaps is not accessible by the intranasal administered vaccine virus and therefore the animals were not protected.

notes

DEFINING REQUIREMENTS AND EXECUTION OF INTERNATIONAL FIELD TRIALS FOR NEXT GENERATION FMD VACCINES AND DIAGNOSTICS

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Introduction

The agricultural defense mission of the U.S. Department of Homeland Security Science and Technology Directorate (DHS S&T) includes development of state-of-the-art countermeasures for high priority transboundary animal diseases. This includes research and development for vaccines and diagnostics in coordination with federal, industry, academic and international partners. The FMD next generation vaccine and diagnostic development project started in 2005 and funded the development of a human Adenovirus vectored (Ad5) FMD vaccine and a FMD 3B cELISA, which can be used to differentiate vaccinated from vaccinated-infected animals. The monovalent vaccine (serotype A24 Cruzeiro) has completed potency, purity and field safety trials in the U.S. and is conditionally licensed for use in cattle in the U.S. While products have been through rigorous testing in the U.S., there is a desire to further evaluate their performance/ efficacy in a field setting in an endemic environment.

Materials and methods

A series of meetings is planned. The first was held in June 2014, with U.S. stakeholders and potentially interested collaborating countries discussing the feasibility and best approach to the design and execution of a field evaluation of an Ad5 FMD vaccine and 3B ELISA assay. Ultimately, the goal is to further evaluate the performance and efficacy of the Ad5 FMD vaccine and the 3B ELISA in an endemic country.

Discussion

Relationships with endemic countries are necessary to evaluate the efficacy of next generation FMD vaccines and performance of diagnostics in field settings. Such research collaborations must be conducted in a well-coordinated, open and transparent manner, with benefit to all parties involved. Successful research collaborations will ideally lead to enduring partnerships which can further advance the state of the art for foreign animal disease vaccines and diagnostics.

notes

DEVELOPMENT OF A NOVEL, RECOMBINANT, POTENT, AND SAFE DIVA VACCINE FOR FOOT & MOUTH DISEASE (FMD)

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Introduction

FMD control and management have enormous implications in the world trade of agriculture and farm animals. Current FMD vaccines (killed virus) are inherently expensive and unsafe, and have poor antigenicity, short duration, and no reliable DIVA tests. Our recombinant FMD vaccine approach includes recombinant (E. coli and yeast) FMD antigenic proteins (VP1s) conjugated with one of the most powerful tetanus toxin mutant in order to eliminate its toxicity and impart its antigenicity to the FMD-vaccine. The toxin, aside from acting as a powerful adjuvant, will produce antibodies in vaccinated animals that will also serve as a novel DIVA test.

Materials and methods

As proof of concepts, we have expressed and purified FMD O type **VP1** together with the mutated tetanus toxin (**VP1-TTX**). The two recombinant vaccines have been tested in animals for antibody titers against the VP1, VP1-TTX, and TTX alone. Current FMD vaccines (killed virus) were also included as a control.

Results

Recombinant VP1-TTX vaccine induced much higher VP1 antibody titers than VP1 alone or FMD-vaccine (killed). Vaccinated animals remained healthy for the duration of this study (6-months) and also produced robust antibodies against the TTX that can be used for DIVA test. Studies are in progress to assess the virus neutralization efficacy of the recombinant vaccines.

Discussion

There are many important issues such as recombinant vaccine doses, frequency and routes of immunizations, adjuvants, duration of immunity, and serotype coverage that will have to be addressed to determine the efficacy of the recombinant FMD vaccine. Novel biodegradable nanoadjuvants will be included to replace unsafe, less potent oil adjuvants.

notes

FOLLICULAR DENDRITIC CELLS (FDCS): A KEY PLAYER IN THE PATHOGENESIS OF FOOT-AND-MOUTH DISEASE VIRUS?

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Introduction

Foot-and-mouth disease (FMD) is still endemic in many countries in Africa, Asia and South America and vaccination is a major tool for disease control. Two fundamental problems impede effective control by vaccination; the FMD virus (FMDV) carrier state and the short-term duration of immunity induced by vaccination, which contrasts with prolonged immunity induced by natural infection. The basis of this discrepancy is unknown and a clear understanding could lead to the design of a new generation of FMD vaccines, capable of inducing such long-term antibody responses.

Follicular dendritic cells (FDCs) are specialised immune cells that are able to retain antigen on their surfaces for prolonged periods and are thought to play a central role in generating and maintaining antibody titres. The role of FDCs in FMDV infection and vaccination is not understood.

Materials and methods

Using a mouse model we aim to explore the role of FDCs in FMDV infection and vaccination. Using FDC-depletion protocols we aim to determine the importance of persisting FMDV antigen and T-dependent immune responses in the generation and maintenance of long-term antibody responses.

Results

Analysis of mouse spleen sections indicate that FMDV genome is retained in germinal centres up to 46 days post infection. In addition, we have shown in these mice that FMDV capsid protein and genome is association with FDCs.

Discussion

Using mouse as a model, we have shown that FMDV capsid protein and genome is detectable within the light zone of GCs in the spleen and lymph nodes, and is associated with FDCs, highlighting a potential role for FDCs in FMDV persistence. The role of FDCs in the maintenance of long-term humoral immune responses to FMDV was also investigated.

notes

VACCINE DEVELOPMENT, CHALLENGES AND STRATEGIES FOR FMD CONTROL IN INDIAN SUBCONTINENT

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FMD is a highly contagious disease of cloven-footed animals. Global framework of FMD control is on way by government of India as FMD-CP before 10 years. Recent wide spread outbreaks in different states of India, in spite of intense vaccination is wakeup call and challenge for the FMD control in Indian subcontinent. Outbreaks and implementation of control measures are to be targeted for surveillance, strain matching and vaccine development suited for the regions in India. Globally vaccines are the only safe and effective intervention to control foot and mouth disease. Efforts are being made for subunit, DNA, live vector, synthetic peptide, marker/DIVA, empty capsid, edible and cloned novel vaccines but yet no vaccine successfully replace traditional vaccine for controlling foot and mouth disease worldwide. Combined vaccine like FMD-HS has also been developed. Biovet has developed Bluetongue pentavalent and Johne's disease inactivated vaccine first time in country for Asian region. Biovet has produced and delivered more than 30 million FMD vaccine out of 300 million doses used in the country. Challenges for FMD control are the identification of suitable antigens, adjuvants delivery methods, large scale production of antigen/Vaccine under closed bioreactor system for coverage of 90% population, cold chain maintenance and awareness to farmers about the benefits of vaccination for strict following of vaccination schedules, coverage of population, quality vaccine and timely delivery. There is requirement of Molecular characterization of Field strains isolated from different outbreaks occurring in different agro climatic zones and comparison of genome sequences of field strain and vaccine strains to see the homology for any emerging strains and coverage of vaccination with strict schedules as per manufactures guidelines. Development of improved test kits, rapid synchronization and harmonization of protocols for testing of vaccines produced globally in compliance with OIE/FAO protocols, independent surveillance to monitor the vaccination program at field level, random surveillance of antibody level of vaccinated animals and prompt reporting to the policy makers for rapid action. Better coordination among stake holders are very much needed to achieve the goal of controlling FMD in India in recognition under OIE/FAO global framework.

LESSONS FROM DELIVERY AND IMPACT ASSESMENT OF HUMAN VACCINATION PROGRAMMES: THE GAVI EXPERIENCE

S. Malvolti

AVAILABLE UPON REQUEST

notes

QUANTIFYING AND PREDICTING ANTIGENIC RELATIONSHIPS: A COMPARISON OF TWO ALTERNATIVE APPROACHES INVESTIGATED USING DATA FROM FMDV AND INFLUENZA A

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Introduction

Genetic and antigenic variants continue to emerge within each of the seven foot-and-mouth disease virus (FMDV) serotypes, with limited cross-protective immunity even between some viruses of the same serotype. Antigenic variation has important implications for the epidemic potential of emerging strains, outbreak severity and vaccine selection. Modelling approaches have used data from assays that characterise antigenic phenotype of strains of FMDV (including the virus neutralisation test (VNT)), and of Influenza (including the haemagglutination inhibition (HI) assay) to quantify and predict antigenic similarity and to explore the relationship between genetic and antigenic evolution.

Materials and methods

We analysed an HI dataset describing antigenic relationships among 506 viruses for Influenza A (H1N1), and three VNT datasets comprising 201 viruses from serotypes A, O and SAT1 using two methods: 1) A Bayesian extension of antigenic cartography where viruses and antisera are positioned in antigenic space; 2) A direct integration of amino acid sequence and serological data that makes quantitative estimates of the antigenic impact of specific substitutions.

Results

The alternative methods are similarly capable of assessing vaccine match for influenza isolates which have already been tested against other reference sera, but the sequence-based approach also allows for the prediction of antigenic relationships for potential new vaccines and for newly sequenced isolates. Applying these methods to FMDV, we will report their relative advantages in assessing vaccine match, predicting potential coverage of vaccine candidates, estimating antigenic similarity among strains and characterising newly sequenced isolates.

Discussion

Through the antigenic characterisation of Influenza and FMD viruses we present a detailed quantitative comparison of two existing approaches for modelling antigenic variation. Analysing multiple datasets and including data from different viruses characterised using distinct assays allows a fair assessment of their relative strengths, extends the generality of conclusions and ensures that these are robust to differences between datasets.

notes

THE INTRODUCTION OF POSITIVELY-CHARGED RESIDUES AT THE FIVE-FOLD AXIS OF THE FOOT-AND-MOUTH VIRUS SAT-TYPE CAPSID ENHANCES INFECTION OF CULTURED CELLS

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Introduction

The epidemiology of foot-and-mouth disease (FMD) in Africa is complicated by the antigenic variability of the South African Territories (SAT) serotypes of FMD viruses (FMDV) and the maintenance of the virus in persistently infected African buffalo (*Syncerus caffer*). Control of the disease relies on effective vaccines that are produced from chemical inactivation of virus grown in large-scale production cultures of BHK-21 cells. However, not all field strains can be adapted to suspension cell cultures. Thus, the production of custom-engineered FMD vaccines can be facilitated using infectious cDNA technology, which makes it possible to engineer chimeric viruses containing the antigenic region of a field strain but also allows for the introduction of cell-culture receptor binding sites and antigen-stabilising mutations. The primary objectives were to introduce positively-charged amino acid changes observed during cell culture adaptation of SAT1 and SAT2 field strains into an intra- and inter-serotype chimeric virus, and to investigate the receptor use in cultured cells.

Materials and Methods

Surface-exposed residues that resulted in a modification of the local surface charge during cell culture adaptation were introduced individually or in combination into infectious intra-serotype (pΔSAT2/SAT2) and inter-serotype (pΔSAT1/SAT2) chimeric genome-length clones. The chimeric viruses were obtained via transfection and their ability to replicate in BHK-21, CHO-K1, CHO-677, CHO-745 and CHO-lec2 cells were investigated by plaque assays.

Results

One vΔSAT2/SAT2 and two vΔSAT1/SAT2 mutant chimeric viruses, containing positively-charged residue substitutions symmetrically arranged around the five-fold axis of the capsid, showed enhanced infection of cultured BHK-21 cells. In addition, the vΔSAT2/SAT2 chimeric virus was able to infect CHO-K1 cells, a cell-line that expresses HSPG receptors. Additionally, this chimera containing the VP1 KGG110-112KRR mutation was shown to grow to high titres in BHK suspension cells utilised for FMD vaccine production.

Discussion

The symmetrical, positively-charged amino acid clusters allowed efficient cell entry and increased

cell culture adaptation, likely via interaction with HSPG molecules on the cell surface. These chimera viruses are also able to infect BHK-21 suspension cells.

notes

A THIAZEPINO[4,5-*a*]BENZIMIDAZOLE DERIVATIVE INHIBITS THE *IN VITRO* REPLICATION OF EURASIAN SEROTYPES OF FOOT-AND-MOUTH DISEASE VIRUS

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Introduction

Several research groups have reported on the use of interferons, poly IC, CpG oligonucleotides, RNA interference or small chemical molecules as direct-acting serotype-independent alternatives to emergency vaccination in case of FMD outbreaks. Here, we evaluate the *in vitro* activity of a thiazepino[4,5-*a*]benzimidazole derivative against FMDV. The class of molecules was first described by Chimirri *et al.* (2000).

Materials and methods

The antiviral activity of the molecule was determined on SK6 cell cultures infected with strains representative of the 7 FMDV serotypes and of SVDV. Time-of-drug addition studies were performed to identify the specific stage of the viral replication cycle at which the molecule may act. To elucidate the mode of action, FMDV was serially passaged in sub-optimal and increasing molecule concentrations to select for antiviral resistance. Resistant FMDV variants were subsequently sequenced.

Results

The thiazepino[4,5-*a*]benzimidazole derivative showed 50% effective concentrations between 8.0 and 51.4 µM for inhibition of viral replication of the Eurasian FMDV serotypes. Similar values were obtained for inhibition of viral RNA replication. No antiviral activity was observed at a concentration of 100 µM for the SAT serotypes or SVDV. The molecule exerted full anti-FMDV activity when added up to 2 hours post infection. At later time-points, the antiviral activity was lost. Comparative consensus sequencing demonstrated unique amino acid substitutions in the 2C protein of resistant FMDV variants.

Discussion

Previously, we demonstrated proof-of-concept for the ribonucleoside analogue 2'-*C*-methylcytidine in SCID mice (Lefebvre *et al.*, 2013) and for the 3-oxo-3,4-dihyro-2-pyrazincarboxamide derivative T-1105 in guinea pigs (De Vleeschauwer *et al.*, in press). Here we report on a novel class of molecules with anti-FMDV activity possibly targeting 2C. This illustrates the potential of small molecule inhibitors to interfere with the different proteins essential in the replication of FMDV. Further research on the antiviral containment strategy for FMD is ongoing.

notes

IDENTIFICATON OF FMDV STRAIN IN MULTIVALENT VACCINES BY USING LOOP MEDIATED ISOTHERMAL AMPLIFICATION

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Introduction

Loop mediated isothermal amplification (LAMP) is a low cost isothermal genomic amplification with numerous advantages such as specificity, efficiency and rapidity.

The aim of this work was to set up a LAMP assay in order to perform an easy and rapid identity test on combo FMDV vaccines.

Material and Methods

LAMP assay was carried out with 6 sets of primers specific for each of the following FMDV strain: O1 Manisa, O1 BFS, O Tawain, A22 Iraq, A24 Cruzeiro, A Turkey14/98 and Asia 1 Shamir.

The vaccine used was Merial's aftovaxpur DOE® , containing inactivated purified antigens in a double oil emulsion. Genomic extraction from vaccine is performed either by using DNA extraction Qiagen kit or by boiling vaccines. Then, Reverse transcription amplification is carried out with RT-thermoscript and LAMP with Bst DNA polymerase. In order to reduce duration of experiment, reverse transcription and LAMP amplification have been combined as well. Genomic amplification is observed after migration on a Ethidium bromide agarose gel electrophoresis or by incubating samples with SYBRgreen.

Results

After several optimization steps, the set of primers selected has shown to be highly specific without any amplification between strains from different serotypes (O, A, Asia) and between strains within the same serotype (ie: O1Manisa and O1 BFS). LAMP assay has been shown to be effective on FMDV genome previously extracted or on heat treated vaccine suggesting that genomic extraction can be removed. Moreover, the reverse transcription step can be combined with LAMP essay without any impact on genomic amplification. Finally, RT LAMP is usable as an identity test on mono, bi or trivalent MERIAL vaccines with the same efficacy.

Discussion

LAMP technique is a new tool mainly used in diagnostic laboratory for its simplicity and rapidity. We adapt this technology in order to identify FMDV strains in MERIAL's aftovaxpur DOE® vaccine with a very high degree of specificity.

notes

IDENTIFICATION OF NOVEL ANTIBODY BINDING DTERMINANTS OF SEROTYPE O FOOT-AND-MOUTH DISEASE VIRUS

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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.

Materials and methods

Comparison of the ability of bovine antisera to neutralize a panel of serotype O FMDV identified three novel putative sites at VP2-74, VP2-191 and VP3-85, where amino acid substitutions correlated with changes in sero-reactivity. The impact of these positions was tested using site-directed mutagenesis to effect substitutions at critical amino acid residues within an infectious copy of serotype O FMDV.

Results

Using reverse genetics technique a series of recombinant viruses were generated in this study out of which two recombinant viruses, (1) by substituting the critical amino acid residues of the five neutralising antigenic sites of a FMDV type O cDNA clone (5M), and (2) by adding two additional substitutions at position VP-74 and VP2-191 (M6), are noteworthy.

Discussion

Serological characterisation of 5M and M6 recombinant viruses revealed 56% and 74% reduction in neutralising antibody titre reflecting the significance of these residues in the antigenicity of the virus. However it is possible that more unidentified epitopes may exist as 100% reduction in neutralization was not observed. Work is on-going in our laboratory to identify additional capsid amino acid residues that could have an impact on the antigenic nature of the virus.

notes

ESTABLISHMENT OF A PERSISTENT FMDV INFECTION IN MDBK CELLS

Kopliku L, Gorna K, Relmy A, Romey A, Allemandou A, Zientara S, Blaise-Boisseau S, Bakkali-Kassimi L

Introduction

Foot-and-mouth disease virus (FMDV) can cause persistent infection in 15-50% of ruminants. Such carriers represent a potential risk for viral transmission to susceptible animals. Mechanisms of FMDV persistence remain unknown. Our aim is to identify viral and cellular factors involved in the establishment and/or maintenance of FMDV persistence. For this purpose a relevant *in vitro* model of FMDV persistence was established.

Materials and methods

MDBK cells were inoculated with different MOI of a plaque purified derivative of the isolate O/FRA/1/2001. Twenty-four hours post-infection, plates were washed to eliminate dead cells. Surviving cells were maintained in culture and passaged twice a week. Characterization of this cellular model was performed using RT-qPCR and immunofluorescence. Persistence of infectious FMDV particles was monitored by virus isolation. Sequencing of IRES, Lpro, VP3, VP1 and 3C regions of FMDVp's genome from passage 23 were performed.

Results

We have established MDBK cells persistently infected with FMDV, named MDBKfmdvp. Viral RNA is indeed detectable up to 38 passages, viral proteins and infectious particles of "persistent" FMDV are detectable up to 36 passages. Initial studies were made on FMDVp 23, revealing a viral titer 2log lower compared to the initial viral clone. Nevertheless, primary sequencing analyses didn't point out specific mutations.

Discussion

The only cellular model of FMDV persistence has been established and studied in BHK cells. Co-evolution of cells and viruses during persistence was observed, but factors involved in FMDV persistence were not elucidated. The newly established cell model of FMDV persistence may give us better information about the determinants of FMDV persistence in its natural host. The phenotype of persistent viruses will be further studied in order to identify modifications linked to persistence. At the cellular level, innate immune response in MDBKfmdvp will be investigated in order to verify its role in FMDV persistence.

notes

COMPARATIVE UTILITY OF THE FETAL GOAT TONGUE CELL LINE ZZ-R 127 AND FETAL BOVINE KIDNEY CELL LINE LFBK-$\alpha_v\beta_6$ FOR VIRUS ISOLATION FROM CLINICAL SAMPLES COLLECTED FROM ANIMALS EXPERIMENTALLY INFECTED WITH A FOOT-AND-MOUTH DISEASE VIRUS

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Introduction

Isolation of foot-and-mouth disease virus (FMDV) from clinical samples is essential for diagnostic work of FMD. Primary bovine thyroid (BTY) cells have the highest sensitivity for FMDV isolation; however, the BTY cells cannot be cryopreserved without decrease in the sensitivity and the sensitivity varies between batches. The fetal goat tongue cell line ZZ-R 127 and fetal bovine kidney cell line LFBK-$\alpha_v\beta_6$ were reported recently and have similar sensitivities for FMDV isolation with the BTY cells. The sensitivities of both the cells were evaluated only using epithelial suspensions, which are generally difficult to collect, and they have never been compared under the same conditions. In this study, virus isolation rates of both the cells were compared using clinical samples collected from animals which were experimentally infected with an FMDV.

Materials and methods

The ZZ-R 127 and LFBK-$\alpha_v\beta_6$ cells were kindly supplied by the Friedrich-Loeffler-Institute (Germany) and Plum Island Animal Disease Center (USA), respectively. The FMDV O/JPN/2010 strain, which were isolated from the 2010 epidemic in Japan, was inoculated to cattle, goats and pigs. Clinical samples such as sera, saliva, nasal swabs, feces and oropharyngeal fluids were collected routinely from the animals. Detection of viral genes by RT-PCR and virus isolation by both the cells were performed as previously described.

Results

Viral genes were detected in 39 of 210 bovine samples, 77 of 292 caprine samples and 57 of 144 porcine samples by the RT-PCR. Viruses were isolated from 12, 31 and 27 of the bovine, caprine and porcine samples by the ZZ-R 127 cells, respectively. They were isolated from 15, 28 and 27 samples by the LFBK-$\alpha_v\beta_6$ cells, respectively.

Discussion

Viruses were successfully isolated from the clinical samples other than epithelial suspensions by both the cells. Virus isolation rates for both the cells were almost the same.

notes

RESULTS OF THE 2013 PROFICIENCY TESTING SCHEME

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Introduction

The Pirbright Institute as the OIE, FAO and European Reference Laboratory for Foot-and-Mouth Disease (FMD) and Swine Vesicular Disease (SVD) carries out an annual proficiency testing scheme for laboratory assays. National Reference Laboratories as well as OIE and FAO Reference Laboratory from all FMD endemic pools and FMD-free countries were asked to participate. Laboratories have received their individual feedback with recommendations, and the purpose of this presentation is to provide an overview of the scheme and results for the 2013 exercise (Phase XXVI).

Materials and Methods

During 2013, four panels were available to each of the participating laboratories:

Panel 1: Infectious materials from pigs with a vesicular condition for FMD/SVD virus detection; 6 samples

Panel 2: Non-infectious materials originating from pigs with a vesicular condition for FMD/SVD antigen and virus genome detection; 8 samples

Panel 3: Non-infectious materials from Asia 1 vaccinated or non-vaccinated cattle for FMD serological diagnosis; 8 samples

A fourth panel was available for SVD but the result of that will not be discussed in this presentation.

The particular diagnostic methods that the laboratories utilise were not specified; rather, it was up to each laboratory to select and use tests that they considered appropriate.

Results

Fifty eight countries participated in the 2013 proficiency testing scheme. The results presented here illustrate how the various laboratories compared to each other and highlight possible areas of improvement.

Discussion

Changes have been made to the way the 2013 proficiency testing scheme are analysed and

presented. During this meeting, feedback will be welcomed from participating laboratories.

notes

DEVELOPMENT AND VALIDATION OF MULTIPLE NON-STRUCTURAL PROTEIN ANTIBODY TESTS TO CONFIRM FOOT-AND-MOUTH DISEASE INFECTION IN VACCINATED ANIMALS

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Introduction

Purified FMD vaccines stimulate antibodies against the structural proteins of the FMDV. In contrast, infection with live FMD virus elicits antibodies to both structural and non-structural proteins (NSP). Therefore, NSP antibody tests are being used to differentiate infection in vaccinated animals (DIVA). Currently the best validated NSP antibody test is the PrioCHECK® FMDV 3ABC NS. However, this assay cannot detect absolute infection in vaccinated herds. Therefore, there is a need of developing and validating confirmatory NSP antibody tests of comparable sensitivity & specificity as PrioCHECK® FMDV NS.

Materials and methods

In this study 6 indirect ELISAs have been developed and validated using bovine sera from experimental vaccinated-challenged animals, a well-established bovine serum panel, field outbreaks and naïve animals. Out of these six assays, 4 were based on recombinant proteins viz 3ABC, 3D, 3CD, 2C and 2 were based on synthetic peptides (2B & 3B). The sensitivity and specificity of these assays have been calculated using ROC and Bayesian analysis.

Results

Three tests (2B, 3B & 3ABC) were found with comparable sensitivity & specificity to PrioCHECK® FMDV NS. Further, combining these 3 tests in parallel & serial testing with PrioCHECK® 3ABC test, the sensitivity & specificity of the detection of antibodies against FMD virus are increased.

Discussion

2B, 3ABC and 3B NSP tests could be used as confirmatory tests in conjunction with Prionics 3ABC test to detect FMD infection in vaccinated population with more confidence.

notes

READY-TO-USE KITS FOR THE DETECTION OF ANTIBODY TO FMDV SEROTYPES SAT1 and SAT2

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Introduction

Given the endemic presence of FMDV SAT strains in Africa, the availability of robust and simple diagnostic tools targeting these serotypes is a priority. We have developed all-inclusive ELISA kits for the serology of SAT1 and SAT2 serotypes that complete the spectrum of stabilised kits for FMDV serotype-specific antibody.

Materials and Methods

Kits are based on the principle of Solid-Phase Competitive ELISA. The reaction is accomplished with only two incubation steps at room temperature before colour development, i.e. delivery of test sera into plates [pre-sensitized with FMDV inactivated antigens trapped by catching monoclonal antibodies (MAbs)], followed by addition of a labelled-competitor antibody. The latter are neutralising MAbs specific for FMDV SAT1 (4C5) and SAT2 (2H6) respectively and directed against linear sites of VP1.Sera analysed are reported in results.

Results

Analysis of 2301 and 2791 naive field sera (bovine, ovine, buffalo) provided specificity estimates of 99.3% ($CI_{95\%}$ 99-99.6%) for Ab-SAT1 kit and 99.6% ($CI_{95\%}$ 99.4-99.8%) for Ab-SAT2 kit.

Studies for evaluation of sensitivity performance of the Ab-SAT1 kit showed:

- detection of 84 out of 93 sera positive by homologous LPBE, sequentially collected up to 425 days-post-infection from two cattle;

- a seroprevalence rate of 86.5% (vs 94.7% by VNT) in 171 sera collected in Zimbabwe from three farms (farm C: SAT1 and SAT2 infected; farm D: SAT1 infected; farm E: SAT1 vaccinated and infected), and a concordance with homologous VNT results of 90.6%.

Studies for evaluation of sensitivity performance of the Ab-SAT2 kit showed:

- seropositivity of 55 out of 59 (93% vs 91% of LPBE) cattle experimentally vaccinated and/or infected with at least five different SAT2 strains;

- a seroprevalence rate of 98% (vs 99.5% by VNT) in 237 sera collected in Zimbabwe from three farms (farm A: SAT2 vaccinated and infected; farm B: SAT2 infected; farm C: SAT1 and

SAT2 infected), and a concordance with homologous VNT results of 98.3%;

- a seroprevalence rate of 59% (vs 65% by LPBE) in 416 sera collected at 13 sampling points with a history of vaccination and SAT2-infection in the Mpumalanga area, South Africa;

- seroconversion in 29 out of 30 (96.6%) cattle, sampled 36 days-post-vaccination with a trivalent O, A and SAT2 vaccine in Libya.

Conclusions

The results suggest that the developed kits have potential applications as rapid and user-friendly tests for serodiagnosis of FMD due to serotypes SAT1 and SAT2, serosurveillance programmes and post-vaccination monitoring.

Acknowledgments

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notes

DESIGN OF A PAN-FOOT AND MOUTH DISEASE DIAGNOSTIC BIOREAGENT

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Introduction

Many ELISA-based serodiagnostics of Foot-and-mouth Disease virus (FMDV) have been developed utilising the non-structural FMDV proteins (Ma et al., 2011). Non-structural proteins are ideal targets for pan-serotype diagnostic bioreagent development due to their high sequence conservation across the seven serotypes of FMDV. This study describes the development of a single 'consensus' bioreagent and its application in a competitive ELISA (cELISA) for the detection of antibodies against FMDV non-structural protein 3B.

Materials and methods

A consensus amino acid sequence for all published FMDV 3B proteins was constructed *in silico* and expressed using a synthetic codon-optimised gene in *E. coli* as a fusion with the solubility tag, maltose binding protein (MBP). The resulting recombinant MBP-FMDV 3B protein was used for the development of a cELISA to detect serum antibodies to the 3B non-structural protein of FMDV across the seven serotypes.

Results

A consensus amino acid sequence for 3B protein was determined and analysed *in silico*. The 3B consensus protein was successfully expressed in a bacterial system as a soluble fusion to MBP. MBP-FMDV 3B was affinity purified on amylose resin and used as coating antigen in a cELISA. Preliminary data indicated the FMDV 3B consensus cELISA to be a promising diagnostic assay and demonstrated the utility of a consensus protein as a pan-diagnostic bioreagent.

Discussion

Herein we describe a novel approach to bioreagent development; *in silico* identification of a consensus antigen at the amino acid level, and its use as a diagnostic reagent for detection of antibodies to FMDV 3B. It is anticipated that the use of the non-structural protein 3B will allow application of the test to the differentiation of infected and vaccinated animals (DIVA). Further assessment is required to validate this assay as a pan-serotype DIVA diagnostic.

notes

VALIDATION OF VIRUS NEUTRALISATION TEST FOR ALL FOOT-AND-MOUTH DISEASE TO DETERMINE SERO-PREVALENCE IN CATTLE IN ERITREA

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Introduction

Information about sero-prevalence of foot-and-mouth disease (FMD) and virus serotypes in Eritrea is unavailable, but are very important as it may guide the choice of intervention measures including vaccination to be implemented.

Material and methods

To validate the results of the virus neutralisation tests (VNT) against all 7 serotype cattle sera (n = 464) collected in 2000 at a Dutch slaughterhouse were used to determine the cut-off values in the VNT. We carried out a cross-sectional study in 2011 in Eritrea with a two-stage cluster design, sampling cattle in 155 villages with the objective of determining the sero-prevalence of FMD in four administrative regions of the country. We analysed cattle sera (n=2429) for FMD virus antibodies using the non-structural ELISA (NS ELISA) and VNT.

Results

We determined the cut-offs in the VNT's using the specificity of the VNT's and the agreement between VNT results and NS ELISA results. Very different cut-offs were observed in the different VNT tests. In Eritrea the overall sero-prevalence was 26% and 30% for the NS ELISA and VNT respectively. FMD virus serotype O (14%) and A (11%) were the most prevalent. The administrative region Gash Barka showed the highest (39%) sero-prevalence both in NS-ELISA and VNT compared to the other three administrative regions.

Discussion

Different cut-offs in VNT tests have been observed in the past but an extensive comparison on specificity in the same laboratory was not available. Our study not only evaluated the specificity on Dutch sera, but also the sensitivity based on the NS ELISA results. The study in Eritrea showed that strategic FMDV vaccination with type O and A (matching circulating strains) in combination of zoo-sanitary measures would be the best control option for Eritrea which could be started in areas where the disease is less endemic.

notes

DEVELOPMENT OF LATERAL FLOW ASSAY FOR ANTIGEN DETECTION AND SEROTYPING OF FMDV

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Introduction

In FMD outbreak, antigen serotyping is important to implement the contingency plan for disease control. Although antigen detection ELISA is one of the method enables serotyping, it needs some apparatus, takes time and has the risk of the virus diffusion in the laboratory. Therefore, we developed the lateral flow (LF) assay using monoclonal antibodies (MAbs), which allows for rapid detection and serotyping of FMDV in the field and local laboratories.

Materials and methods

Anti-FMDV MAbs against each serotype O, A, C and Asia1, respectively, and multi serotype reactive MAb were applied on the nitrocellulose membrane. Colloidal gold conjugated multi-serotype reactive MAb (G-MAb) was freeze-dried in the tube. FMDVs (O1 BFS 1860, A15 TAI 1/60, C PHI 7/84, Asia1 Shamir and recent several strains) were used for antigen detection experiment. These strains were diluted with PBS and added into the tube including the freeze-dried G-MAb followed by dipping the LF strip into the tube. After 10 minutes, the mABS of the appeared lines were read by mABS reader.

Results

The LF strips were able to detect the FMDVs and distinguish serotype O, A, C and Asia1, respectively. Their sensitivities are almost equal to from 10^3 to 10^4 $TCID_{50}$ of each virus stain.

Discussion

This LF strip enables rapid antigen detection and serotyping of FMDV without extra-devices. This method will be useful in not only FMD-free countries but also FMD infected countries, especially where it could not be conducted laboratory diagnosis. As a result, we will be able to share more precise information about FMD epidemics by using this method. Further study is ongoing for applying for seven serotypes, sensitizing and manufacturing with private companies.

notes

DEVELOPMENT AND COMPARISON OF VARIOUS ELISA FOR THE DIAGNOSIS OF FOOT & MOUTH DISEASE (FMD)

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Introduction

FMD control and management requires continuous monitoring of the disease. Current FMD vaccines (killed virus) provide protection by inducing antibodies to viral structural proteins, most prominently FMD VP1. The most current vaccines contain varying but significant amounts of various NSPs (NSP2A-C and NSP3A-D). Antibodies to non-structural proteins (NSPs), such as FMD2-3ABC, are produced during active viral replication. Therefore, the presence of 3ABC is used for DIVA tests. Thus, there is a need for additional commercial ELISA tests (NSP2ABC and NSP3ABC) for more robust FMD DIVA test and disease control.

Materials and methods

ADI has produced recombinant structural protein VP1 from various FMD serotypes (O, A, A1, and SAT1-3) and NSPs (FMD2A, 2B, 2C, 3AB, 3C, and 3D), and developed antibody ELISA kits for individual or serotype combinations (O+A+A1 and SAT1-3 antibodies). Reference antisera from animals experimentally infected with a given FMD serotypes were used to assess the efficacy of various ELISA kits.

Results

VP1 ELISA kits for various FMD serotypes individually or in combination (O+A+A1 and SAT1-3) detected antibodies to VP1 in experimentally infected animals. There was significant but variable crossreactivity of various serotype antibodies to any given VP1. Higher levels of antibodies to 2ABC, 3D protein were detected when 3ABC showed weak antibody response in experimentally infected animals. Therefore, additional ELISA kits based upon FMD-2ABC and FMD-3D should allow better DIVA Tests.

Discussion

FMD VP1 and NSP2-3 proteins show variable but significant conservation across the FMD serotypes. The significance of antibody crossreactivity, as detected by various ELISA kits, in FMD detection of vaccinated and infected animals will be presented and discussed.

notes

DIAGNOSTIC OBSERVATIONS WITH IZSLER ANTIGEN ELISA KITS FOR DETECTION AND SEROTYPING OF FMDV SEROTYPES O, A, SAT1 AND SAT2 IN SEVERAL AFRICAN COUNTRIES

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Introduction

Two FMDV antigen detection ELISA kits have been developed by the IZSLER institute in collaboration with the World Reference Laboratory in Pirbright, UK. The assays are performed with selected combinations of catching and conjugated anti-FMDV monoclonal antibodies (MAbs). The test can be applied for detection and typing of FMD viruses in vesicles epithelium homogenates and fluid. One kit is designed for detection and typing of FMD viruses of type O, A, Asia1 and C. . Another kit, tailored for African countries, is designed for FMD viruses of type O, A, SAT1 and SAT2. A pan-FMDV test, detecting any isolate of type O, A, C and Asia1 and in addition some isolates of the SATs serotypes, is included in both kits to complement the specific typing. The second kit was produced on request of EUFMD during the FMDV SAT2 epidemic in Egypt in 2012. Since then these kits have been delivered to 24 different countries in Africa, of which Egypt, Kenya and Nigeria have used the kits most extensively.

Materials and methods

ELISA results were analysed for a subset of tests performed between 2012 and 2014 by the NRLs in Egypt, Kenya and Nigeria (163, 158, and 102 samples, respectively). Mainly vesicular samples were tested, but sometimes also swabs from vesicular lesions and myocardial tissues collected from cases of neonatal mortality were included.

Results

In the samples from Egypt 38% scored positive (A: n=4, O: n=30, SAT2: n=28). All type O positive samples did not cross-react with Mab 4D12 (a type A Mab that may cross-react with certain type O strains), and all SAT2 positive samples were not detected with the pan-FMD Mab. Four out of 28 SAT2 positive samples cross-reacted with SAT1 Mabs. In the samples from Kenya 61% scored positive (A: n=13, O: n=47, SAT1: n=15; SAT2: n=21); 19% of the type O positive samples cross-reacted with Mab 4D12. For 10/15 (67%) and 3/21 (14%) of the SAT1 and SAT2 positive samples, respectively, a positive result was obtained with the "pan-FMD" MAb. Three out of 21 SAT2 positive samples cross-reacted with SAT1 Mabs. In the samples from Nigeria 50% scored positive (A: n=14, O: n=5, SAT1: n=6; SAT2: n=27) 40% of the type O positive samples cross-reacted with Mab 4D12. All SAT1 and SAT2 positive samples scored negative with the "pan-FMD" MAb.

Discussion

This FMDV antigen detection ELISA is robust, user friendly and mainly yields unequivocal typing results. Also for those samples that were also sent to WRL Pirbright the agreement in typing results was very good. Conjugate B, used for the SAT1 and SAT2 rows of the plate, tends to give a higher background. Therefore the washing procedure should be followed strictly. Type O cross-reactions only occur with one type A Mab, which does not influence the interpretation. For few SAT2 strains significant cross-reactions may occur with SAT1 Mabs that is worth to be further investigated. Sample type and quality (use of transport media, transport conditions) clearly influence the typing rate.

notes

NEW COMPETITIVE ELISAs FOR FMD DIAGNOSIS BY DETECTION OF NON-STRUCTURAL OR STRUCTURAL ANTIBODIES AGAINST FMDV

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Introduction

FMD serology is mainly based on the use of NSP and SP-serotype specific ELISAs. Robust and easy-to-use ELISA kits are thus essential to monitor and control FMDV infections. Validation data obtained for different Solid Phase Blocking ELISAs (competitive ELISAs, cELISA) will be presented.

Materials and methods

Samples from animals from non-endemic areas, as well as from vaccinated and/or infected animals, were tested .

ID Screen® FMD Type O Competition ELISA specifically detects antibodies against FMDV serotype O with serum or plasma from cattle, swine or other susceptible species, whereas ID Screen® FMD NSP Competition ELISA detects anti-3ABC antibodies.

A FMD Pan-serotype Competitive ELISA, detecting anti-structural antibodies for all serotypes, is also available as a prototype.

Results

For the Type O and NSP cELISA kits, analytical sensitivity, sensitivity and specificity will be presented and discussed. Depending on the species tested, the specificity of these kits ranged from 99.0% to 99.9 %. Sensitivity was excellent, and results showed high agreement with other techniques. Preliminary validation data for the Pan-serotype competitive ELISA will also be presented.

Discussion

These new cELISAs, allowing for Type O serotype-specific and NSP antibody detection, show excellent test performance. These tests are particularly user-friendly, as all reagents are supplied ready-to-use (not freeze-dried) and results are obtained in 90 minutes or 4 hours. IDvet welcomes proposals for collaborative validation work on this disease.

The document once it's finished it will be send, the collect of some data is ongoing (until now there are some FMD outbreaks)

notes

OUTBREAKS OF FOOT-AND-MOUTH DISEASE VIRUS IN LIBYA AND SAUDI ARABIA DURING 2013 DUE TO AN EXOTIC O/ME-SA/IND-2001 LINEAGE

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Introduction

Each serotype of foot-and-mouth disease virus (FMDV) has a different geographical range, with type O being the most widespread. Phylogenetic analysis of outer capsid polypeptide 1 (VP1) coding region identifies eleven geographically restricted genotypes (topotypes) within serotype O and further genetic lineages within topotypes which also may be restricted geographically. The O/ME-SA/Ind-2001 lineage is normally restricted to India, Nepal, Bhutan and Bangladesh but it has occasionally spread westwards causing limited outbreaks.

Methods

FMD viruses were collected from recent FMD field cases in North Africa and the Middle East. The sequence of the genome region coding VP1 of a number of type O virus isolates was determined and phylogenetic analyses were performed. Additionally, an O-Ind-2001-specific real-time RT-PCR (rRT-PCR) assay was developed to target conserved regions within the VP1 coding sequence.

Results

Viruses from outbreaks in Libya (September to December 2013) and Saudi Arabia (August to November 2013) were found to belong to the ME-SA/Ind-2001 lineage. Phylogenetic analyses showed these viruses are most closely related to isolates from India and Bhutan collected in 2013 and provide evidence that the FMD outbreaks in Libya and Saudi Arabia have arisen through separate introductions of this virus strain into these two countries. Representative RNA from 55 virus isolates were tested by the developed "Ind-2001" lineage specific rRT-PCR and the pan-specific assay detecting the 3D region of the genome (Callahan et al., 2002). All samples including representative serotype O FMDV lineages (O/EA-3 and O/ME-SA/PanAsia-2) that might be present in the region were identified as FMDV positive with the "3D" assay while only those 35 samples from the O/ME-SA/Ind-2001 lineage were also amplified by the "Ind-2001" specific assay.

Discussion

Based on the previous occurrences of O/ME-SA/Ind-2001 outside of the Indian sub-continent, it is possible to speculate that these outbreaks in Libya and Saudi Arabia will only seed a limited number of onward outbreaks, such like the recent outbreak in Tunisia. However, if this new sub-lineage was able to establish itself in the region it could potentially out-compete indigenous FMDV O/ME-SA lineages such as O/ME-SA/PanAsia-2.

notes

MASS VACCINATION, IMMUNITY AND COVERAGE:MODELLING POPULATION PROTECTION AGAINST FMD IN TURKISH CATTLE

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Introduction

Efforts to achieve sustained herd immunity through mass FMD vaccination of cattle are undermined by rapid population turnover and waning protection.

Materials and methods

A dynamic model of the Turkish, Anatolian cattle population was created, incorporating changes in age structure over time. Vaccine status by number of doses and time since vaccination was calculated and used to estimate population immunity. Coverage estimates were based on field data. The model was fitted to data from a field study that measured structural protein antibody titres after vaccination with the standard Turkish ≥3PD50 vaccine in 2012.

Results

With biannual mass-vaccination with a single-dose primary course, six months after the last round of vaccination only 50% of cattle will have been vaccinated more than once, with the last dose received <7 months ago. Five months after the last vaccination round half to two-thirds will have a low antibody titre (<70% protection level). A two-dose primary course reduces the proportion of 6-12 month old cattle with low titres by 25-40%.

Discussion

Higher potency vaccines and a two-dose primary course should significantly improve population immunity. However, immunity gaps would still exist, as each round of vaccination may exclude a quarter of all cattle. Therefore, effective zoo-sanitary measures are likely to be required in addition to vaccination. Since spring 2014, ≥6PD50 vaccines have been used routinely throughout Anatolia, with a two-dose primary course given in the West. As sustained high coverage is crucial, long-term vaccine production capacity has been increased.

notes

MODELLING ENDEMIC FOOT-AND-MOUTH DISEASE IN TURKEY

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Introduction

Mathematical models of livestock disease are being increasingly used as a tool to aid policy makers. These models can be used to test out a variety of control measures and help to reduce the disease burden.

Traditional models of disease assumed that populations mixed homogeneously, such that all members of a population contact each other with equal probability. However, this assumption will not capture the demographic heterogeneity that exists in many real world systems. A natural way to incorporate heterogeneous mixing is to use a network approach, where members of the population act as nodes and potential infectious contacts between them are edges. This idea extends intuitively to livestock disease with nodes being farms and edges being livestock movements between them.

Materials and methods

We build a bottom up, compartmental model of endemic FMD in Turkey utilising a network approach to simulate disease transmission through the livestock movement network. Transmission through other mechanisms is incorporated into the model using a distance dependent transmission kernel approach that accounts for the demographic characteristics of susceptible and infectious farms.

Results

This model utilises detailed movement records supplied by TurkVet and reported outbreak data. We parameterise the model in a Bayesian framework which allows for priors informed by expert opinion. We then investigate the impact of intervention strategies such as movement control and targeted vaccination upon reducing disease burden.

notes

D HEALTH SITUATION IN TUNISIA

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Introduction

The Foot and mouth disease FMD is a part of the list of regulated diseases in Tunisia. It appeared in the end of April 2014 in the North East of the country in a cattle trader. The disease was distributed in practically all the territory. In this paper we describe the health status of the disease, its evolution over the time and space and its epidemiological and clinical characteristic. Control measures implemented by the Veterinary Services to deal with the spread of the disease are detailed in the second part. A third part will be focused on the prospects to implement and further investigations planned and the modifications to bring to the control plan in order to adapt its compounds to the new health situation. (EN)

La fièvre aphteuse maladie figurant dans la liste des maladies réglementées en Tunisie est apparue fin avril 2014 dans le Nord Est du pays chez un marchant de bovins. La maladie a diffusé dans pratiquement tout le territoire. Dans cette communication on décrit la situation sanitaire de la maladie, son évolution dans le temps et dans l'espace ainsi que ses caractéristiques épidémio-cliniques. Les mesures de lutte instaurées par les Services Vétérinaires pour faire face à la propagation de la maladie sont détaillées dans la deuxième partie. Une troisième partie sera consacrée aux perspectives à mettre en œuvre, aux investigations supplémentaires programmées et aux modifications à apporter au plan de contrôle de la maladie afin de l'adapter à la nouvelle situation sanitaire (FR).

Materials and methods

Investigations in outbreaks have been done and that were used in the data analysis in order to look for the infection origin, epidemio-clinic characteristics and determine the lesions age.

The data are analyzed by the Excel software

Results and the discussion

The disease appeared in April 2014, the last episode was in 1999 (14years ago), the disease affected all species but particularly the bovine. The origin of infection, in the majority of cases, is the introduction of sensitive animal which the health statue is unknown. The laboratory investigations show that the serotype responsible of the disease is O and it has 99,98 % of resemblance with the Libyan serotype that has appeared in 2013. The crisis management shows many weak points like the animal identification, animal movement, animal (flocks) immunity statue …

The control plan must be reviewed and modified in order to adapt its compounds to the new

health situation notably the surveillance. The immunity statue and the immunity threshold of the livestock must be determined. The prophylaxis campaign must be re-assessed .

notes

SEROLOGICAL SURVEY IN LIBYA TO ASSESS FMD VIRUSES CIRCULATION AND VACCINE IMMUNE RESPONSE

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Introduction

FMD is known to be present in Libya (currently qualified in stage 1 of the Progressive Control Pathway). Mass vaccination of large (trivalent vaccine O, A and SAT2) and small ruminants (bivalent O and A) is carried out since late 2012 when a collaboration with IZSLER was established. In the framework of this collaboration serological surveys have been undertaken to: (i) evaluate the immune response of vaccinated animals; (ii) assess the level of virus circulation and identify serotypes.

Materials and Methods

During spring 2013 a total of 164 and 30 serum samples were randomly collected from small and large ruminants (SR and LR) respectively to assess the immune response at 30 days post-vaccination. In parallel a country-wide serosurvey has been carried out through which a total of 2542 and 1273 sera were collected from SR and LR respectively Sera were tested by NSP and SP ELISA (against O, A and SAT2) using IZSLER kits. Serotypes prevalence and between-serotype cross-reactivity was studied through the evaluation of both antibody titers against the three serotypes and a stratified Odds Ratio.

Results

In SR immunity at 30 DPV was 82% and 93% against type O and A respectively while in LR the proportion of seroconversion was 100% for both type O and A and 97% against SAT2. After 5-6 months post-vaccination the seroprevalence dropped to about 50% in NSP seronegative animals. Results of the nation-wide serosurvey stratified in each of the following age-category: 0-12, 12-24 and > 24 months showed a NSP antibodies prevalence of 4%, 14% and 23% in SR and 10%, 14% and 21% in LR respectively. Significantly higher titres of antibodies to serotype O or A in NSP-seropositive animals were detected in certain zones. Of note that among SR (not exposed to SAT2 vaccine) 5.74% of the sera tested positive for antibodies against SAT2. A significant cross-reactivity was found in SR between type O and SAT2 (Mantel-Haenszel OR_{mh} = 4.8) stratified over type A status.

Discussion

The NSP serologic profile observed in both small and large ruminants supports the hypothesis of an endemic level of FMDV. Evidence of SAT2 presence was not found and most of the SAT2-positive results can be explained as cross-reactivity of type O-antibody with SAT2 antigens. While in clinical samples type O (Ind/2001 lineage) was the only serotype detected, a deeper evaluation of SP-antibody titers suggests for the concurrent circulation of type A in some areas.

notes

SEROEPIDEMIOLOGICAL STUDY OF FOOT AND MOUTH DISEASE IN LIVESTOCK IN TRIPOLI, LIBYA

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Introduction

Foot-and-mouth disease (FMD) in Libya is endemic in many parts of the country. The economic impact of FMD is not readily apparent since the country does not export livestock and livestock products and the productivity of animals is low. The study aimed to establish an accurate comprehension of the epidemiology of FMD in Tripoli, and the outcome of the results will help in the surveillance and control of FMD in Libya.

Methods

A cross sectional seroprevalence and questionnaire survey was undertaken between June, July and August 2013 for sheep and goats, and in April and May 2014 for cattle, in Tripoli area. Ten percent of sheep and goat herds which have 100 heads or more were randomly selected , and 48 animals from the selected herds were also randomly selected from different age groups (16 samples from each of the following age groups: < year, 1 year- 2 year, and > 2 year). Ten percent of cattle herds were selected randomly, and all animals in the herd if less than 5 animals will be selected, and if the herd has more than 5 animals, then only 5 animals will be selected. The preferred age is 6 – 18 months.

Results

Sera samples were diagnosed using a commercial NSP kit, PrioCHECK® FMDV NS, from Prionics AG (Switzerland). The herd prevalence of FMD was 100% and 72% for small ruminant and cattle respectively. Mean within-herd prevalence for small ruminant was found to be 15% (95% CI: 8.8% - 21.3%) with FMD prevalence in an infected herd ranged from 3.8% to 45.8%, whereas for cattle it was 42% (95% CI: 32.9% - 51.1%) with FMD prevalence in an infected herd ranged from 0% to 100%. Some risk factors at the animal level and herd level were analysed during the study.

Discussion

Mass vaccination was used in the country for small ruminants and cattle once per year (2012 and 2013). Starting from this year 2014 cattle will be vaccinated twice per year in order to control FMD in Libya.

notes

FMD IN LIBYA AND THE CONTROL STRATEGY

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Introduction

FMD is known to be present in Libya (currently qualified in stage 1 of the Progressive Control Pathway (PCP-FMD)). In 2012-2013 a mass vaccination campaign has been initiated (against A and O for small ruminants and O, A and SAT2 for large ruminants). A cooperation agreement was signed with the Italian Government in 2012 to gain a better understanding of the epidemiological situation and specific activities designed and conducted in collaboration with IZSLER.

Materials and Methods

Since early 2013 a surveillance system has been designed and implemented structured into three main components:

1: Collection of samples from clinically affected animals in FMD suspected outbreaks: more than 300 samples (vesicular epithelium, swabs, blood) have been collected from suspected cases during the second half of 2013 from different areas.

2: Evaluation of immune response of vaccinated animals by collecting blood samples at the vaccination day and 30 days post vaccination.

3: Investigation on the level of FMD virus circulation (anti-NSP antibodies) and the serotypes present (anti-SP serotype-specific antibodies): more than 5000 sera samples collected all over the country.

Most of the lab work for the 3 components was carried out in IZSLER, Italy. More recently, for field diagnosis, the Veterinary Rapid Response Teams (RST) are using the Pen-Side tests (provided by EuFMD as part of the component 2.3 of EuFMD workplan (REMESA)).

Results

Results of the component 1 are presented here. Initial diagnosis was carried out in Tripoli lab using IZSLER ELISA kits whereas confirmation and virus isolation was carried out in IZSLER lab.

Only serotype O was found in suspect samples, phylogenetic analyses carried out in Pirbright lab indicated first detection in Libya of a strain belonging to O/ME-SA/Ind-2001 lineage. Vaccine matching estimates suggested that the vaccine strain O-3039 should provide protection against it better than vaccine strain O-Manisa.

Discussion

Means of introduction of the new strain O/ME-SA/Ind-2001 into the country are not known yet. The activities carried out so far would suggest that FMD is endemic in Libya and the working hypothesis is that the virus is maintained mainly through the small ruminant population. To further progress along the PCP and with the objective of reaching stage 2 additional studies are on-going (supported by Eu-FMD) to better characterize risk hotspots and transmission pathways. Same 3 components are ongoing for this year 2014.

notes

PROMOTING A RISK BASED STRATEGIC PLAN FOR FMD CONTROL: IMPROVEMENT OF RISK ASSESSMENT THROUGH REAL TIME TRAINING

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Introduction

EuFMD, under a contract between the FAO and the government of Australia, conducts "Real Time" (RT) training courses for veterinarians and associated stakeholders. RT courses involve field investigation of an FMD outbreak.

Under an agreement between FAO and the Department of Livestock Services, Nepal (DLS), training takes place in Nepal, and support is provided to DLS in the development of a risk-based strategic plan (RBSP) for FMD control.

In this study, we show how outbreak investigations, carried out during the RT courses, have been instrumental in guiding the development of this RBSP.

Materials and methods

Between June 2012 and June 2014, 12 RT courses were conducted, training a total of 112 Australian, 12 New Zealander and 59 Nepalese participants. Participants conducted a full outbreak investigation including clinical examination, laboratory diagnosis and an epidemiologic investigation, including local area survey. Findings from each RT course were collated to identify common risk factors and recommendations.

Results

A number of risk factors were identified including poor biosecurity, animal movement without quarantine and lack of vaccine. Key recommendations included awareness-raising and education, particularly through groups such as dairy cooperatives, and the use of enlightened farmers to educate their peers. Another recommendation was for the DLS to make quality vaccine available for private use and to establish a nationwide record keeping and reporting system.

Discussion

Outbreak investigation goes beyond sampling FMD cases for confirmation and virus typing. When including epidemiologic investigation it allows understanding of risk-factors for FMD introduction and spread within a local community. This understanding has supported the development of a national control plan based on the risks identified.

notes

SEROSURVEILLANCE AND THE PCP-FMD: WHY ARE SEROSURVEYS USEFUL, HOW ARE THEY BEING APPLIED AND WHAT ARE THE GAPS?

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Introduction

Serological surveys to measure antibody to non-structural protein (NSP) in FMD-susceptible animals are an important tool in the Progressive Control Pathway for Foot-and-Mouth Disease (PCP-FMD). These surveys provide a measure of the level of FMD virus circulation in both clinically and subclinically infected animals.

Materials and methods

Information about implemented NSP serosurveys was gathered through a literature search and contact with colleagues working in FMD endemic countries. The objective, design and results of these serosurveys were compared.

Results

Reports of NSP surveys implemented between 2006-2010 were obtained from 28 countries. The survey objectives included estimate prevalence of NSP antibody, evaluation of diagnostic tests and identification of risk factors. A 2 stage design was most commonly used, with sampling at farm and animal levels. Results were usually reported at animal level; the reported animal level NSP antibody prevalence ranged from 8-80%. Most serosurveys were done in context of international projects, and all but 2 were funded by donors.

Discussion

Serosurveys are resource intensive, however in some situations they provide information that may be extremely useful to guide FMD control and eradication. Comparison of serosurvey results to the annual number of reported clinical outbreaks suggest a high level of subclinical disease and/or under-reporting in many countries. Complementary log log link binomial regression models may be useful to translate data on NSP prevalence into FMD incidence rates. Understanding FMD incidence in different populations is critical to develop national risk-based control strategies. It is also important for FMD-free countries to assess the risk posed by virus circulating in endemic countries, and to estimate impact of FMD at national, regional and global levels. Guidelines on serosurveys would be a valuable addition to the PCP-FMD toolbox, describing the situations in which serosurveys would provide valuable information, and appropriate designs to achieve the particular objectives.

notes

VACCINATION OF CATTLE ONLY SEEMS TO BE SUFFICIENT TO STOP TRANSMISSION IN MOST MIXED POPULATIONS OF CATTLE AND SHEEP

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Introduction

For understanding transmission of FMDV under field conditions where different animal species coexist, quantification of the reproduction ratio *R* for heterogeneous populations (e.g. consisting of sheep and cattle) is necessary. And although the effect of vaccination is quantified for homogeneous populations, this has not yet been quantified for heterogeneous population. It is e.g. not clear whether emergency vaccination of all susceptible species is necessary to control an epidemic or if targeting vaccination to certain species (e.g. only cattle) could be sufficient. In the current study we developed a method that allowed quantification of *R* for mixed populations consisting of different proportions of cattle and sheep and the effect of different vaccination strategies.

Materials and methods

We quantified $R_{\text{cattle-to-cattle}}$, $R_{\text{sheep-to-sheep}}$, and partial $R_{\text{sheep-to-cattle}}$ for non-vaccinated animals and of $R_{\text{vac cattle-to-cattle}}$ and $R_{\text{vac sheep-to-sheep}}$ for vaccinated animals using results from previously published transmission experiments. A 4 by 4 table was constructed using these estimates. By assuming separable mixing i.e. assuming that the (partial) *R*'s are the product of a relative infectivity f_i and a relative susceptibility g_i (where i is either non-vaccinated cattle, vaccinated cattle, non-vaccinated sheep, or vaccinated sheep), we calculated the missing values in the table. Subsequently, a next generation matrix was constructed where the elements of the matrix are functions of the relative infectivity, the relative susceptibility, the proportion of cattle in a population, and the proportion of vaccinated cattle and that of sheep. The dominant eigenvalue of the NGM, the *R* for a mixed population, was determined depending on different proportions of cattle and sheep. Finally, for these populations for 3 different vaccination strategies (vaccination of both cattle and sheep, vaccination of cattle only, vaccination of sheep only) the effect was determined.

Results and Discussion

In non-vaccinated populations the higher the proportion of cattle in a mixed cattle-sheep population, the higher *R* for the mixed population is. After vaccination of all animals *R* = 0.1 independent of population composition. In mixed cattle-sheep populations with at least 14%

cattle, vaccination of cattle only seems sufficient to reduce *R* such that it is < 1. The NGM technique can be a valuable tool to determine the impact of control measures for heterogeneous populations.

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ELITE: AN ELECTRONIC LABORATORY INFORMATION TRACKING ENVIRONMENT FOR SUPPORTING THE PROGRESSIVE CONTROL OF FMD IN PAKISTAN

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Introduction

The ELITE technology was developed as a robust platform for disease monitoring and management within developing countries. It pairs a Laboratory Information Management System (LIMS) supporting day-to-day laboratory operations with a biosurveillance module for aggregating, monitoring, analyzing, and reporting diagnostic test results for program diseases such as FMD. The LIMS is an extensible information management system supporting the full workflow of the diagnostic testing process, from sample receipt through reporting of results. The system can be configured to support different test types, procedures, workflows, forms/reports, and hardware/network infrastructure, and can be defined locally or distributed throughout a network of laboratories. ELITE streamlines information flow within and between these diagnostic laboratories, allows for sharing of standard operating procedures with laboratory personnel, ensures consistent structured data entry across all users in the network, and enhances overarching biosurveillance efforts.

Materials and methods

Working in close collaboration with the Food and Agriculture Organization of the United Nations (FAO), the United States Department of Agriculture (USDA), and the National University of Sciences and Technology (NUST), ELITE is being piloted in Pakistan within both the National Veterinary Laboratory (NVL) and the provincial laboratory network. This system is a key component to FAO's strategy for the progressive control of FMD in Pakistan.

Results

ELITE has been deployed to NVL and all provincial laboratories in Pakistan, allowing automatic aggregation of FMD test result data by FAO. The research team has been providing ongoing training and technical support to laboratory end-users, as well as system refinements to ensure it adequately meets their day-to-day needs.

Discussion

The biosurveillance capabilities of ELITE fully integrate with the LIMS, and automatically detect

and report program disease cases to a centralized server. The system provides visualization and analytical tools for working with the aggregated testing data. Designed to function robustly under complex real-world conditions, ELITE can withstand intermittent network connectivity and power instability and provides a robust platform that could easily be used by other developing countries.

notes

MOLECULAR VARIABILITY WITHIN THE FOOT-AND-MOUTH DISEASE VIRUS O-PANASIA-2 LINEAGE

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Introduction

Foot-and-mouth disease virus is antigenically classified in seven serotypes and genetically characterized on basis of the region coding the VP1 capsid protein into a number of topotypes, lineages and sub-lineages. Molecular sequences are used to survey the transboundary movements of the virus as well as a starting point for vaccine matching analysis. In this study, a database of VP1 sequences from isolates representing all of the O/ME-SA/PanAsia-2 viruses collected worldwide by the FAO World Reference Laboratory for FMD (WRLFMD) were analysed in order to: (i) understand the spatial and temporal distribution of this lineage; (ii) to develop approaches to predict the emergence of new viral lineages and sub-lineages in endemic countries.

Material and Methods

A total of 1,129 VP1 sequences from the Middle East and Eurasia from viruses collected mostly from cattle (63%), but also small ruminants, pigs and wildlife (such as water buffalo, wild boar, gazelle and oryx) from 2002 to 2014 were aligned and analysed using different bioinformatics and statistic computing programmes.

Results and Discussion

The viruses were classified and distributed into the following sub-lineages within the PanAsia-2 lineage: ANT-10 (50.58%), FAR-09 (8.06%), BAL-09 and PUN-10 (0.62% each) and SAN-09 (0.44%). The sub-lineages of the rest of the viruses (39.50%) are still unnamed. More than two thirds of the sequences were obtained from countries within the Middle East and distributed in two main waves of outbreaks caused by unnamed (2007) and ANT-10 (2010) viruses. The analysis of the sequences showed nucleotide changes at 364 sites which corresponded to 110 amino acid substitutions. Despite the high variability within this segment of the genome, four conserved regions of 20 to 47 amino acids length were found. Conserved and non-conserved regions need to be further analysed for practical and theoretical purposes on the variability of FMDV.

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KNOWLEDGE AND PERCEPTIONS OF COMMUNAL FARMERS CONCERNING FOOT-AND-MOUTH DISEASE AT THE WILDLIFE/LIVESTOCK INTERFACE OF THE KRUGER NATIONAL PARK

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Introduction

Foot-and-mouth disease (FMD) and its control negatively impact the livelihood of resource poor communal farmers at the border of the Kruger National Park (KNP), South Africa. The KNP is an FMD infected zone and communities surrounding the KNP are part of the protection zone with vaccination.

Materials and methods

A structured questionnaire was administered to livestock farmers as they presented their cattle at inspection units (dip tanks) within the Mnisi Tribal Area of Bushbuckridge, Mpumalanga, South Africa. The questionnaire was written in English but in-person interviews were performed using the local language (Shangaan). Questions addressed owner demographics, herd management practices, general disease control and knowledge of FMD epidemiology.

Results

One hundred and four farmers responded to the questionnaire with 73% (76/104) being cattle owners while the remainder being hired cattle handlers. The majority of respondents (79%, 95%CI: 70-80%) indicated a high level of satisfaction with the current animal health programmes in practice at the dip tanks. The education level of farmers varied over levels of satisfaction, with the median education level being standard 9 (IQR: 2-12) for non-satisfied respondents, standard 3 (IQR: 0-6) for the little satisfied and standard 7 (IQR: 2-11) for the very satisfied respondents (P=0.036). The non-satisfied respondents were more likely to treat sick animals themselves rather than seek veterinary assistance (P=0.002). The majority of respondents identified the African Buffalo as a risk factor for FMD outbreaks (92%, 95%CI: 85-96%).

Discussion

The knowledge and perceptions of farmers concerning disease control programmes will help the implementation of disease control policies. Communal farmers at the interface with the KNP have some knowledge concerning FMD epidemiology and control, however this knowledge varied with education

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SEACFMD ROADMAP: A RISK-BASED APPROACH TO FMD CONTROL IN SE ASIA AND CHINA

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Abstract

The regional initiative to control foot and mouth disease (FMD) in South-East started in 1994 when countries in the region agreed to set-up an OIE Sub-Commission for FMD control. In 1997, the members formally launched the South East-Asia FMD (SEAFMD) Campaign to coordinate a sub-regional programme to control the disease. In 2010, China joined the Campaign, thus it was renamed as South-East and China Foot and Mouth Disease (SEACFMD).

To provide long-term guidance to the Campaign, a SEAFMD Roadmap 2020 was launched in 2007. The strategy was focused at progressive zoning approach to ensure effective use of limited resources from the donors and national governments. With the expansion of SEACFMD in 2010 and new developments in the socio-political arena of the region, new findings on the epidemiology of the disease, the SEACFMD 2020 Roadmap was revised. The 2nd edition of SEACFMD Roadmap launched in 2011 gave more emphasis at the reducing the overall FMD prevalence by targeting hotspots and critical nodes along movement pathways, and the progressive zoning approach was also continued but in areas with the most advance stage of FMD control. Maintenance of FMD free countries and zones was also highlighted in the 2nd edition.

Four years after the publication of the 2nd edition, many changes have transpired on the technical as well as socio-political aspects in the sub-region. Significant amount of scientific and epidemiological information on the status and behaviour of FMD have been acquired the past years. More detailed information on the animal movement pathways and other main risk factors involved in the trans-boundary spread of FMD have also been acquired.

A 3rd edition of the Roadmap is being developed to further implement a risk-based approach to FMD control. The principal objectives of this risk-based approach are to identify areas which are possible sources of FMD viruses and areas of higher risk of FMD introduction. A risk assessment will help identify major contributors to the risk of FMD incursion in a particular area or population and define potential targets for intervention. A risk-based approach is useful approach to support both strategic and operational decision-making. In scarce-resource settings, human and financial resources available to support government veterinary services and livestock disease control can be limited. Therefore, issues that present higher risks should merit higher priority so that resources and investments can yield higher benefit-cost ratios.

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EPIDEMIOLOGICAL INVESTIGATION OF FMD INCIDENCES IN SOUTHERN PENINSULAR INDIA DURING 2013

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Vaccination based Foot-and-mouth disease (FMD) control programme is in operation in India since 2003-04 for progressive control and eventual eradication of the disease. Since then, clinical FMD have reduced in these areas. However, sudden increase in FMD incidences was observed in the country in the year 2013. In this study, FMD incidences were epidemiologically investigated with the objective to find out the contributing factors responsible for the outbreaks.

Between January- 2013 and February- 2014, 631 FMD outbreaks/ incidences were reported in the country of which 472 were confirmed by the laboratory diagnosis. Most of these incidences (228) were confirmed in the four states (Karnataka, Tamilnadu, Kerala, and Andhra Pradesh) of southern peninsular region of the country which were totally covered under the control programme. Whereas, only sporadic cases of FMD were observed in other areas covered under the control programme. Virological examination revealed that Ind2001d lineage of serotype O was responsible for all the incidences of FMD in the country except for a few outbreaks which were caused by serotype A and Asia1.

Epidemiological data collected from every incidence were analysed and plotted up to the village level on GIS maps. Expected duration of protection from vaccination and serological status of animals were integrated with the time line of FMD incidences to determine the temporal window for FMD infection in these states. Differential pattern of temporal and spatial distribution of FMD incidences were observed in these areas. It was determined that decline in the protection level of the immunity provided the infection window. Course of the disease spread was traced by the GIS maps and was correlated with time line of herd immunity. Areas/ district with higher herd immunity had much lesser occurrence of the disease.

notes

USING RISK ASSESSMENT TO INFORM FMD POLICY IN MONGOLIA

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Introduction

In Mongolia there are no indications that FMD is endemic but four separate incursions have been recorded since 2010. A qualitative risk assessment was conducted to assess the effectiveness of existing prevention and control measures and to inform policy making.

Materials and methods

The risk assessment was conducted for the Western region of Mongolia during two separate workshops in May and September 2013. Local experts from central and regional offices prepared the risk pathways, collected and analyzed necessary data and estimated the risk for each pathway under the guidance of two international experts. An electronic audience response system was used to record individual risk estimates and measure uncertainty.

Results

The participants clearly assigned different levels of risk to different pathways. Very high risk was associated with exposure through direct contact between livestock and with spreading of infection before first detection through livestock trade and mixing of herds on pasture. High uncertainty mostly existed in the entry assessment and in the assessment of effectiveness of outbreak control measures. Participants concluded by providing valuable policy recommendations.

Discussion

The participants maintained a high level of interest throughout the entire process. The electronic audience response system allowed capturing individual risk estimates, and the level of disagreement among participants was used as a proxy to measure uncertainty. The Western region of Mongolia has experienced very few outbreaks and this was reflected in high levels of uncertainty in the assessment of entry and effectiveness of control measures. During a national FMD conference in spring 2014, the results and policy recommendations of the risk assessment were used to redefine the Mongolian FMD control strategy.

notes

PIG, CATTLE AND BUFFALO VALUE CHAIN AND SOCIAL NETWORK ANALYSIS IN XAYABURY PROVINCE OF LAO PDR

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Introduction

Foot and Mouth Disease (FMD) is endemic in the Lao People's Democratic Republic (Lao PDR) and the provinces of Xayabury, Champasak and Vientiane Capital are considered as FMD high risk areas. A self assessment of the progressive control pathway for FMD (PCP-FMD) in 2012 showed that Lao PDR was validated as PCP stage 1 with remaining knowledge gaps about FMD hotspots and risk factors along livestock value chains. Therefore a study was conducted in a high risk area for FMD, Xayabury Province, to determine economic drivers and risk factors influencing FMD transmission.

Materials and methods

A total of 484 stakeholders were interviewed for the value chain study by using structured questionnaires for producers and traders. Social network data was subsequently collected by interviewing a total of 189 stakeholders with questionnaires. Descriptive statistical and social network analysis tools were used.

Results

The value chain study found that the main sources of traded livestock is from small holders. About 71% of pigs are kept in traditional production systems. Native cattle and buffalo are kept in a free-ranging system on public land and forests. Most of the producers (59%) keep only cattle while 11% keep only buffalos and 30% keep both cattle and buffalo. Livestock keepers perceived Classical Swine Fever, diarrhea in piglets, Hemorrhagic septicemia (HS) and FMD as the major health related problems for cattle and buffalo production. The livestock value chain consists of three actors: livestock collectors, livestock buyer-slaughter-meat sellers and importers. Most of the animals are slaughtered at slaughter points and the carcasses transported to fresh markets. Traders use vans to transport on average about 3-4 heads of cattle or buffaloes and 6-8 pigs per one load.

Logistic regression analysis reveals that nodes experiencing HS were associated with FMD. The average number of in-degree and out-degree centrality is only 1.4 which means there are few links for animal moving in or out among nodes. Nodes with the highest value of indegree centrality were animal collectors who were also involved in livestock raising and slaughtering. One giant component with 297 nodes was identified.

Discussion

The movement of breeds, animal feeds, infected animals and traders were considered as multiple risk factors for FMD transmission. The structure of the giant network component suggests that veterinary authorities focusing control measures on several important nodes would be more cost- and time- effective than a blanked approach. Nodes with high degrees of betweenness and centrality should be focused for disease surveillance. These nodes are potential hubs of FMD spread. In case that the disease would be detected early, egocentric tracing from the infected nodes and ring vaccination from the nodes would appear to be effective.

Acknowledgement

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notes

ISOLATION AND IDENTIFICATION OF FMDV TYPES AND ITS SEQUENCE ANALYSIS ON THE BASIS OF *P1* (CAPSID PROTEIN GENE) IN PAKISTAN

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Introduction

Foot-and-mouth disease (FMD) is an infectious, highly communicable disease of cloven footed animals with huge economic impacts golbally. The disease is endemic in Pakistan incurring great economical losses (direct and indirect) to the country's livestock industry. Indeed, most phylogenetic analyses of FMDV are done on the basis of the VP1 gene. Now it has been established that sequences of P1 are providing a more reliable picture of phylogenetic analysis due to the presence of other factors responsible for the production and pathogenesis of FMDV.

Materials and methods

Swab and tissue samples (n=80) from bovines were collected from eight outbreaks of FMD that occured in four different districts of Punjab province, Pakistan. Samples were subjected to conventional reverse-transcription PCR (RT-PCR) and real-time RT-PCR (rRT-PCR) for confirmation of presence of FMDV. A new universal FMDV LPro-P1 RT-PCR and sequencing method (Xu, et al., 2012) was employed to obtain the complete sequnce of the capsid coding P1 region of FMDV isolates. Phylogenetic analysis of the sequences was carried out in MEGA *5.0.*

Results

The amplified products (*P1*) of these samples were sequenced and three distinctive sequences (two for type O and one for type A) were identified and submitted to the NCBI GenBank database. A list of forward and reverse primers was used for sequencing and P1 coding region sequences were awarded accession nos GU384684, GU384685 and GU384686. Phylogenetic analysis on the basis of *P1* sequences revealed close relation of these viruses to previous strains of FMDV O and A circulating in this area.

Discussion

Pakistan's livestock is facing FMD outbreaks through out the year with different frequencies. Phylogenetic analysis of the sequences of isolated FMDV from outbreaks occuring throughout Pakistan revealed that the same viruses with minor nucleotide changes are responsible for recurring disease outbreaks.

Reference

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PROMOTING A RISK-BASED STRATEGY PLAN FOR FMD CONTROL IN INFECTED COUNTRIES: DEVELOPMENT AND EXAMPLES FROM 4 COUNTRIES

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Introduction

In the framework of the Progressive Control Pathway for Foot-and-Mouth Disease (PCP-FMD), countries are required to develop an FMD control plan targeting high risks for FMD virus transmission and situations where FMD has great impact on livelihoods and livestock acknowledging that resources are limited. Upon request from the FAO/OIE Working Group, EuFMD has developed a template for such risk-based strategy plan (RBSP) based on concurrent facilitation of RBSP development in a range of countries.

Materials and methods

Using the principles of the logframe approach and related project management approaches, guidelines (and a template) on RBSP were written in 2013. Through a series of workshops, the RBSP template was used to guide qualitative risk-assessments. The outcomes of such assessment, were used for developing component objectives for the RBSP which were subsequently elaborated into tactics and activities as these from key elements of the RBSP.

Results

Workshops were conducted in Iran, Turkey, Georgia, Nepal, Palestine and Libya, resulting in definition of so-called risk-hotspots and risk pathways, and allowing for the development of the component objectives of the RBSP. Guidelines and template are now included as integral parts of the PCP-FMD review for countries to move from PCP-FMD Stage 1 to Stage 2 and slightly adapted versions are currently being developed as guidelines for countries wanting to more into Stage 3.

Discussion

In this presentation we will provide examples of identified risk hotspots and subsequently defined component objectives. Between countries there are recurrent risk hotspots (animals movements between provinces, incursion of new strains from neighbouring countries) and also recurrent needs for strengthening the capacity of the competent authority on disease management issues (monitoring & evaluation, need for data management and epidemiology to inform evidence-based policy making).

notes

EPIDEMIOLOGICAL ANALYSIS OF FMD OUTBREAKS IN SOUTH-EAST ASIA AND CHINA (2010-2014)

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Abstract

The South-East Asia and China belongs to FMD virus pool 1, as classified by the Global network of OIE/FAO FMD reference laboratories. Present in this pool are serotypes O, A and Asia 1. For serotype O, three topotypes have been identified – Myanmar 98, Panasia and Cathay. For serotypes A and Asia 1, no distinction of topotypes was classified.

There are significant epidemiological changes in the circulation of FMD viruses in South-East and China over the last four years. During the second semester of 2010 until the first quarter of 2011, increase number of outbreaks of FMDV serotype O was reported in Cambodia, Vietnam, Lao PDR and China. Most of the isolates from these outbreaks were classified as Panasia O, although a few Myanmar 98 were also isolated. This is a significant change from previous outbreaks, such as the 2006 epizootics wherein the predominant topotype was Myanmar 98. This could be considered a recurrence of Panasia O after it caused epizootics in the region in 1999 and 2000.

A significant change in the epidemiology of serotype A was observed in 2013. While serotype O has been predominant the FMDV isolates prior to 2102, this was overtaken by serotype A in 2013. In 2012, 8 % of the FMD outbreaks were due to serotype A, 29% due to serotype O and 63% were untype. But in 2013, 33 % were due to serotype A and 28% were due to serotype O. From January to June 2014, the initial data shows that serotype A is 21 % and serotype O 18%. All the rest are untype or no samples were submitted to the laboratory.

There were observations of a possible change in the antigenic structure of the current serotype A virus circulating in the region. Thailand found that 16 isolates of serotype A collected from March to December 2012 did not strongly match with the A/Sakolnakorn/97 vaccine strain. However, 14 of these isolates have r-values greater than 0.4 when matched with a new serotype A Lopburi 2012 strain. China found that the serotype A 2013 isolates is slight different from serotype A that caused outbreaks in 2009, hence they classified it as serotype A Generation 2.

In terms of spatial distribution, an increase of serotype A was observed in Thailand, Vietnam and China. All the 2013 serotype A isolates from these countries were genetically closely related.

No serotype Asia 1 was isolated since 2010. The last outbreak of serotype Asia 1 was reported in China in 2009.

notes

DEVELOPMENT OF PROBE-BASED REAL TIME RT-PCR ASSAYS FOR DETECTION AND SEROTYPING OF FMDVs CIRCULATING IN WEST EURASIA

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Introduction

Rapid and accurate diagnosis of foot-and-mouth disease and virus typing is of paramount importance for control of the disease in endemic areas where vaccination is used. Ideally this virus characterization should be performed without the need for virus amplification in cell culture. Due to the heterogeneity of FMDVs in different part of the world, region specific diagnostic tests are likely to be required. This study builds on the use of such systems described previously (Reid et al., 2014; Ahmed et al, 2012).

Materials and methods

Hydrolysable probe-based real time reverse transcription polymerase chain reaction (rRT-PCR) assays were developed for specific detection and serotyping of FMDVs currently circulating in West EurAsia. These assays were evaluated using field samples (both epithelial and oral swabs) belonging to different sublineages within the O-PanAsia, A-Iran05 and Asia-1 Group- VII/Sindh-08 viruses originating from Pakistan and Afghanistan.

Results

All the three primer/probe sets, each designed to be specific for serotypes O, A and Asia-1 FMDVs, detected the RNA from homotypic viruses with cycle threshold (Ct) values comparable to those obtained with serotype-independent assays targeting the 3D and/or 5' UTR genome fragment. No cross-reactivity was observed with heterotypic viruses circulating in the region.

Discussion

FMD is endemic in the majority of the countries within the West EurAsian region. Serotypes O, A and Asia-1 FMDVs are circulating within this region, all belonging to pool 3 viruses. Spread of the disease from East towards the West within West EurAsia is well established involving migration of viruses from Pakistan/Afghanistan towards the Middle East and Europe reaching Turkey and Bulgaria (Jamal et al., 2011a,b,c; Valdazo-González et al., 2012; Brito et al., 2012; Jamal et al., 2013). The present study describes the development of serotype-specific rRT-PCR assays which can help in the early detection and typing of FMDV serotypes O, A and Asia-1 circulating in West EurAsia.

notes

DEVELOPMENT AND EVALUATION OF A MULTIPLEX CONVENTIONAL 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 the outbreaks of serotypes O, A, SAT1 and SAT2 have been reported. Early diagnosis of FMD is crucial for implementation of the adequate outbreak management. This study describes the development of a multiplex conventional RT-PCR for both detection and typing of these occurring serotypes.

Material&Methods

RT-PCRs were developed by using primer sets targeting 3D, VP1 (O/A/SAT1/SAT2-specific) and β-actin genes. These sets of primers were designed to give the following amplicon sizes: A(600bp)/O(500bp)/SAT1(400bp)/SAT2(250bp)/β-actin(186bp)/3D(100bp). Eight FMDV strains namely: O/BEN/1/2010, O/BEN/26/2010, A/BEN/19/2010, A/BEN/36/2010, SAT1/KEN/2/2011, SAT1/BOT/1/68, SAT2/LIB40/2012, SAT2/EGY3/2012 and two negative, porcine and bovine epithelial suspensions were used to develop and evaluate multiplex RT-PCRs. The 6-plex prototype was then tested with additional FMDV strains and 20 positive/negative field samples.

Results

Primer sets were first assessed in simplex protocols. The intermediate triplex/4-plex protocols such as: O/A/3D, SAT1/SAT2/3D, O/A/3D/β-actin, SAT1/SAT2/3D/β-actin, and O/A/SAT1/SAT2 were evaluated. All of them were specific for tested panels. The 6-plex (O/A/SAT1/SAT2/3D/β-actin) detected all targets with none improper amplifications and it's specific towards to other FMDV strains. It detected and typed indeed successfully FMDV positive field samples and confirmed the negatives as well.

Discussion&Conclusion

We have developed and evaluated a 6plex RT-PCR allowing both detection and typing of FMDV strains. We will further validate this method on a larger panel of field samples. This multiplex conventional RT-PCR developed for rapid molecular detection of O, A, SAT1 and SAT2 serotypes of FMDV in West Africa is a promising method that can be used for early detection. The conventional RT-PCR method is well known, and moreover doesn't need expensive laboratory equipment and reagents. It could be thus easily implemented in diagnostic laboratories in developing countries, providing an improvement for rapid detection and typing of FMDV strains.

notes

DEVELOPMENT OF TAILORED SPECIFIC REAL-TIME RT-PCR ASSAYS FOR DETECTION OF FMDV SEROTYPES A, O, SAT 1 and SAT 2 CIRCULATING IN EAST AFRICA

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Introduction

Foot-and-mouth disease is endemic in countries of East Africa with four serotypes (O, A, SAT 1 and SAT 2) causing regular outbreaks of the disease. To facilitate identification of currently circulating virus serotypes a set of real-time RT-PCR assays each detecting a specific FMDV serotype was designed and validated. The assays were developed primarily to identify FMDV strains circulating in Uganda, Tanzania and Kenya.

Methods

VP1 sequence data, including historical and current strains of FMDV circulating in East Africa, were aligned and analysed and conserved regions were identified as targets for individual serotype-specific assays. Multiple primers and probes for each of the assays were designed according to TaqMan specification and tested for diagnostic sensitivity and performance. Best assay candidates were selected and validated with a panel of field samples at the Pirbright Institute, UK and the Technical University ofDenmark.

Results

Five different serotype-specific RT-PCR assays for detection of FMDV strains circulating in East Africa were designed; each to detect FMDV serotype O, A and SAT 1 and two respective assays to cover genetic variability within SAT 2 viruses. These assays were shown to correctly identify FMDV serotypes in a panel of field and tissue cultured samples originating from East Africa. Mixed serotype samples could also be identified using the system. In addition, FMDV RNA positive samples which could not be propagated in tissue culture were investigated and, in many cases, the serotype of the samples could be determined. All assays developed have been shown to be of a similar efficiency to the “gold standard” 3D assay (Callahan at al., 2002).

Discussion

A set of real-time RT-PCR assays was developed able to determine the serotype of FMD viruses circulating in East Africa, particularly in Tanzania, Kenya and Uganda. This set of assays can aid studies on molecular epidemiology of FMDV and help to inform FMDV control policy in

the region. The system could be also adapted to field diagnostic platforms aiding transfer of technology and use in the countries concerned.

notes

REAL-TIME RT-PCR FOR THE RAPID DETECTION OF FMDV IN MILK

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Introduction

There is a need for rapid diagnostics and surveillance plans to enable early detection of FMDV in cattle. FMDV detection in milk presents unique opportunities to use non-invasive sampling for surveillance and early detection both prior to and during a FMD outbreak. The aim of this study was to evaluate the effectiveness of preclinical indicators of FMDV infection and to evaluate a high-throughput screening tool using real-time(r)RT-PCR which could be scaled up for use with bulk tank milk.

Materials and Methods

Samples were collected from experimentally infected cattle to evaluate the diagnostic window of detection in milk and to gauge the impact of FMD infection on milk production. Four Jersey dairy cows were infected via direct contact with two cattle that had been inoculated with FMDV (PanAsia strain, O/UKG/2001). Diagnostic tests were carried out in real time on fresh samples collected over 28 days. Virus isolation and routine pan-serotypic rRT-PCR targeting 3D used in the WRLFMD were compared with new optimised rRT-PCR protocols for milk.

Results

FMDV was detected in milk before the onset of characteristic clinical signs. The greatest window for virus detection was by rRT-PCR in the milk up to 21 days post contact and both rRT-PCRs detected virus for a longer period than seen in virus isolation. In addition to the *in* vivo study described, samples collected from field cases (including 12/13 milk samples from the UK 2007 outbreak) were used as a positive cohort to evaluate diagnostic sensitivity.

Discussion

The data implies that rRT-PCR of milk from a bulk tank in a large herd could detect a single infected cow in the early stages of infection. This suggests that milk could be an excellent sample type for the detection of FMDV and could be used for the development of a national FMD surveillance plan in the event of an outbreak.

notes

REALISING THE POTENTIAL OF SIMPLE ISOTHERMAL MOLECULAR TOOLS FOR FIELD DIAGNOSIS OF FOOT-AND-MOUTH DISEASE

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Introduction

Accurate, timely diagnosis is essential for control, monitoring and eradication of foot-and-mouth disease (FMD). Currently, samples are tested at reference laboratories, a lengthy process which delays critical decision making. Reverse transcriptase loop-mediated isothermal amplification (RT-LAMP) provides a realistic option for rapid, sensitive, *in situ* detection. Here we describe the development of a robust "field-ready" lyophilised RT-LAMP assay, compatible for use with clinical samples without the need for RNA extraction.

Materials and Methods

A concordance study was undertaken using existing foot-and-mouth disease virus (FMDV) RT-LAMP primers combined with commercially available RT-LAMP master mixes (wet and lyophilised). Analytical sensitivity was defined using fluorescence and molecular LFDs and compared to rRT-PCR. Clinical samples from experimentally and naturally infected animals were used to develop simple sample preparation methods, enabling direct RT-LAMP to be performed on the optimised assay. The final RT-LAMP protocol and associated lyophilised reagents were field tested in an endemic setting combining the Genie® II platform (OptiGene, UK) and visualisation on molecular LFDs.

Results

Analytical sensitivity of the lyophilised RT-LAMP was equivalent to the wet reagents and rRT-PCR. Robust sample preparation methods for serum, oesophageal-pharyngeal fluid and epithelial suspensions were developed to negate the need for RNA extraction prior to RT-LAMP. When trialled in a field setting, the lyophilised RT-LAMP assay detected active and persistent FMD infection consistent with rRT-PCR.

Discussion

Robust RT-LAMP protocols have been developed for lyophilised reagents, reducing difficulties associated with field deployment. The ability of RT-LAMP to utilise simple sample preparation, amplification and detection methods offers promise for rapid *in situ* FMD diagnosis.

notes

DEVELOPMENT AND EVALUATION OF MULTIPLEX REVERSE TRANSCRIPTION LOOP MEDIATED ISOTHERMAL AMPLIFICATION ASSAYS COMBINED WITH LATERAL-FLOW VISUALISATION FOR THE DISCRIMINATION OF FOOT-AND MOUTH-DISEASE FROM OTHER VESICULAR DISEASES

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Introduction

Foot-and-mouth disease (FMD) Virus (FMDV) is an economically important, highly contagious picornavirus that affects both wild and domesticated cloven hooved animals. Because of the clinical similarity of FMD to other vesicular diseases such as Swine Vesicular Disease (SVD) and Vesicular Stomatitis (VS) there is a pressing need for rapid, sensitive and specific differential diagnostic assays that are suitable for decision making in the field. Singleplex reverse transcription loop mediated isothermal amplification assays (RT-LAMP) combined with lateral-flow visualisation (RT-LAMP-LFD) have been developed for vesicular diseases such as FMD and SVD but there are currently no available multiplex RT-LAMP-LFD assays which would permit rapid discrimination between 'look-a-like' less economically important diseases *in situ*.

Materials and Methods

The objectives of this study were i) to develop two multiplex RT-LAMP assays combined with molecular lateral-flow detection RT-LAMP-LFD); one to detect FMDV and/or SVDV and the other to detect FMDV and/or VSV, ii) to evaluate these assays against the equivalent real time RT-PCR assays and iii) to develop simple sample preparation methods compatible with field use.

Results

The limit of detection of both multiplex assays was demonstrated to be equivalent to that of the equivalent laboratory based real time RT-PCR assays when visualised using fluorescence of molecular LFD. Importantly, this study demonstrated that FMDV, SVDV and VSV RNA could be reliably distinguished from a range of epithelial suspensions without the need for prior RNA extraction.

Discussion

This study describes an approach that could be used as the basis for a rapid and low cost assay for differentiation of FMDV from other less economically important vesicular disease viruses in the field.

notes

FROM SEQUENCE TO PREVALENCE: PHYLODYNAMICS OF FOOT-AND MOUTH DISEASE VIRUS

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Introduction

RNA viruses, such as foot-and-mouth disease (FMD) virus (FMDV), are characterised by high mutation rates, large population sizes and short generation times, which means that epidemiological and evolutionary processes occur on a similar time scale. Modern coalescent theory enables viral demography to be reconstructed from whole genome sequence data and, therefore, suggests that sequence data might be used to better understand the epidemic and endemic dynamics of FMD. The UK 2001 FMD outbreak provides an excellent opportunity to explore how to integrate both epidemiological and genetic data, because the outbreak was observed closely epidemiologically (the temporal patterns in incidence and prevalence) and a complete genetic dataset will become available (through whole genome sequencing of viruses isolated on infected farms) in the near future. Here analyses of both data types have been developed and inferences from viral genome and space-time incidence data compared with each other.

Materials and Methods

Epidemiological data derived from the UK 2001 FMD outbreak were used to reconstruct infectious periods for each infected premise (IP) by means of a space-time kernel-based approach developed to reconstruct transmission trees relating who-infected-who. The transmission trees were then used to simulate an FMDV genome sequence for each IP using a discrete-time Markov chain model. A Bayesian analysis framework was then employed on the simulated sequence alignment to estimate the effective population size () through the Bayesian skyline plot, and then testing the results by comparison to the observed incidence and prevalence data.

Results

Transmission trees comprising 2026 IPs were reconstructed from the space-time incidence data. The average generation time () of an IP was estimated as 7.2±2.7 days. Using a molecular clock rate estimated from real FMD virus (FMDV) isolates (*n*=39) collected during the UK 2001 FMD outbreak of 2.37×10^{-5} nt/site/day (95%HPD 1.99×10^{-5} - 2.74×10^{-5}), the average number of nucleotide substitutions per transmission link reported for the full-simulated data was 1.3±1.2 nucleotides. Re-analyses of the clock rate from simulated data closely matched the input value as should be expected. The dynamics of the effective population size of the viral population, as predicted by the Bayesian skyline plot, closely reflected that of the epidemic curve, capturing the exponential increase period, the timing of the peak and the decrease period of the outbreak. However, no simple transformation of the effective population size accurately

predicted the observed peak incidence or prevalence, with the simple effective population size underestimating both incidence and prevalence.

Discussion

The model developed for reconstructing the UK 2001 FMD transmission tree and simulating the corresponding genetic component was able to capture the crude dynamics of the outbreak. Parameters estimated from the simulated data matched those extracted from the UK 2001 FMDV field isolates and previously published, lending confidence to the consistency of the modelling process. The demographic analysis of the simulated alignment has shown a correlation with the UK 2001 FMD epidemic size, although the population size was underestimated. The study shows that sequence data can be used to obtain understanding of epidemiological dynamics and this approach will be modified in future to examine the most appropriate transformation to map effective population size to prevalence, and anticipate the biases most likely to be encountered in working with partially-observed genetic data sets.

notes

BEYOND THE CONSENSUS: INVESTIGATING INTRA-HERD VARIABILITY OF FOOT-AND-MOUTH DISEASE VIRUS USING THE ILLUMINA MISEQ

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Key words – Next generation sequencing, beyond the consensus, Foot and mouth disease virus.

Background

RNA viruses such as foot-and-mouth disease virus (FMDV) exist as genetically heterogeneous populations known as 'quasispecies' comprising of closely-related but non-identical genomes. Such complexity is due to their high replication rate, large population size and error-prone replication. With current polymerase error rate estimates, it can by hypothesised that at least one nucleotide change occurs in each genome during each transcription event. While consensus level sequencing identifies dominant viral species, minor variants remain uncharacterised; the importance of which remains unclear with relation to both transmission and quasispecies evolution. We used the Illumina MiSeq platform to deep sequence the population of viruses within a herd of animals, from a single premises, during the UK 2007 outbreak.

Materials and Methods

Twenty milligrams of epithelial tissue was used to produce an original suspension (OS). Total RNA was extracted using the RNeasy mini kit (QIAGEN) from each OS and tested for presence of FMDV using qRT-PCR. Of those samples positive for FMDV, total RNA was converted into cDNA using gene specific primers. A novel long-PCR employing a high fidelity polymerase was optimised to amplify the FMDV L-fragment. PCR products were purified and sequenced on the Illumina MiSeq using the Nextera XT protocol.

Results

All eight samples yielded viral loads between 2 x 10^3 and 5 x 10^5 viral copies/µl. All samples tested with long-PCR produced a 7.6 Kb band and were sequenced in duplicate using the MiSeq. Analysis revealed the consensus sequence for each of the samples, with coverage between 6.45 x 10^2 and 1.13 x10^4 across the L-fragment. Further analysis will be performed investigating; entropy, substitutions and SNPs. Data will help characterise polymorphism frequency, mutational spectra and determine the evolutionary dynamics of minority variants

Discussion

Findings will provide an important insight to the strategies for reconstructing patterns of transmission and monitoring future outbreaks.

notes

PROBABILITY OF INFECTION OF CATTLE, SHEEP AND PIGS EXPOSED TO FOOT-AND-MOUTH DISEASE VIRUS AEROSOLS

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Introduction

Aerosol transmission of foot-and-mouth disease virus (FMDV) has been given extensive consideration. Yet the likelihood of susceptible animals (cattle, sheep or pigs) exposed to different amounts of virus-carrying air becoming infected has not been fully quantified. The objective of this study was to quantify the relationship between the virus-aerosol dose and the probability that a susceptible animal becomes infected and develops clinical signs of FMD.

Materials and methods

Published and unpublished experiments, where cattle, sheep and pigs were exposed to FMDV aerosols delivered via a face-mask were reviewed and their data collated in a database. These data was used to quantify the dose-response relationship between the amount of inhaled virus (expressed as $TCID_{50}$) and the probability of subclinical-infection and of disease. In addition, the effect of virus serotype on the dose-response relationship and the length of the incubation period (*IP*) as a function of dose and serotype were also estimated.

Results

Dose-response curves for cattle, sheep and pigs were generated. A lower dose is required to cause a subclinical-infection than that required to cause disease. The probabilities (95% confidence intervals) of infection per virus $TCID_{50}$ were 0.027 (0.016 – 0.045), 0.024 (0.012 – 0.044) and 0.0003 (0.0001 – 0.0007) for cattle, sheep and pigs respectively. The probabilities of disease were 0.005 (0.0030 – 0.009), 0.006 (0.003 – 0.011) and 0.0001 (0.0000 – 0.0003) for cattle, sheep and pigs respectively. With this information, the median infectious dose that leads to infection or disease was also quantified. The length of the *IP* was dependent on virus serotype and dose.

Discussion

The dose-response and infectious period after aerosol challenge was quantified. This information could be used for: 1) risk assessment of airborne transmission, 2) improving our understanding of the transmission mode of FMDV and 3) use of aerosol-challenge for experimental studies including potency tests.

notes

A NOVEL PROTOCOL TO GENERATE CONSENSUS LEVEL GENOME SEQUENCES FOR FOOT-AND-MOUTH DISEASE VIRUS AND ITS APPLICATION TO SEQUENCING A LARGE OUTBREAK

Graham Freimanis[1], Grace Logan[1], David King[1], Begoña Valdazo-González[1], Katarzyna Bachanek-Bankowska[1], Richard Orton[2], Nick Sanderson[1], Nick J. Knowles[1], Dan Haydon[2], Eleanor Cottam[1], Donald King[1] and the Epi-Seq consortium

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Background

Next Generation Sequencing (NGS) has facilitated the adoption of whole genome sequencing (WGS) in both diagnostic and epidemiological settings for a variety of viral pathogens. Previous sequencing protocols have been subject to biases such as those encountered during PCR amplification and cell culture or restricted by the requirement for large quantities of starting material. We have developed a simple and robust methodology for the generation of whole genome sequences on the Illumina MiSeq and adapted this protocol to process large numbers of samples in a high throughput environment and validated it using samples submitted during the UK 2001 FMDV outbreak.

Materials and Methods

Tissue samples were used to produce original suspensions in lysis buffer using the Fastprep bead-mill (MP). Total RNA was extracted from original suspensions and subjected to DNase treatments. cDNA was synthesised using a combination of random and specific priming. Libraries for sequencing were produced using the Nextera XT kit (illumina). Subsequent reads were trimmed and aligned using Bowtie2.

Results

WGS of FMDV was completed from samples down to a viral load of 10^7 virus copies/µl. The protocol was successfully validated using six FMDV positive clinical samples from the 2001 epidemic in the United Kingdom, in addition to a panel of representative viruses from all seven serotypes. Genome sequences from three other non-FMDV polyadenylated RNA viruses (EMCV, ERAV, VESV) were also obtained with minor protocol amendments. This workflow was then applied to samples from the UK 2001 archive in which data was used to produce consensus level sequences for each IP allowing mechanisms of virus evolution to be investigated.

Discussion

We have developed a universal WGS method for FMDV and other polyadenylated RNA viruses. This method works successfully from a limited quantity of starting material and eliminates the requirement for genome-specific PCR amplification.

notes

NON-INVASIVE SAMPLING SYSTEMS FOR THE DETECTION OF FMDV IN WILD BOAR

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Introduction

While wild boar have not been regarded as an important reservoir for FMDV in the past, wild boar played an important role in the outbreaks in Bulgaria 2011 and the wild boar density in several parts of Europe is much higher nowadays than during most of the previous large FMD epidemics. Two recent FMDV infection experiments in wild boar published by Mohamed et al. (2011) and Breithaupt et al. (2012) provide further evidence that wild boar have a significant potential to spread the disease as infected animals may shed virus at high titers before clinical signs become apparent. Even after vesicles have developed on their feet, mobility may not be significantly impaired. Adequate surveillance schemes which facilitate early detection of FMD in the wild boar population are an essential basis for risk assessment and risk control measures in ruminants and domestic pigs. However, collection of a statistically significant number of samples for early disease detection is dependent on hunting activities which in Europe are seasonally limited. Therefore, we developed a non-invasive sampling method ''pSWAB'' (pathogen sampling wild animals with baits) and tested it´s suitability to detect FMDV infection in wild boar (Mouchantat et al. 2014).

Materials and Methods

Two animal experiments were carried out. In the first experiment, five ten-months-old wild boar weighing about 70 kg and in the second experiment, five domestic pigs of about 30 kg were used. In both trials, all animals were kept in the same stable. Two animals (donors) were inoculated into the bulb of the heel with FMDV isolated from a wild boar in Bulgaria in January 2011 (O/BUL/1/2010) while the other animals were infected by contact.

The pSWABs were produced in the form of a standardized product by embedding a 10 cm long cotton rope in a cereal-based bait matrix. Baits based on maize ears were produced by replacing 6 kernels by cotton swabs (Q-tips®). While in the first experiment, pSWABS were compared with saliva samples taken individually from sedated animals. In the second experiment, pSWABS and sampling baits based on maize ears were used. Furthermore blood samples were taken about twice a week. RNA was extracted from all samples using standard commercial products and the viral RNA load was determined by a FMDV specific real-time reverse transcription PCR (RTqPCR) (Callahan et al., 2002). Some of the wild boar samples were also tested by virus isolation (VI) in cell culture.

Results

Viral RNA was detectable in pSWABS from infected wild boar between 1 dpi and 23 dpi and in pSWABS from infected domestic pigs between 1dpi and 9 dpi (afterwards the last domestic pig

had to be euthanized for humane reasons). Viral RNA in pSWABs was identified already 24 h after infection during the incubation period. Generally, results obtained with pSWABS, maize ear swabs and conventional saliva samples were similar. However, usually the cotton ropes inside pSWABS were chewed by several animals and therefore have to be considered as collective sample of the group. In pSWABs from taken during the first days after infection and tested by VI, high viral loads ($10^{4.5}$–$10^{5.0}$ TCID50 per 50 ml) were detected.

Discussion

While non-invasive sampling of wild boar has been attempted before using baits based on ears of maize (Khomenko et al., 2013), such improvised baits could hardly be produced commercially and the tiny Q-tips® may be difficult to collect from the mud or even be eaten by larger pigs. In our trials, it was demonstrated that FMDV genome can be isolated from infected wild boar with comparable sensitivity using pSWABs, facilitating a cheap sample collection system that can be commercially produced and is independent of hunting activities. Further studies are needed to evaluate the practical use of pSWABs in the field, particularly related to the distribution of pSWABs and collection of the ropes. Distribution and collection might be dependent on different factors which have to be considered, e.g. group size, landscape or climate. Investigations on the impact of time lag between chewing and sample collection, temperature and environmental conditions are ongoing.

notes

PERFORMANCE OF DIAGNOSTIC TESTS FOR FOOT-AND-MOUTH DISEASE IN RED DEER.

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Introduction

During an incursion of FMD into New Zealand, a country with 1.1 million farmed red deer, testing of large numbers of deer samples would potentially be required. Currently, the accuracy of available diagnostic tests for detecting FMD in red deer is unknown.

Materials and methods

In a collaborative research project between the National Centre for Foreign Animal Disease, Winnipeg, Canada, and the Ministry for Primary Industries in New Zealand, the following FMD test methods were evaluated: 3D and IRES rRT-PCR, four commercial NSP antibody ELISAs, three commercial serotype O antibody ELISAs and an antibody penside test. Sensitivity of these test methods was determined by experimentally inoculating ten red deer with a serotype O FMD virus, regularly collecting samples over four weeks, and testing the samples in the various test methods. Virus isolation, virus neutralization test and two in-house ELISAs were used as reference tests. Specificity of the test methods was determined by using samples from 950 non-infected New Zealand red deer.

Results

Of 10 experimentally infected red deer, one developed generalized infection and clinical disease. Six animals gave a strong antibody response after re-inoculation, without clinical signs. RT-PCRs performed as expected, detecting infected animals sensitively and with 100% diagnostic specificity. Of the seven commercially available antibody ELISAs, only some performed satisfactory.

Discussion

Details of the project and detailed performance characteristics of the FMD diagnostic test methods will be presented and discussed.

notes

EVALUATION OF TWO AIR SAMPLERS FOR THE DETECTION AND QUANTIFICATION OF AIRBORNE FOOT AND MOUTH DISEASE VIRUS

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Introduction

Air samplers, especially liquid impingers, have been commonly used to quantify Foot-and-mouth disease virus (FMDV) in aerosols from infected animals. However, the efficiency of virus collection and recovery can vary between different types of samplers and different sample extraction protocols. This study aimed to develop laboratory methods of known efficiency for the recovery and quantification of sampled virus from the collection plates of two samplers; the AirScan MD8 (Sartorius) and the Electrical Low Pressure Impactor (ELPI$^+$, Dekati).

Materials and methods

Two aerosol collection plates were evaluated: 1) gelatine filters, which filter particles from air drawn through the AirScan MD8 and 2) aluminium foils, which collect particles by impaction from air drawn through the ELPI$^+$.

Collection plates were spiked with decreasing concentrations of FMDV O UKG 34/2001. M25 phosphate buffer, PBS and EMEM were tested over a range of volumes, from 5ml to 20ml, using qPCR and virus isolation to assess their ability to isolate virus from the collection plates.

Results

AirScan filters: Gelatine filters have an acid pH, which prevents isolation of live virus from samples other than at high levels (>10^5 $TCID_{50}$). The volume of buffer used to dissolve the filters had a significant effect on the amount of virus detectable by PCR. Reliable qPCR results were achieved using 10ml of EMEM. The limit of detection by PCR, using EMEM, was 10^2 $TCID_{50}$.

ELPI foils: EMEM and PBS were better than M25 for recovering virus/genome from the collection foils. However, live virus was only detected when EMEM was the eluting buffer. The limit of qPCR detection using this buffer was 10^3 $TCID_{50}$.

Discussion

This work provides standardised laboratory methods for detection and quantification of FMDV from two air samplers. The information on the limits and accuracy of these methods need to be considered for data analysis.

notes

EVOLUTION OF FOOT-AND-MOUTH DISEASE VIRUS DURING PERSISTENCE IN AFRICAN BUFFALO

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Introduction

The near-symbiotic association between foot-and-mouth disease virus (FMDV) South African Territories (SAT) serotypes and African buffalo (*Syncerus caffer*) is unique and possibly represents the evolutionary base from which other FMDV lineages evolved and adapted to domestic livestock. African buffalo are considered the primary maintenance host for the SAT serotypes and present a considerable barrier to FMDV control or eradication in Africa. Following acute infection, most buffalo become carriers and it has been assumed that transmission from carriers is responsible for ensuring FMDV persistence in buffalo populations. However, most attempts at demonstrating transmission between carrier and susceptible buffalo or cattle under experimental conditions have been unsuccessful. The objective of this study was to explore the evolutionary mechanisms that may contribute to FMDV persistence and transmission during the carrier phase in African buffalo.

Material and Methods

We established sixteen FMDV-carrier buffalo that were inoculated with SAT serotypes and maintained for 400 days at Kruger National Park (South Africa). Probang and serum samples were collected one a month. Four buffalos were killed at 1, 3, 6 and 12 months post-infection. Laser micro-dissected (LMD) Germinal Centre (GC) and Epithelium (Epi) samples were screened by cloning and massive sequencing to determine the spectrum of virus isolates detected during the acute and carrier phases in several lymphoid tissues from slaughtered animals: Pharyngeal Tonsil (PhT), Palatine Tonsil (PtT) and Dorsal Soft Palate (DSP). Besides, sera from different time points were analysed with homologous and heterologous virus neutralisation (VN) assays and the changes in the virus were tracked with Sanger sequencing.

Results and Discussion

Positive results from LMD were obtained from all analysed tissues, individuals and time points. Nucleotide diversity estimations from GC and Epi showed comparable levels of diversity. Population analyses showed mixed results at 35Dpi, with increasing levels of differentiation at later time points. Homologous and heterologous VN assays reported differential results, though all buffalos were protected against FMD. Several marker positions differentiated the challenge virus and samples isolated at several time points.

notes

GENETIC CHARACTERISATION OF CIRCULATING FOOT-AND-MOUTH DISEASE VIRUSES FROM AFRICAN BUFFALO (*SYNCERUS CAFFER*) AND CATTLE IN KENYA: EVIDENCE FOR INDEPENDENT VIRUS POPULATIONS

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Introduction

Foot-and-mouth disease (FMD) is endemic in Kenya. Disease control is by vaccination plus animal movement control and is hampered by limited knowledge on the emerging and re-emerging strains plus roles played by different hosts in FMD epidemiology. Wildlife, particularly the African buffalo (*Syncerus caffer*) is a known reservoir for the SAT serotypes that commonly intermingles with livestock, yet previous studies have focused on FMD in domestic livestock. The purpose of this study was to enhance knowledge on the impact of wildlife on FMD in livestock.

Materials and Methods

The study used antigen detection ELISA, reverse transcription-PCR and sequencing to analyse archived epithelium samples from cattle in various parts of Kenya from 1964 to 2013 and probang samples from 102 buffalo in selected wildlife ecosystems in 2012.

Results

Four serotypes (O, A, SAT 1 and SAT 2) were found circulating among cattle but no evidence for a recent occurrence of serotypes C and SAT 3 in cattle and buffalo was found. Analysis of 35 serotype O viruses collected between 2008 and 2013 revealed four independently evolving lineages. Among 38 serotype A field isolates collected between 1964 and 2013, four genotypes within the Africa topotype and a fifth apparently emerging lineage were detected. Analysis of the 102 buffalo samples collected in 2012 found that buffalo harbour SAT 1 and SAT 2 serotypes that were genetically divergent from those found in cattle.

Discussion

Though FMDV serotypes O, A, SAT 1 and SAT 2 were recently found circulating among cattle in Kenya, only SAT 1 and SAT 2 viruses were successfully isolated from buffalo. Control efforts should focus primarily on reducing FMDV circulation among livestock. Exhaustive studies on the roles of buffalo in FMD epidemiology, including more comprehensive sample material and

information, plus deliberate efforts to isolate and characterise FMDVs from buffalo is needed. Continuous disease surveillance and more research to assess the effectiveness of the current FMDV vaccine strains were recommended.

Keywords: *African buffalo, cattle, control, epidemiology, foot-and-mouth disease, lineages.*

notes

MULTIPLE FMDV SEROTYPES IDENTIFIED IN UGANDA DURING 2010-2013

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Introduction

Studies of FMDV in Ugandan cattle during 2006-2009 pointed towards a major outbreak of FMDV serotype O with less significant presence of the SAT serotypes of FMDV. Moreover, although SAT 1 and SAT 2 FMDVs were isolated from the African buffalo (*Syncerus caffer*), it was not possible to establish the role of wildlife, especially these buffalo, in the epidemiology of FMD in domestic animals. Thus, further studies were carried out in Ugandan cattle and buffalo during 2011-2013.

Materials and methods

Blood, swab, epithelial and/or probang samples were obtained from Ugandan cattle in three different surveys: (1) A temporal random survey in two subcounties bordering Queen Elizabeth National park (QENP), (2) A temporal survey of two sentinel herds grazing within QENP during 2012-2013 and (3) samples from investigation of Ugandan outbreaks during 2011-2013.

Sera were assayed for antibodies to the FMDV NSPs, positive sera were tested for serotype specific antibodies by SPBE and sera with high SPBE titres were tested by virus neutralization test. RNA was extracted from the probang samples and analyzed for the presence of FMDV genomes using real time RT-PCR assays (RT-qPCR). FMDV was isolated on BTY cells from probangs, epithelial suspensions or swabs, and analysed by serotype specific antigen ELISA and sequencing.

Results

Isolates of serotypes O, A, SAT 1, SAT 2 and SAT 3 FMDV were obtained from Ugandan cattle, and two different epidemics of serotype O FMDV were proven.

Discussion

The geographical distribution of these viruses, their relatedness to earlier Ugandan and East

African FMDVs and implications for spread between wildlife and cattle are being studied.

notes

IDENTIFICATION OF NOVEL GENOTYPES OF FOOT-AND-MOUTH DISEASE VIRUS RECOVERED FROM AFRICAN BUFFALO IN MARROMEU, MOZAMBIQUE

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Introduction

Foot-and-mouth disease (FMD) is endemic in Africa and impacts upon the productivity and rural livelihoods of farmers in sub-Saharan Africa. The epidemiology of FMD in the region is complicated by involvement of wildlife in transmission and spread of FMD virus (FMDV). African buffalo (*Syncerus caffer*) can also play an important role as a wildlife reservoir of the virus. In order to explore the extent of genetic diversity for FMDV that exists in sub-Saharan Africa, we have analysed samples collected from a recent field survey in livestock-wildlife interface areas around Marromeu National Park in Mozambique.

Methods

Oesophageal/pharyngeal (OP) scrapings and sera collected from cattle and African buffalo around Marromeu National Park (NP) in 2011 were investigated. The investigation was undertaken using NSP and antigen-detection (ag) ELISA, virus isolation, RT-PCR, VP1 sequencing and phylogenetic analysis.

Results

The exposure status to FMDV infection was significantly higher ($P < 0.05$) in buffalo than cattle in the same ecosystem. Ag-ELISA revealed the existence of serotype SAT 1, SAT 2 and SAT 3 from buffalo. The VP1 sequence analysis revealed the closest nucleotide identities to FMD viruses isolated from African buffalo and cattle in southeast Zimbabwe collected during the early 1990's (~16-20% nt identity). Phylogenetic reconstructions of VP1 sequences of 5 isolates collected from buffalo in Marromeu NP provided evidence for the presence of novel lineages for all three FMDV SAT serotypes which were distinct from the topotypes previously described in the region.

Discussion

The presence of significant sequence diversity of these viruses provides an indication of the extent of unsampled FMD viruses that may exist in wildlife populations in other National parks and game reserve across Africa which are epidemiologically discrete from each other. Further studies are required to investigate factors responsible for the occurrence of such genetic diversity and its implication in the epidemiology and control of FMD in endemic settings of Africa.

notes

EMERGENCE OF ANTIGENIC VARIANTS OF SAT2 FOOT-AND-MOUTH DISEASE VIRUSES AT THE WILDLIFE/LIVESTOCK INTERFACE IN SOUTH AFRICA

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Introduction

The South African Territories (SAT) type foot-and-mouth disease viruses (FMDV) are endemic to the greater Kruger National Park (KNP) area in South Africa, where it is maintained through persistent infections of African buffalo. The occurrence of FMDV within the KNP constitutes a continual threat to the livestock industry. To expand on knowledge of FMDV diversity, the genetic and antigenic relatedness of SAT2 type viruses isolated from cattle in 2013/2014 were investigated.

Materials and Methods

Molecular epidemiological relationships of the viruses were determined by sequencing and phylogenetic analysis. Genetically disparate viruses were chosen to determine neutralisation titres between outbreak viruses using virus neutralisation tests (VNT) against four reference sera. Differentiation of variants was determined by nucleotide sequence comparison of the outer capsid proteins to verify amino acids contributing to antigenicity. In addition, the antigenic relatedness (r_1-values) of the outbreak viruses and the most suitable vaccine match was determined.

Results

Phylogenetic analysis of the recent outbreak viruses revealed their genetic relatedness to other SAT2 isolates from topotype I (South Africa, Zimbabwe and Mozambique). The SAT2 outbreak viruses are genetically distinct from previously isolated viruses (2011 and 2012). High neutralisation titres were observed for all outbreak viruses tested against the reference sera representative of viruses from the endemic area in South Africa, as well as Zimbabwe. However, cross-neutralisation data for the outbreak viruses yielded different antigenic profiles. Comparison of the outbreak viruses with reference sera indicated a good vaccine match with 75% (12/16) of r_1-values > 0.4, 13% (2/16) of r_1-values between 0.3 and 0.4, and 13% (2/16) of r_1-values < 0.3.

Discussion

These results confirm the genetic and antigenic variability of SAT2 viruses, emphasising that continuous characterisation of field viruses is important with regards to determining the occurrence of new virus strains, epidemiological surveillance aspects and vaccination.

notes

CHALLENGES FOR FMDV DIAGNOSIS - CASE STUDY OF OUTBREAK CONFIRMATION IN UGANDA (2011 – 2014)

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Introduction

FMD is endemic in Uganda and confirmation is by antigen ELISA or PCR and sequencing. This paper studied the timelines between outbreak reporting and serotype identification/ confirmation.

Materials and methods

Data on FMD outbreak investigation and confirmation in Uganda (2011 – 2014) was reviewed.

Results

Year	No. of districts affected	No. of outbreaks investigated (Districts)	Stage of outbreaks	No. of outbreaks confirmed (Districts)	Serotype (s) identified by Ag ELISA	Serotype(s) Confirmed – RT-PCR & Sequencing	Time to confirmation
2011	22	7	Midway	4	-	4 (O)	> 4weeks
2012	15	3	Resolving	-	-	-	-
2013	8	6	Onset	3	A, SAT 2	2 (A), 5 (SAT 2)	Ag ELISA- < 1 week, PCR/ sequencing > 4 weeks
2014*	23	8	Midway	4	O	†	< 1 week

*: By 14th July, 2014; -: No result, †: Not yet (3rd – 14th July samples)

Discussion

FMD diagnosis is limited by delayed investigations and lack of appropriate samples. Antigen ELISA results can be obtained in less than 1 week compared to RT-PCR and sequencing where the results may take over 1 month due to cell culture and sequencing services abroad. More than 50% of the FMDV outbreaks were neither investigated nor confirmed. Availability of affordable, robust pen side serotype-specific tests may offer lasting solutions to FMD diagnosis in endemic countries.

notes

A VACCINE MATCHING STUDY FOR SAT2 VIRUSES IN SOUTHERN AFRICA

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Introduction

Foot-and-mouth disease (FMD) is widely considered an economically important disease of livestock, and is a compulsory OIE notifiable disease as it remains a global threat to national and international trade in livestock and livestock products. FMD is of particular importance in Africa where the disease is endemic and six of the seven immunologically distinct serotypes occur, with South African Territories type 2 (SAT2) being responsible for most of the FMD outbreaks in domestic animals in southern Africa. Control of FMD by vaccination is complicated due to inadequate reporting of outbreaks, uncertainty surrounding vaccine efficacy and low vaccine coverage.

Materials and methods

The antigenic cross-reactivity of SAT2 viruses in southern Africa has been tested against sera from four reference viruses using *in vitro* virus neutralisation tests. The capsid protein amino acid variation and one-way antigenic relationships (r1-values) were compared.

Results

Examining recent SAT2 outbreak viruses, the 38% variable amino acid residues in the outer capsid proteins stongly agreed with surface-exposed structural loops and known antigenic sites. For two reference sera a weak antigenic relationship has been observed with the field viruses in southern Africa, with only 14% of the viruses cross-reacting to the sera, an indication of poor vaccine match. However, antisera for the two remaining reference viruses had strong antigenic relationships with 68% and 61% of the field viruses being neutralised, respectively.

Discussion

From this initial in vitro cross-reactivity data it is clear that the SAT2/ZIM/7/83 antisera does not provide adequate cross-reaction and thus protection against majority of the field strains in southern Africa, whereas SAT2/ZIM/14/90 and SAT2/SAR/3/04 antisera cross-reacted sufficiently to the selected SAT2 field isolates. Furthermore, the genetic diversity in the capsid proteins of the SAT2 viruses are reflected in the antigenic properties of these viruses and therefore highlights the importance for the selection of vaccine strains that would provide the best vaccine match against emerging and re-emerging viruses.

notes

ANTIGENIC AND GENETIC CHARACTERIZATION OF FOOT-AND-MOUTH DISEASE VIRUS SEROTYPE O CIRCULATING IN EAST AFRICA

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Introduction

Foot-and-mouth disease (FMD) is one of the most economically important livestock diseases. The disease is endemic across Africa, with five of the seven known FMDV serotypes circulating in East Africa. Despite this, there is no effective control policy except ring vaccinations in selected dairy farms. The vaccine strains used in the region are out of date and do not match. Here we report the genetic and antigenic characterization of serotype O FMD viruses circulating in East Africa with a view to recommending suitable vaccine strains for use in the region.

Materials and methods

Two-dimensional virus neutralisation tests (VNT) were carried out using four different bovine post-vaccinal sera including one current vaccine strain and three putative vaccine strains and 50 FMDV serotype O viruses isolated from six East African countries and three neighbouring and livestock-trade-related countries, and results represented as antigenic relationship (r_1) values. In addition, full capsid sequence data was generated for all the viruses used in this study.

Results

Phylogenetic analysis revealed circulation of mainly East Africa (EA) topotype viruses in East African countries. In addition Middle East and South Asian (ME-SA) topotype viruses are also circulating in Libya and Egypt. Within East Africa topotype all the four sub-lineages (EA-1 to 4) of FMDV serotype O were detected. Preliminary vaccine matching results indicate all the three putative novel vaccine strains were broadly protective with East African serotype O FMD viruses compared to the locally produced O-KEN 78 vaccine strain. There was no linear correlation between r_1 values and no. of capsid amino acid changes.

Discussion

The serology and capsid sequence data will now be analysed further to predict the vaccine match. This may lead to identification of sequence motifs contributing to the loss of cross-reactivity with the antisera that can be tested in a reverse genetics system to study their impact on the antigenicity of the virus.

notes

SIMULTANEOUS IMMUNIZATION OF CATTLE WITH FOOT-AND-MOUTH DISEASE (FMD) AND LIVE ANTHRAX VACCINES.

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Introduction

FMD-vaccination in Argentina is performed under the control of the regulatory authorities and conducted by certified veterinarians. These organized campaigns may facilitate the controlled application of other vaccines against other endemic diseases, like Anthrax, which is controlled with a live vaccine. There is no information on the interference of immunity against FMD vaccines when applied together with a live bacterial vaccine. In this study we evaluated if the simultaneous application of Bacillus anthraces vaccine with the commercial tetravalent oil-based FMD vaccine used in Argentina modifies the antibody booster responses against FMDV.

Materials and methods

Two groups of 15 bovines each; with comparable Liquid Phase Blocking ELISA (LPBE) titers at 0 days post vaccination (dpv) were immunized with the currently used tetravalent FMDV vaccine (FMD-V) and a commercial attenuated bacterial vaccine (ABV) containing not encapsulated non-virulent spores (Sterne strain of Bacillus anthraces F234; 18,000,000 spores per dose). Both vaccines were applied simultaneously in different sides of the neck. Serum samples were obtained at 0, 25, 60 and 90 dpv and antibody titers against the vaccine O1/Campos strain were measured by LPBE and isotype-ELISA.

Results

Bovines immunized with FMDV-V or FMDV-V and ABV responded with a boost in the LPBE antibody titers at 25 dpv and remained within similar levels up to 90 dpv. However, animals vaccinated with FMD-V had significantly lower LPBE titers at 25 and 90 dpv, compared to those immunized simultaneously with both vaccines. The difference in total Ab titers at 25 dpv was due to an increase in IgG2 titers. Overall, kinetics of antibody titers was comparable.

Conclusions

The combined application of a live vaccine with the current FMD tetravalent vaccine used in Argentina did not interfere with the antibody FMDV-booster responses. Antibody titers were similar in both groups and followed comparable kinetics over time.

notes

REVIEW OF PCP-FMD IN GENE POOL 2 SOUTH ASIAN COUNTRIES

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Introduction

The South Asian countries of gene pool 2 FMD virus embarked on a PCP-FMD road map 2011 – 2020 by holding a regional meeting in November 2011. In that meeting Bangladesh, Bhutan, Nepal and Sri Lanka placed themselves at stage-1 of PCP-FMDwhile India placed itself at stage-3. Present study reports the review of progress and current status ofPCP-FMDroad map 2011-2020 of the South Asian countries.

Materials and methods

Information was collected and analysed from above countries in the check list comprising of questions regarding required and recommended activities for stage-1 of PCP-FMD. The countries developed activity plan for 2013 and 2014 to pursue the agreed road map.

Results

The check list based information indicated that Bhutan and Sri Lanka fulfilled all the requiredactivities of stage-1 of PCP while respectively meeting 92.1 % (n=35) and 60.5% (n=23) of recommended activities. The self assessment made by Bangladesh indicated that the country has met 90% (n=18) of required and 84.2% (n=32) of recommended activities. Nepal has yet to prepare a comprehensive plan which is required to enter in stage-1.
The activities not yet initiated or partially completed under PCP stage-1 were related to outcome 4 in Bangladesh and outcome 1-7 in Nepal.

Discussion

Sri Lanka is ready to move on to stage-2 of PCP-FMDin 2014 with Bhutan following in 2015. Bangladesh ishowever, required to enhance reporting and characterization of FMD outbreaks in addition to work on the legislation;study the movement of small ruminants and wild life to assess potential risks of FMD in the country.
Nepal prepared an activity plan for PCP-FMD stage-1 to move on to next stage in 2016.
India claimed to be at stage-3 and aimed to be at stage-4 by 2015 following its vaccination program in 221 selected districts in the country. In conclusion, Bangladesh, Bhutan and Sri Lankaare pursuing their roadmaps 2011-2020 to move on to next stage. India has prepared a FMD Control Programme for onward submission to OIE for its official endorsement.

notes

TOWARDS THE DEVELOPMENT OF A PEN-SIDE DIAGNOSTIC STRATEGY FOR CONTROLLING FOOT AND MOUTH DISEASE VIRUS WITHIN THE CONTROL ZONES OF THE SOUTHERN AFRICAN DEVELOPMENT COMMUNITY (SADC) COUNTRIES PHASE 1.

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Introduction

Livestock farming is a way of living for many people in the Southern African Development Community (SADC) countries and outbreaks of viral disease, such as Foot-and-Mouth disease (FMD), results in the imposition of stringent food-safety regulations that negatively affects the national economies of such states. Despite the accumulation of extensive knowledge of the disease as well as the availability of vaccines, attempts at eradicating FMD have remained unsuccessful. To that end, our study aims to integrate pen-side diagnosis into an emergency disease control programme.

Materials and methods

Phase 1 of this strategy is based on the development of a Loop-mediated isothermal amplification (LAMP) method for the SAT-specific amplification of FMD viral RNA fragments using a custom-engineered instrument suitable for use in the field. LAMP PCR primers were designed based on the conserved 3D polymerase gene of all three SAT virus serotypes. The ability of the primers to amplify viral nucleic acid was tested using SAT viruses representative of each of the three virus serotypes. The sensitivity and specificity of the assay was further evaluated by comparing results to those obtained using published LAMP and quantitative TaqMan real-time PCR assays.

Results

The SAT-specific LAMP assay could successfully amplify viral nucleic acid from each of the three SAT virus serotypes. The assay was 10 fold more sensitive than a previously published LAMP assay but not as sensitive as a previously published TaqMan real-time PCR assay.

Discussion

The results obtained in Phase 1 of this study indicate that the developed diagnostic platform is sensitive enough for the pen-site detection of FMD infections. This paves the way forward for the successful implementation and execution of the next phase of the study, which involves validation and integration of the developed assay into a surveillance and emergency response strategy in the field.

notes

CASE SERIES OF CLINICAL FORM OF FMD WITH LESIONS IN BASE OF HORNS IN IRAN

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Keywords: FMD, horn lesions, , cattle, Iran

Introduction

Foot and Mouth Disease (FMD) is a severe, highly contagious viral disease of livestock with significant economic impact. FMDV is an aphthovirus of the family Picornaviridae. The clinical signs are vesicles on the nose, tongue, oral cavity, toes, hooves and teats. During outbreaks in 2008 and 2013, multiple animals affected with FMD were seen with extensive horn lesions. This study is the first to describe these horn lesions in Iran.

Methods and Materials

Affected beef and dairy 24 herds in Qom province in FMD epidemics were inspected by local veterinary officers. Routine epithelium samples were submitted for FMD confirmation to the Central Veterinary Laboratory (CVL). In addition, samples of vegetative tissues of horn and tissues of base of affected horns were submitted for histopathology to the CVL and RAZI Institute.

Results

Affected cattle ranged between 8 and 12 years of age. Morbidity was 40 to 100 % and all cases were acute. In 5-10% of cases, vesicles and lesions in base of horns were seen whereas in fewer cases, horns were completely separated. Affected animals were not vaccinated against FMD before.
FMDV isolation and PCR tests were positive for all samples, Elisa tests showed FMDV serotypes as below: 10 herds with A05 in 2013 , 9 herds with Opanasia 2008 and 10 herds with Asia 1 in 1998. Histopathology showed aggregation of inflammatory cells in epithelial tissue of base of horns.

Discussion

There have been few cases of FMD virus with horn lesions due to FMD virus infection reported in the world before. These cases were the first reported in Iran. Occurrence of this lesion form in acute FMD cases related to three different serotypes of FMD showed that lesions in base of horns weren't related to serotype of FMDV but related to virulence and pathogenicity of FMD virus.

notes

ENHANCED SENSITIVITY OF FMDV ANTIGEN DETECTION ELISA BY NOVEL SIGNAL AMPLIFICATION SYSTEMS

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Introduction

FMD-antigen detection ELISA is a simple and strategic diagnostic tool, also enabling virus serotyping, though it may suffer from insufficient sensitivity when compared to virus isolation and RT-PCR, which provide for a preliminary amplification of the analyte to be detected. To achieve a desirable improvement of ELISA sensitivity we explored strategies aimed at increasing the signal of the detection system.

Materials and methods

The sandwich ELISA based on catching and detector monoclonal antibodies (MAbs), available as ready-to-use kit, was used as the reference assay for the evaluation of two amplification systems, which were both integrated in the ELISA kit platform.
The so called ANANAS (Avidin-Nucleic-Acid-Nano-Assembled-System, University of Padua) system is based on poly-avidin nanoparticles (about 100 nm) that improve the performance of avidin-biotin-based technologies: increasing the number of binding sites in avidin allows bringing together many more signal generating molecules. The analytical detection system is composed of biotinylated MAbs (the same detector MAbs that are conjugated with peroxidase in the ELISA kit), poly-avidin nanoparticles and biotinylated peroxidase.
ELAST (ELISA Amplification system, Perkin-Elmer), based on Tyramide Signal Amplification technology, amplifies the signal generated by immobilized peroxidase in ELISA assays. A biotinyl-tyramide reagent is activated by peroxidase resulting in covalent binding to tyrosine residues on the solid phase. Subsequent reaction with streptavidin-peroxidase results in the binding of additional peroxidase to the biotinylated solid-phase.
With both systems the colorimetric reaction was developed as for the unamplified ELISA.
Reference FMD viruses and negative controls were used for preliminary evaluation.

Results

Serial dilutions of reference strains of four FMDV serotypes (O, A, C and Asia-1) were analysed by the antigen detection and serotyping ELISA kit and the reaction was developed using ANANAS and ELAST amplification technologies in parallel to the conventional unamplified assay. Enhanced signals (absorbance values) were obtained with both amplification systems, with on average 15 to 20-fold improvement of detection limits for all the tested serotypes using ELAST and ANANAS methods respectively.

Discussion

The two signal amplification strategies significantly increased ELISA sensitivity without

background noise, overcoming one main limit of the test though maintaining typical advantages. The encouraging results lead to undertake further optimization and validation on field samples.

Acknowledgments: research was supported by national grants PRC2011/014 and PRF2010/203.

notes

PATHOLOGICAL CHANGES IN PIGS EXPERIMENTALLY INFECTED WITH FOOT-AND-MOUTH DISEASE VIRUS ISOLATED FROM THE 2010 EPIDEMIC IN JAPAN VIA THE INTRANASAL AND INTRAORAL ROUTES

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Introduction

There are a limited number of reports on pathology in pigs infected with Foot-and-mouth Disease Virus (FMDV) within Southeast Asia (SEA) topotype. To deepen our understanding of pathogenesis of FMDV SEA topotype in pigs, we examined pathological changes in pigs experimentally infected with FMDV isolated from the 2010 epidemic in Japan via the intranasal and intraoral routes.

Materials and methods

Each three pigs were inoculated intranasally with 106 and 103 TCID50 of FMDV O/JPN/2010 isolate. Four and three pigs were inoculated orally with 106 and 103 TCID50 of FMDV O/JPN/2010 isolate, respectively. Infected pigs were examined clinically and euthanized at 14 days post inoculation (dpi). At necropsy, tissue samples were collected from each pig, fixed in 10% neutral buffered-formalin and embedded into paraffin wax for histology. Sections were stained with hematoxylin and eosin.

Results

Vesicular lesions were induced to pigs by intraoral inoculation with both 106 and 103 TCID50 of the virus from 2 and 7 dpi, respectively. By intranasally, vesicular lesions were induced to pigs with 106 TCID50 from 3 dpi, however, were not induced with 103 TCID50. Histologically, subcorneal vesicles with dermatitis were observed in the skin of the coronet and heel in pigs inoculated orally with 103 TCID50. In pigs inoculated with 106 TCID50 via both the intranasal and intraoral routes, only resultant erosions healed of the vesicles were seen in the skin of feet, the snout and inner lip. Laminitis was appeared in pigs which showed resultant erosions on the feet. The histological lesion seen in pigs inoculated intranasally was milder than those in pigs inoculated orally.

Discussion

The result showed that oral cavity may be a major entry route of an FMDV in naturally exposed pigs. Although the vesicular lesion heals, secondary laminitis may be developed and lead to lameness in pigs infected with FMDV.

notes

DETERMINATION OF THE POTENCY (PD50) OF A FMD O PANASIA 2 VACCINE IN CATTLE

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Introduction

To address FMDV antigenic variation and broaden the width of vaccine antigenic coverage, a new strain from the serotype O, ME-SA topotype Panasia-2 lineage was developed into a vaccine. We relate here the determination of the potency of this vaccine in cattle.

Materials and methods:

Vaccine : purified, inactivated O Panasia 2 antigen formulated in double oil emulsion
Animals : 22 bovines (> 6 months), free from FMD antibodies

Study design

On D0, animals were allocated to 5 groups and vaccinated as follows :
Group 1 : 5 animals, 2 mL vaccine IM (full dose)
Group 2 : 5 animals, 0.5 mL vaccine IM (1/4 dose)
Group 3 : 5 animals, 0.125 mL, vaccine IM (1/16 dose)
Group 4 : 5 animals, 0.033 mL vaccine IM (1/64 dose)
Group 5 : 2 animals, unvaccinated controls
on D28, all animals were challenged with approximately 10.000 cattle ID50 of a virulent O Panasia 2 isolates, intradermally in the tongue. Animals were then monitored daily for 8 days for rectal temperature and clinical signs. All animals were inspected for FMD lesions (feet, mouth & lips, tongue and nose) on D31 and D36. Animals presenting feet lesions on D31 were euthanized on that date. All other were euthanized on D36.

Results

Detailed results will be presented.
Both controls had developed FMD lesions on all feet, on D31, thus validating the challenge. There was a strong relation between the vaccine dose injected and the protection against feet lesion, with 100 % protection in the groups 1 and 2 and 80% protection in group 3. The potency of the vaccine was estimated ≥ 30 PD50.
This study thus demonstrated that the vaccine tested provides a very high level of protection against a O Panasia 2 challenge.

notes

SAFETY EVALUATION OF AFTOPOR® VACCINE FOLLOWING ADMINISTRATION TO DAIRY COWS

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Introduction

Aftopor® is an inactivated, purified, FMD vaccine in a double oil emulsion. It has been used routinely in all categories of cattle for several years.
Side effects of vaccination are a constant concern for high-producing dairy farmers, who have to balance the potential losses with the benefits of annual vaccination.
The goal of this trial was to substantiate the impact of vaccination on milk production and gestation.

Material and Methods

240 dairy cows were included in the study. They originated from three commercial dairy farms situated in different climatic regions in Israel, 80 cows per farm. The cows were randomly allocated (by parity and day of gestation) into two study groups of 40 animals in each farm:

- G1 vaccinated group, vaccinated with trivalent Aftopor® vaccine (O, A, Asia1)
- G0 non-vaccinated control group, received a placebo (sterile saline solution)

The animals were clinically followed pre- and post-vaccination on the day of treatment, and for 4 consecutive days post-vaccination. Local and general clinical signs, temperature, daily milk production, and parturition were recorded.

Results

- None of the pregnant cows in the trial aborted.
- Rate of calf mortality, at birth or shortly after birth, were within normal range for each farm.
- No systemic adverse effects were observed, when compared to control.
- Local reactions were mild and not significantly different between vaccinated and control groups.
- A moderate increase of 0.4°C in rectal temperatures was observed in vaccinated animals in the 3 days after vaccination, significantly different from the controls only on the day after on vaccination.
- A minor milk drop (on average 2.59kg on the first day) was observed for a transient three day period following vaccination. Milk yield resumed to normal values 3 days post vaccination.

Discussion

Aftopor® is well tolerated in dairy cattle, with moderate temperature increase and minor drop in milk production for two days after vaccination.

notes

THE EXTERNAL QUALITY CONTROL OF ASSAYS' RESULTS FOR DIAGNOSIS AND SURVEILLANCE OF VESICULAR DISEASE OF ANIMALS

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Introduction

National Reference Laboratory for FMD and SVD of the Institute for Diagnosis and Animal Health has participated to the proficiency tests organized by the EuRL Pirbright since 2004. Proficiency testing is an essential element in the frame of accreditation policy, providing strong evidence of the activities of the quality management system.

Materials and methods

FMD and SVD Combined Proficiency Testing Scheme - 2013
Panel of samples for virus isolation, antigen detection and genome detection
- Virus isolation on cell cultures;
- Antigen detection by indirect sandwich ELISA and SVD;
- Viral genome detection by Real Time RT – PCR and conventional nested RT-PCR
Panel of samples for antigen detection and genome detection (non-infectious material from cattle or pig);
- Antigen detection by indirect sandwich ELISA for FMD serotypes and SVD;
- Viral genome detection by Real Time RT – PCR and conventional nested RT-PCR
Panel of samples from suspected FMD cattle for serology diagnosis
¨ SP antibody detection by:
- LPB ELISA for FMD serotypes;
- competitive ELISA
¨ NSP antibody detection by:
- blocking ELISA;
- indirect ELISA;
¨VNT
Panel of samples from pigs suspected for suspected SVD cases
- competitive ELISA;
- LPB ELISA;
- VNT.

Results

- Carrying on the diagnosis methodology and obtaining of individual results;
- Overall interpretation of results for each panel of samples – healthy animals, infected animals (identifying the serotype / virus) or vaccinated animals;
- supplying of detailed information about tests and standard operating procedures used, reference materials, level of accreditation, data about national surveillance programs.

"The results presented were comparable with most of the labs".

Discussion

Using the diagnosis methodology on panel of samples of exotic diseases of animals is a very useful exercise for free countries. Therefore is possible the optimization of diagnostic assays, assimilation of new methods and maintaining of working capabilities of the laboratory, according to SR EN ISO 17025.

notes

SEROLOGICAL RESPONSES OF CATTLE INOCULATED WITH AN INACTIVATED TRIVALENT FOOT-AND-MOUTH DISEASE VACCINE

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Introduction

The routine prophylactic vaccination of cattle is a method of choice for the control of Foot-and-mouth disease (FMD) in endemic countries. Cattle within the FMD protection zone with vaccination of the Kruger National Park (KNP) are routinely vaccinated for FMD using a trivalent (SAT 1-3) inactivated vaccine.

Materials and methods:

Four dip tanks from a list of 16 community dip tanks in the Mnisi communal area were purposively selected based on the scheduling of weekly animal inspection at the dip tanks. Cattle aged >6 months were selected from available herds after obtaining informed consent from owners. Cattle were vaccinated using a trivalent inactivated FMD vaccine (SAT 1-3) and longitudinally followed for a period of 112 days. Blood samples were collected on a fortnightly bases and analysed for FMD-specific antibodies using a liquid phase blocking ELISA.

Results

A total of 293 cattle were sampled and few cattle had evidence of pre-existing antibody responses to SAT viruses at the beginning of the study. However, 14 days post-vaccination, the proportion of seropositive cattle (≥2 log10 titre) to the three SAT type viruses varied between 39% - 77% with SAT 2 having the highest proportions. Antibody responses peaked up to 98%, 98% and 65% at 42 days post-vaccination for SAT 2, SAT 3 and SAT 1 respectively until starting to decline at 56 days post-vaccination.

Discussion

Sampled cattle had serological responses to vaccination of limited duration and this in combination with few cattle having evidence of pre-existing antibody responses suggests that poor serological responses might be a risk factor for FMD outbreaks.

notes

THE DIAGNOSTIC UTILITY OF STABILIZED BLOOD FOR DETECTION OF FOOT-AND-MOUTH DISEASE VIRUS RNA BY RT-qPCR

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Introduction

In Europe, clinical signs indicative of foot-and-mouth disease (FMD), would immediately lead to collection of blood and relevant organ material for further laboratory examination for this vesicular virus disease. Today, the first line system for detection of virus in the sample material is real time RT-PCR (RT-qPCR). The aim of this study was to investigate the diagnostic utility of stabilized blood for detection of FMDV RNA in this system.

Materials and Methods

EDTA-stabilized and unstabilized blood (serum) samples were collected from pigs and cattle during experimental studies. The cattle experiment included 13 animals (5 inoculated and 8 contacts) infected with FMDV serotype O. The pig experiment included 14 animals (4 inoculated and 10 contacts) infected with FMDV serotype A. At days 0-15 post inoculation (dpi), blood was collected from the cattle and pigs. All samples were analysed after robotic extraction of RNA using RT-qPCR and the sensitivity of detection of FMDV RNA in the two different types of blood samples was compared.

Results

In the cattle experiment, 13/13 animals developed clinical signs (indicative of FMD) and viral RNA was detected in serum as well as in EDTA-stabilized blood samples from all animals. In the pig experiment, 10/14 animals developed clinical signs and viral RNA was also detected in both sample types. Results from these experiments showed a similar profile of RNA detection but with, in general, reduced sensitivity for EDTA-stabilized blood compared to serum.

Discussion

In this study, viral RNA from FMDV could be detected in both unstabilized and EDTA-stabilized blood, however, for EDTA-stabilized blood with lower sensitivity. In the present set-up, it can therefore not be advised to use EDTA-stabilized blood for individual monitoring of infection in FMDV infected animals, however, on a herd basis (large sample size) this material will provide a clear picture of the FMDV infection status.

notes

PRODUCTION AND CHARACTERIZATION OF MONOCLONAL ANTIBODIES AGAINST FOOT-AND-MOUTH DISEASE VIRUS TO DEVELOP DIAGNOSTIC ASSAYS

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Introduction

Sensitive and specific FMD laboratory diagnostic as well as rapid identification of the serotype involved is essential in case of outbreak. Furthermore serological surveillance is crucial in FMD free country or in a country with sporadic outbreaks. This study aims to produce and characterize monoclonal antibodies (mAbs) against FMDV to use them to develop diagnostic assays.

Materials and methods

A mAbs panel has been produced in mice immunized with vaccinal antigen O1 Manisa, A5 Allier or C1 Noville. The mAbs panel was first screened by ELISA using vaccinal antigens from the seven serotypes. mAbs which gave a specific positive result were further tested by IF. Immunofluorescence (IF) assays were carried out on either infected IBRS-2 cells (positive control) or on IBRS-2/BHK-21 cells transfected with expression vectors (pIRES2AcGFP1, Clontech) encoding FMDV structural proteins.

Results

Among the 27 mAbs analysed, 12 were O1 Manisa-specific, 4 were A5 Allier-specific and 11 were C1 Noville-specific. Further analysis with IF assays indicated that among the 12 O1 Manisa-specific mAbs, 5 recognize the VP2. Among the 4 A5 Allier -specific mAbs, 3 recognize the VP1.

Discussion

Up to now, a part of the mAbs panel has been already characterized and the work is underway concerning the others, necessitating the construction of eukaryotic expression vectors encoding viral proteins of interest. To complete these data, we will carry out Western Blot analyses to ascertain if the epitope recognized is conformational or linear. We will also study the virus-neutralizing capacities of the mAbs.

notes

USE OF LATERAL FLOW DEVICE FOR SAFE AND LOW COST SHIPMENT OF FMDV SUSPECTED SAMPLES

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Introduction

An essential step towards the global control and eradication of FMD is to identify circulating strains in endemic areas. However, biological risk and shipment under freeze conditions are two of major obstacles to submission of suspected samples to Reference Laboratories. Penside tests based on immune-detection method on strip are used on field to detect FMDV. In this study, we aim to develop a low cost and safe method for shipment of FMD samples, based on the inactivation of FMDV on the lateral flow device, allowing its subsequent detection by real-time RT-PCR and recovery of live virus upon transfection.

Materials and methods

FMDV strains were deposited onto penside tests (Svanodip® FMDV-Ag). Different concentrations of citric acid and sodium hydroxide solutions were tested to inactivate virus on the strip. Strips were then disassembled and grounded. Monolayer cells (IBRS-2 and ZZ-R127) were then incubated with grinding supernatant for 48 hours. A second passage in cell culture was realized in the same conditions. In parallel, viral RNA was extracted from grinding supernatant by using the QIAamp Viral RNA mini kit. Real-time RT-PCR targeting FMDV genome (3D and IRES) and the endogen cellular gene (β-actin) were performed in duplex.

Results

After treatment of “FMDV collector strips” in a 0.2% citric acid bath during 15 minutes, FMDV was found to be inactivated. Indeed no CPE was observed after two passages in cell cultures. Viral RNA was however detected by 3D and IRES real-time RT-PCR.

Discussion

After live FMDV collection onto penside strip and adequate chemical treatment, FMDV is inactivated but viral RNA is still detectable by real time RT-PCR. To determine if live virus can be rescued from treated FMDV collector strips, viral RNA transfection assays will be performed. We will finally evaluate and validate this process on field samples.

notes

BIOLOGICAL SURVEILLANCE OF FOOT AND MOUTH DISEASE IN THE REPUBLIC OF AZERBAIJAN

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Introduction

Foot and mouth disease outbreaks have occured in Azerbaijan in different years. In neighboring states, such as Iran and Turkey, foot and mouth disease epizooty is reported every year. Under FAO project twice a year livestock and small cattle is vaccinated against the disease as a preventive measure Biological surveillance of foot and mouth disease in the country for the years 2010-2011. The purpose were biological surveillance of foot and mouth disease in the country for the years 2010-2011 vaccination supervision and quality control of vaccines, identification of asymptomatic animals and managing control over animals passing through the border.

Materials and methods

Assays were conducted with new diagnostic kits- trapping-indirect ELISA for the non-structural polypeptide 3ABC of FMD virus in serum samples of large and small ruminants and a solid phase competitive ELISA, using a selected neutralizing anti-FMDV monoclonal antibodies (MAbs).

Results

Total 4,500 serum samples were collected across the country as a result of this study. Assay result:
From 2,300 livestock serum samples: 1, 919 were negative and 381 were positive.
From 598 small cattle serum samples: 497 were negative and 101 were positive.
From 80 serums isolated from livestock brought from Germany: 80 were negative.
From 200 sheep serum samples collected in bazar(market): 116 were negative and 84 were positive.
From 120 serum samples isolated before and after vaccination in Absheron: all 120 were negative.
10% positive results were obtained across the country

Discussion

As a result of seromonitoring, it was defined that the epizootic state against foot and mouth disease was stable. The high percentage of positive results among animals passed through the border and sold in bazars indicates the necessity for imposing a quarantine on imported animals. The positive results obtained shows the need to continue the vaccination.

notes

TESTING THE EFFICACY OF A MALAYSIA 97 HIGH POTENCY VACCINE AGAINST CHALLENGE WITH A/VIETNAM/2005 (A SEA-97 STRAIN) IN PIGS

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Introduction

A high potency A Malaysia 97 vaccine was tested in pigs for its ability to provide early protection against heterologous challenge. In addition, to determine whether vaccination decreases virus excretion to prevent disease dissemination, unvaccinated pigs were kept in close, indirect contact.

Materials and Methods

Pigs were vaccinated 4 (n=8) or 7 (n=8) days prior to needle challenge with a pig adapted A/Vietnam/2005 (A SEA-97) strain. Five unvaccinated, uninfected pigs were kept in the same room, but without direct contact, as each of the vaccinated/challenged groups, or unvaccinated controls. Clinical scores were recorded daily and virus levels in excretions measured using qRT-PCR. Serological responses to non-structural and structural viral proteins were determined using ELISA.

Results

All pigs challenged 4 days post-vaccination (dpv) were protected whereas one animal challenged 7 dpv showed disseminated disease. The swabs taken from the vaccinated and challenged animals remained positive for viral RNA up to 10 days post-challenge (dpc) in some cases. The in-contact pigs for both groups remained clinically normal, while viral RNA was detected intermittently in nasal swabs of only those in contact with pigs challenged 4 dpv. Although all the unvaccinated control pigs were infected and excreted virus for 2–9 days, disease was not transmitted to the in-contact pigs and viral RNA was not detected in the secretions of these pigs. Three pigs vaccinated 7 days before challenge had antibodies to FMDV structural proteins on the day of challenge, while only one pig in this group sero-converted to FMDV non-structural proteins (NSP) 14 dpc. In the group challenged 4 dpv, three pigs sero-converted to NSP 7–14 dpc.

Discussion

Only one vaccinated pig showed clinical disease, although virus replicated in more pigs, as

demonstrated by sero-conversion to NSP. Therefore, the high potency A Malaysia 97 vaccine successfully protected pigs from heterologous challenge as early as 4 and 7 dpv.

notes

CONCENTRATION AND QUANTIFICATION OF FOOT AND MOUTH DISEASE WHOLE VIRUS PARTICLE

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Introduction

The outbreaks of foot-and-mouth disease (FMD) type A and type O occurred in South Korea in the first half of 2010 and type O was re-occurred in the second half of 2010. In order to discriminate between infected and vaccinated animals, it is of primary importance that the FMD vaccines should not contain nonstructural protein (NSP). The present study was conducted to find the optimal concentration of polyethylene glycol (PEG) for concentrating FMDV type Asia 1 (Mongolia strain).

Materials and methods

FMD Asia 1 virus was obtained from Institute for Animal Health (Pirbright, UK). The FMD viruses were grown in BHK-21 monolayer cell culture, collected 1-2 days after inoculation and clarified by centrifugation. The FMD virus suspensions were inactivated with binary ethyleneimine (BEI) at 3mM for 24 hours. The inactivated FMD virus was concentrated by various PEG 6000 (Sigma-Aldrich, Aldrich Chemical Co., Milwaukee, WI, USA) concentrations (2.5%, 5.0%, 7.5%, 10%, and 12.5%) for 18 hours at 4ºC. The pellet was suspended using Tris-NaCl buffer. The sucrose gradient ultracentrifugation (25,000rpm for 4 hours) was used to obtain purified 146S particles followed by collecting fractions to quantify protein concentration at 250 nm.

Results

In 7.5% PEG 6000 concentration, the FMDV 146S particles were best recovered with a concentration of 146μg/ml. The NSP was not detected in all PEG-treated groups as measured by Prionics FMD 3ABC NSP ELISA (PrioCHECK; Prionics, Schlieren, Switzerland).

Discussion

We investigated the optimal PEG concentration to concentrate FMDV 146S particle which is important in the vaccine production process. It was observed that 7.5% PEG was optimal concentration for recovering FMDV 146S particles.

notes

ANTIVIRAL EFFECT OF THE ANTIVIRAL AGENT (T-1105) ON VARIOUS SEROTYPES OF FOOT-AND-MOUTH DISEASE VIRUS

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Introduction

Effective antiviral agent is required for Foot-and-mouth disease (FMD) outbreak control because the current FMD vaccine could not be able to produce protective antibodies against FMDV until 7 days post vaccination. Hence we evaluated in vitro the antiviral effect of T-1105, which is a FMD viral RNA-dependent RNA polymerase (3D polymerase) inhibitor.

Materials and methods

We measured the 50% cytotoxic concentration (CC50) and the 50% effective concentration (EC50) for the T-1105 (Toyama Chemical Co., Ltd., Japan) compound. For measuring CC50, swine kidney (IBRS-2) cells were treated with the compound without FMDV for 72 hours at 37□. For measuring EC50, IBRS-2 cells were treated 100 TCID50 of FMDV for one hour and then removed supernatant and treated with the compound for 72 hours at 37□. We measured optical density (OD) using MTS assay for evaluation of cell proliferation and calculated selectivity index (SI) based on the results of OD of CC50 and EC50 to find out the optimal concentration of compound. Also, we carried out real-time RT-PCR to quantify FMD viral RNAs after treating the compounds in the cell.

Results

We tested antiviral effect of T-1105 on nine serotypes of FMDV which are O/SKR/2000 (ME-SA/PanAsia), O/SKR/2002 (ME-SA/PanAsia), O/Andong/SKR/2010 (SEA/Mya-98), A/Pocheon/SKR/2010, A22/Iraq 24/64, Asia1/MOG/05, Asia1/Shamir/89, A/Malaysia/97, and O1/Manisa/Turkey/69 (ME-SA/PanAsia). T-1105 had lower SI than ribavirin, which is the commercial antiviral agent against FMDV. T-1105 was effective against seven serotypes of FMDV based on SI, except for two serotypes of O/SKR/2002 and O/Andong/SKR/2010.

Discussion

We evaluated T-1105 as an antiviral agent against several serotypes of FMDV in vitro. On the basis of our results, T-1105 showed the antiviral effect against some serotypes of FMDV. Further study is required to confirm the antiviral effect of T-1105 based on animal experiments.

notes

THE IMPACT OF A FOOT-AND-MOUTH DISEASE OUTBREAK IN SOUTH AFRICA

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Introduction

Foot-and-mouth disease (FMD) has a profound effect on agriculture in South Africa. Whilst seldom fatal, it hampers production and prohibits trade in animal products. The disease is highly contagious and affects cloven hoofed animals. The major host (carrier of FMD virus) in South Africa is the African buffalo. The eradication of the disease is practically impossible given the importance of the host species in terms of conservation policies. Practically, the control of FMD consist of maintaining an infected area from which no export is allowed, and vaccinating domestic animals (especially smallholder farmers cattle and goats) within the buffer zone.

Materials and Methods

This study aims to quantify the economic effects of FMD and in particular a ban on trade, on the local economy, which strengthens the argument for maintained investment in vaccine development.

Results

A FMD outbreak in the FMD-free zone effectively precluded any trade in these animals and their products as during 2011 in KwaZulu-Natal. Beef exports to the European Union had been banned, costing the country according to industry estimations roughly R4 billion annually, which had a negative impact on jobs and economic growth. South Africa regained its FMD-free status on 14 February 2014.

Discussion

This study indicated the economic effects of FMD and in particular that of a ban on trade, on the local economy. A preliminary assessment was completed followed by a more detailed assessment of the impact to date. The ARC-OVI will continue to contribute to efforts aimed at controlling FMD in collaboration with national, regional and international agencies.

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